ANTIOXIDANT, GENOPROTECTIVE AND HEPATOPROTECTIVE ACTIVITIES OF *PANUS GIGANTEUS* (BERK.) CORNER

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FACULTY OF SCIENCE
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ABSTRACT

Panus giganteus, a culinary and medicinal mushroom consumed by selected indigenous communities in Malaysia is currently being considered for large scale cultivation. This study was performed to investigate the medicinal potential of *P. giganteus* fruiting bodies and wheat grains fermented by *P. giganteus* including antioxidant, genoprotective and hepatoprotective properties.

Ethanol extracts of *P. giganteus* fruiting bodies, wheat grains fermented by *P. giganteus* and unfermented wheat grains exhibited moderate antioxidant properties by virtue of DPPH free radical scavenging activity, reducing power, antioxidant capacity and inhibition of lipid peroxidation. The extracts also contained moderate amounts of phenolic compounds. Fruiting bodies were more potent than fermented and unfermented wheat grains in protecting DNA of peripheral blood mononuclear cell (PBMC) against hydrogen-peroxide (H_2O_2)-induced damage. However, all the extracts had comparable activities to repair DNA damaged by H_2O_2.

Hepatoprotection studies indicated that *P. giganteus* fruiting bodies were able to prevent and treat liver injury induced by thioacetamide (TAA). Administration of *P. giganteus* lowered the elevated liver body weight ratio, also restored the levels of serum liver biomarkers and oxidative stress parameters comparable to the standard drug silymarin. Gross necropsy and histopathological examination further confirmed the hepatoprotective effects of *P. giganteus.*

This is the first report on the medicinal properties of locally grown *P. giganteus*. Overall, consumption of *P. giganteus* fruiting bodies or wheat grains fermented by *P. giganteus* have genoprotective and hepatoprotective effects against injury induced by oxidative stress.

ABSTRAK

Panus giganteus merupakan cendawan yang digunakan dalam masakan dan untuk tujuan perubatan. Ia digunakan oleh masyarakat asli di Malaysia dan sedang dipertimbangkan untuk penanaman secara besar-besaran. Kajian ini telah dijalankan untuk mengkaji nilai-nilai perubatan *P. giganteus* termasuk antioksidan, potensi untuk melindungi DNA dan hati.

Ekstrak etanol dari cendawan *P. giganteus*, bijirin gandum yang ditapaikan oleh *P. giganteus* dan bijirin gandum yang tidak ditapaikan mempunyai nilai antioksidan yang sederhana. Mereka berupaya untuk menghapuskan radikal bebas DPPH, mempunyai kuasa penurunan, menunjukkan kapasiti pengoksidaan serta dapat merencatkan oxidasi lipid. Semua ekstrak juga mempunyai jumlah sebatian phenol yang sederhana. Ekstrak etanol dari cendawan lebih berpotensi daripada ekstrak lain dalam perlindungan DNA. Walaubagaimanapun, semua ekstrak adalah setanding dalam pemulihan DNA selepas dicederakan oleh H_2O_2.

Panus giganteus juga menunjukkan keupayaan untuk mencegah dan merawat kecederaan hati yang diinduksikan oleh thioacetamide (TAA). Penggunaan *P. giganteus* bukan sahaja menurunkan nisbah berat badan dengan hati, malah ia memulihkan tahap penanda biologi hati di serum dan parameter tekanan oksidasi ke paras yang setanding dengan silymarin. Ini seterusnya disahkan oleh ujian nekropsi kasar dan pemeriksaan histopatologikal.

Laporan ini merupakan kajian pertama ke atas nilai-nilai perubatan *P. giganteus* yang ditanam di Malaysia. Secara keseluruhannya, penggunaan cendawan *P. giganteus* atau bijirin gandum yang ditapai oleh *P. giganteus* berpotensi untuk melindungi DNA dan hati daripada kecederaan yang diinduksi oleh tekanan oksidasi.

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LIST OF SYMBOLS AND ABBREVIATIONS

A_{blank}	Absorbance of blank
A_{sample}	Absorbance of sample
$ABTS^{\bullet*}$	2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)
ANOVA	Analysis of variance
AOAC	Association of Analytical Communities
BHT	Butylated hydroxytoluene
℃	Degree Celcius
$C_2H_3NaO_2 \bullet 3H_2O$	Sodium acetate trihydrate salt
cm	Centimetre
dH_2O	Distilled water
DMSO	Dimethyl sulfoxide
DPPH	1,1-diphenyl-2-picrylhydrazyl
EC_{50}	50% effective concentration
EtBr	Ethidium bromide
Fe^{2+}	Ferrous
Fe^{3+}	Ferric
$FeCl_3.6H_2O$	Ferric trichloride hexahydrate
$FeSO_4.7H_2O$	Ferrous sulfate heptahydrate
FRAP	Ferric reducing antioxidant power
g	Gram
GAEs	Gallic acid equivalents
GYMP	Glucose-Yeast-Malt-Peptone
HCl	Hydrochloric acid
H_2O_2	Hydrogen peroxide
$(HOCH_2)_3CNH_2$	Tris(hydroxymethyl)aminomethane

IC_{50}	Concentration to scavenge 50% free radicals
i.p	Intraperitoneal injection
KH_2PO_4	Potassium dihydrogen phosphate
K_2HPO_4	Dipotassium phosphate
$K_2O_8S_2$	Potasium persulfate
mA	Milliampere
MDA	Malondialdehyde
mg	Milligram
mg/kg	Milligram per kilogram
mg/ml	Milligram per millilitre
mg of GAEs/g	Milligram of gallic acid equivalents per gram
$MgSO_4.7H_2O$	Magnesium sulfate heptahydrate
min	Minute
ml	Millilitre
mM	Millimolar
NaCl	Sodium chloride
Na_2CO_3	Sodium carbonate
$Na_2EDTA.2H_2O$	disodium EDTA titriplex
$NaHPO_4$	Sodium hydrogen phosphate
NaOH	Sodium hydroxide
NH_4Cl	Ammonium chloride
nm	Nanometre
$O_2^{\cdot -}$	Superoxide radical
PBS	Phosphate buffered saline
PDA	Potato dextrose agar
po	Oral feeding

psi	Pound per square inch
R^2	R-squared
RDA	Recommended daily allowance
rpm	Rotation per minute
SDS	sodium dodecyl sulfate
S.E.M	Standard error of mean
TAA	Thioacetamide
TBA	Thiobarbituric acid
TBARS	Thiobarbituric Acid Reactive Substances
TCA	Trichloroacetic acid
TEP	1,1,3,3,-tetraethoxypropane
TPTZ	2,4,6-tripyridyl-s-triazine
Trolox	6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid
µ	Micro
µg	Microgram
µg/ml	Microgram per millilitre
µl	Microlitre
µM	Micromolar
µmol of $FeSO_4.7H_2O$ equivalents/g	Micromole of ferric reducing antioxidant power equivalents per gram
V	Voltage
v/v	Volume per volume
w/v	Weight per volume
±	Plus-minus
8-OH-dG	8-hydroxy-2'-deoxyguanosine
%	Percent

CHAPTER ONE: INTRODUCTION

According to Chang and Miles (1992), mushrooms are "macrofungi which have distinctive fruiting bodies, which can be hypogeous or epigeous, large enough to be seen with the naked eye and to be picked by hand". Since ancient times, mushrooms have been essential food for human due to its unique fragrance and delicate flavour (Manzi *et al.*, 1999). Mushrooms are high in proteins, vitamins and minerals but low in fats and calories (Jayakumar *et al.*, 2009). Hayes and Haddad (1976) stated that all essential amino acids needed by an adult can be obtained from mushrooms. As the amino acid composition of mushroom protein are equivalent to animal protein, mushrooms carry high dietary importance due to high health risks associated with consumption of animal foods and high protein demand due to the increasing of human population (Guillamon *et al.*, 2010).

Mushrooms have been long recognized for its profound health properties. According to Lambert (1938), the consumption of mushrooms is much earlier in eastern civilisation than the European. Chinese have used mushrooms as folk medicine for thousand years although the active ingredients and health boosting mechanisms of mushrooms are actively studied by scientists recently. In Chinese traditional medicine, the mushroom extracts are usually mixed with different herbs in various ways to treat medical disorders (Diyabalanage *et al.*, 2008). The remarkable effects of mushrooms in promoting good health have been supported by some previous studies.

Nowadays, mushrooms are regarded as functional food as they accumulate several physiologically active compounds with no side-effects (Sadler, 2003). The compounds isolated from mushroom such as polysaccharides, polysaccharide–protein complexes, triterpenes, phenols, peptides and lectins possess antitumor, immunomodulatory, antiviral and antimicrobial properties. Mushrooms hold great potential in drug and nutraceutical development (Lindequist *et al.*, 2005). The dried fruiting bodies and extracts of mycelia grown in submerged or solid substrate fermentations are marketed as supplements in the form of tablets, capsules or powders (Wasser, 2005).

Panus giganteus is a known edible mushroom and widely consumed by the indigenous communities in Malaysia. It was introduced by local mushroom growers and Department of Agriculture Malaysia in 2003 for cultivation purpose. Previously, it was known as ‘cendawan perut lembu’ (cow’s stomach mushroom) and has been renamed as lowland shiitake or ‘cendawan seri pagi’ (morning glory mushroom). Although *P. giganteus* is relatively new in Malaysia, it is popularly consumed in China and overseas (Deng *et al.*, 2006). Preliminary studies showed that *P. giganteus* can be easily cultivated in Malaysia although it was originally come from China. Currently, large scale cultivation of this species looks promising with a kilogram of mushroom priced at USD 7 (Nabil, personal communication, March 15, 2011). However, poor knowledge and paucity of scientific studies on the benefits of this mushroom as compared to other species including *Lentinula edodes* (Wang *et al.*, 1999) and *Ganoderma lucidum* (Boh *et al*., 2007) may affect consumer acceptance of *P. giganteus* grown in Malaysia.

The endogenous oxidation processes and exogenous sources produced reactive oxygen species (ROS) and free radicals. According to Barros *et al*., (2008), these ROS and free radicals pose harmful effects and involved in the onset of cancer, rheumatoid arthritis, liver cirrhosis, arterioscleorosis besides of degenerative diseases related with ageing. However, nearly all living organisms equipped with antioxidant defense system which mainly comprised of antioxidant enzymes which include catalase (CAT), glutathione peroxidise (GSHPx), superoxide dismutase (SOD), or chemicals including ascorbic acid, a-tocopherol, carotenoids, glutathione and polyphenol substances (Niki *et al*. 1994). However, this may only prevent certain degree of damages caused.

Recently, there is a great interest in research regarding natural antioxidant of plants and mushrooms origin which could help to reduce oxidative damage. Many studies have proved that edible mushrooms contain appreciate amounts of antioxidants. Phenolic compounds are the most common antioxidants found in mushrooms subsequently followed by tocophenols, ascorbic acid and carotenoids (Vaz *et al.*, 2010). Many of these compounds were quantified from a number of mushroom species especially from Finland, India, Korea, Poland, Portugal, Taiwan and Turkey (Ferreira *et al*., 2009). According to Cheung *et al*. (2003), antioxidant properties of water crude extracts of Shiitake mushroom (*L. edodes*) and straw mushroom (*Volvariella volvacea*) were closely associated with their total phenolic content. Further, a number of locally grown mushrooms which include *Auricularia auricular*-judae, *G. lucidum*, *Hericium erinaceus*, *Pleurotus sajor-caju* were also shown to exhibit antioxidant properties (Noorlidah *et al*., 2012).

Single cell gel electrophoresis or popularly known as comet assay is a common method used to investigate the prevention and repairing of DNA damage. Comet assay is cost effective, sensitive, rapid and easy to perform (Tice *et al*., 1990). Physiological processes and exposure to environmental mutagens and carcinogens can cause DNA damage up to one million individual molecular lesions per cell daily. These molecular lesions could trigger mutations and affects the next generations (Lodish *et al*., 2004). Thus, there is of great interest to search for potential genoprotective compounds especially from natural sources. Edible mushrooms have caught attention and popularity these years as hepatoprotective agent due to their safety and are inexpensive which is beneficial to developing countries. There were several reports on the hepatoprotective potential of mushrooms including *Agaricus bisporus* (Shi *et al*., 2002) and *Agaricus blazei* (Angeli *et al*., 2009).

Liver is a vital organ playing several important physiological roles (Saleem *et al*., 2010). Liver cirrhosis and hepatitis are major health problems worldwide. Despite medical advances, conventional medicine lack efficiency, have unfavourable side effects and are often not cost-effectiveness (Stickel & Schuppan, 2007). Prevention and the treatment of complications are the most common approaches in the treatment of liver cirrhosis (Sorensen *et al*., 2003). There is an urgent need to search for alternative medicine to treat this disorder. Herbal medicine has been used to treat hepatic diseases for a very long time. There are as much as 160 substances isolated from plants that are claimed to have hepatoprotective properties (Saleem *et al*., 2010). One of the most important herbs is *Silybum marianum* (milk thistle). The value of silymarin (extract of the seed of *S. marianum*) as excellent hepatoprotectant has been validated both *in vivo* and *in vitro*. It prevented glutathione depletion and possessed antifibrotic activity (Stickel & Schuppan, 2007). Further, some edible mushrooms which include *G. lucidum*

(Gao *et al*., 2003), *Pleurotus ostreatus* (Jayakumar *et al*., 2006) and *Antrodia camphorate* (Ao *et al*., 2009) have been shown to possess significant hepatoprotective effects. The present study was carried out to evaluate the antioxidant and genoprotective effects of *P. giganteus* fruiting bodies and mycelia fermented by *P. giganteus*. Hepatoprotective effects of *P. giganteus* fruiting bodies were also studied.

The objectives of the present study were to

a) prepare fermented and unfermented wheat grains by solid substrate fermentation.
b) prepare crude ethanol extracts from fruiting bodies, fermented and unfermented wheat grains.
c) evaluate antioxidant properties of crude ethanol extracts.
d) determine genoprotective effects of crude ethanol extracts.
e) determine hepatoprotective effects of *P. giganteus* fruiting bodies.

CHAPTER TWO: LITERATURE REVIEW

2.1 Mushroom

Mushrooms existed on earth earlier than human being evident by the fossil records of lower cretaceous period (Wani *et al*., 2010). Basically, they are found everywhere on paper, leather, fur, wood, straw and etc (Yiliz, 1999) and play vital ecological roles in the environment (Wani *et al*., 2010). Since ancient times, mushrooms are valuable food and folk medicine for human (Guillamon *et al*., 2010). Eastern civilizations have older history in the consumption of mushrooms than Europeans do (Lambert, 1938). Chinese use mushroom extracts combined with various herbal preparations to treat various medical disorders (Diyabalanage *et al*., 2008). Nowadays, edible mushrooms have gained popularity in human diet although not as main constituent (Valentao *et al*., 2005).

Generally, mushrooms possess good nutritional value with high protein, fibre, vitamin and mineral content but low in fat (Barros *et al*., 2008). The high protein and low fat composition make mushrooms good as low calorific diets (Barros *et al*., 2007). In concern of increasing protein demand due to rapid growing of human population, mushrooms with comparable protein content to animals can replace animal protein that may be harmful to our health (Guillamon *et al*., 2010). According to Bano and Rajarathnam (1982), the content of mushroom protein is largely affected by the mushroom species, combination of substratum, pileus size and harvest period.

Mushrooms were also reported to contain carbohydrate content of 50-90% with polysaccharides or glycoproteins as the main constituent (Nada *et al*., 2010). Polysaccharides in mushrooms are mostly comprised of b- and a- glucans, xylans, galactans, mannans, hemicelluloses and chitin (Manzi & Pizzoferrato, 2000). Low fat content is another key nutritional feature of mushrooms. Lipids as large water insoluble molecules have various important biological functions. They make up the essential hormones and act as major energy storage in our body (Ribeiro *et al*., 2009). Besides, they are the major constituents of phospholipid cellular membrane, myelin sheath and make great thermal insulators (Burtis & Ashwood, 1996). Omega-3 and omega-6 are two important families of essential fatty acids (EFAs). Polyunsaturated fatty acids (PUFA) from these two families played vital biological functions in low concentrations (Gibney *et al*., 2002). They are precursors to biosynthesize eicosanoids (i.e. prostaglandins) (Ribeiro *et al*., 2009) which are signalling substances to regulate many body functions (Voet & Voet, 2004). However, intake of diets that high in saturated fat and cholesterol has serious consequence leads to hypercholesterolemia, cardiovascular diseases and diabetes (Kuller, 2006; Layman *et al*., 2008).

In addition, mushrooms contain all essential amino acids, water soluble vitamins and all essential minerals needed by human (Buigut, 2002). According to Murcia *et al*. (2002), mushrooms have plentiful of vitamin A, vitamin C and β-carotene that exhibited protective effects due to their antioxidant properties. Several investigations also indicated that they have vitamins B1, B2, C and D2 (Manzi *et al*., 1999; Mattila *et al*., 2000).

Numerous studies have revealed that mushrooms contain pharmacological potential to prevent and treat a variety of diseases (Wasser & Weis, 1999; Wani *et al*., 2010). They accumulated multitude of secondary metabolites such as phenolics, polyketides, steroids and terpenes (Cheung *et al*., 2003). The bioactive compounds with therapeutic effects had been isolated from mushrooms and were known to have antiallergic, antiatherogenic, anti-inflammatory, antimicrobial, antitumor, antiviral, hepatoprotective, hypoglycemic, immunomodulating and central activities (Lindequist *et al*., 2005). Several scientific studies even suggested that mushrooms are potent to fight with HIV (King, 1993). The bioactive metabolites of mushrooms can be used for drug production similar to plants (Lindequist *et al*., 2005). The good nutraceutical and pharmaceutical properties of mushrooms make them potential to be used as functional food. Health supplements of dried fruiting bodies and extracts from mycelia of fermentations can be packaged into forms of powders, capsules or tablets (Wasser, 2005).

When compared to plants, mushrooms have advantages to supply bioactive compounds due to the fruiting bodies can be harvested in a shorter time, and the culture media for liquid or solid substrate fermentation can be manipulated in order to obtain optimum amount of bioactive compounds (Ferreira *et al*., 2009). Growing mycelia in a culture media is an efficient way to harvest fungal biomass of controlled quality (Yang & Liau, 1998).

Mushrooms are able to bioconvert agricultural and industrial wastes into nutritious food (Ingale & Ramteke, 2010). There are plenty of land wastes including sawdust, straws, leaves, stems, roots and etc. These wastes were decomposed by fungi and the nutrients absorbed used in mushroom formation. Bioconversion of these wastes into useful matter greatly reduces the environmental pollution. In addition, mushroom cultivation brings benefits to the poor farmers due to its labour intensive, short cultivation period and land saving (Shah *et al*., 2004).

Mushroom cultivation as a promising industry is welcomed by the small scale farmers and helps to diversify crops planting in Malaysia. In Asia, mushrooms are mainly cultivated by using low-cost facilities without expensive equipment, but several companies in Japan, Korea and Taiwan have used large scale mechanized systems in the mushroom production (Yamanaka, 2005). Most of the studies have been particular focused on the mushrooms in the northern hemisphere, while there were limited researches done on edible mushrooms from Southeast Asia, notably Malaysia (Wong & Chye, 2009). Our studies into the nutritional and medicinal properties of *P. giganteus* could encourage mushroom cultivation and helps to boost the local mushroom industry.

2.2 *Panus giganteus* (Berk.) Corner

Panus giganteus (Berk.) Corner is a relatively new species in Malaysia introduced by local mushroom growers and Department of Agriculture Malaysia in 2003. It was previously known as ‘cendawan perut lembu’ (cow’s stomach mushroom) and has been renamed as lowland shiitake or ‘cendawan seri pagi’ (morning glory mushroom). *Panus giganteus* is a known edible mushroom and widely consumed by the indigenous communities in Malaysia. There were other synonyms for *P. giganteus* including *Lentinus giganteus* Berk. and *Velolentinus giganteus* (Berk.). Based on the

morphological and molecular verifications, the locally cultivated *P. giganteus* is similar to Zhudugu mushroom (Chinese commercial name) in China. Zhudugu (pork bellies mushroom or pig stomach mushroom) is one of the several new developed mushrooms that caught attentions in China and overseas (Deng *et al*., 2006). *Lentinus giganteus* (*P. giganteus*) is also cultivated in Lao PDR and locally called 'Hed Thongfone' (Tapingkae, 2005).

A recent study done by Deng *et al*. (2006) revealed that *Clitocybe maxima* was widely misused as the scientific name for Zhudugu in China and should be replaced with *P. giganteus*. Based on the modern taxonomic system (Kirk *et al*., 2001), there were apparent differences between the genera of *Panus* and *Clitocybe* (Deng *et al*., 2006). In China, *P. giganteus* was named as 'Zhudugu' due to its creamy-like pig stomach taste. It was also called 'shoot mushroom' when the stipe was removed, as it tastes like bamboo shoots. *Panus giganteus* has good nutritional value, unique in texture and flavour (Yang *et al*., 2011).

Deng *et al*. (2006) described that *P. giganteus* has thick walled and unbranched skeletal hyphae. *Lentinus giganteus* (*P. giganteus*) usually grows on the ground and has a deeply radicating stipe originates from the buried wood (Corner, 1981). According to Deng *et al*. (2006), research and standardization of methods to cultivate *P. giganteus* have been carried out in Fujian, China and this species showed promising commercial prospect in China. On the other hand, early studies indicated that *P. giganteus* can be easily cultivated in Malaysia although it came from China of temperate climate. There were several reports on *P. giganteus* including extraction of polysaccharides (Yang *et al*., 2011) and genetic breeding study (Dong *et al*., 2010). Fu and Chen (2010) isolated

and purified the polysaccharides extracted from the *P. giganteus* fruiting bodies and showed that it inhibited lipid peroxidation in the mouse liver homogenate study.

Due to the misidentifation of Zhudugu as *C. maxima*, there were possibilities that literatures on the *C. maxima* in China indeed refer to *P. giganteus*. Wang and Ng (2004) isolated a ribonuclease from the fruiting bodies of *C. maxima*. The ribonucleolytic potency was suggested to be greater than ribonucleases from straw mushroom (Wang & Ng, 1999) and *Russula virescens* (Ng & Wang, 2004). A recent investigation by Zhang *et al*. (2009) showed that laccase isolated from edible mushroom *C. maxima* in mainland China exhibited anti-tumor activity against human hepatoma Hep G2 cells and MCF-7 human breast cancer cells. The laccase reduced the human immunodeficiency virus-1 (HIV-1) reverse transcriptase activity. Laccase is a lignin-modifying enzyme important for bioremediation and have wide biotechnological applications (Kunamneni *et al*., 2008). Little studies have been done on this species when compared to other popular mushrooms including *P. ostreatus* (Jayakumar *et al*., 2009), *L. edodes* (Wang *et al*., 1999), and *V. volvacea* (Wang & Ng, 1999). To the best of our knowledge, there is no report pertaining the locally cultivated *P. giganteus*. More studies should be done on this species to discover its medicinal importance and further assure the consumer acceptance.

2.3 Antioxidant

2.3.1 Free radicals and oxidative damage

The endogenous oxidation processes and exogenous sources produced reactive oxygen species (ROS) and free radicals. The ROSs are produced *in vivo* in respiratory chains of mitochondria, activated polymorphonuclear leukocytes, macrophages and peroxisomes (Hu *et al*., 2009). Exogenous sources of these harmful radicals are such as tobacco smoke, pollutants, pesticides, ionizing radiation and organic solvents (Kumar, 2011).

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) have both beneficial and detrimental effects to the living systems (Valko *et al*., 2006). Free radicals originated from oxygen are the most important family of radical species in living organisms (Grassi *et al*., 2010). Superoxide anion radical ($O_2^{\bullet -}$) arose via metabolic functions or exogenous processes including physical irradiation is recognized to be 'primary' ROS, it is subsequently generated 'secondary' ROS by reacting with other molecules (Valko *et al*., 2005). Most of the superoxide production occurs in cellular mitochondria (Cadenas & Sies, 1998). Nitric oxide radical ($NO^{\cdot}$) is the key member of RNS family and contains one unpaired electron (Valko *et al*., 2007). According to Ghafourifar & Cadenas (2005), nitric oxide synthetase (NOSs) metabolize arginine to citrulline via a five electron oxidation give rise to nitric oxide.

Scientific research revealed that both ROS and RNS are biological significant secondary messengers to regulate various normal body physiological functions (Valko *et al*. 2007). However, overproduction of the free radicals may induce oxidative stress and nitrosative stress which further resulted in biological damage (Valko *et al*., 2001;

Ridnour *et al*., 2005). Oxidative damage to DNA, protein and other macromolecules was known to be accumulated by age (Fraga *et al*., 1990).

Many studies had suggested that oxidative stress and physiopathology of various diseases are interelated. Oxidative stress caused by free radicals involved in the onset of cancer, rheumatoid arthritis, liver cirrhosis, arterioscleorosis and degenerative diseases related with ageing (Barros *et al*., 2008). Nearly all living organisms have evolved a complicated antioxidant system mainly comprised of antioxidant enzymes which include catalase (CAT), glutathione peroxidise (GSHPx), superoxide dismutase (SOD), or substances including ascorbic acid, a-tocopherol, carotenoids, glutathione and polyphenol subatances to protect the cells and organ systems (Niki *et al*. 1994). However, the production of natural antioxidants under normal physiological conditions are insufficient to prevent all damages caused (Sarikurkcu *et al*., 2010). Consumption of antioxidant supplements or foods possess antioxidative activies may reduce the oxidative damage.

2.3.2 Synthetic antioxidants

As antioxidants protect human against harmful effects of free radicals, they may hinder lipid peroxidation and oxidative stress related diseases (Ferreira *et al*., 2009). Butylated hydroxyanisole (BHA), butylated hydroxyltoluene (BHT), tert-butylhydroquinone (THBQ) and propyl gallate are synthetic antioxidants in common use nowadays (Jayakumar *et al*., 2007). The synthetic antioxidants were applied in food industry, in fatty/ oily foods to hinder oxidative degradation (Loliger, 1991).

However, several reports suggested that synthetic antioxidants promote tumour formation (Botterweck *et al*., 2000; Bauer *et al*., 2001). As synthetic antioxidants may bring undesirable harmful effects to our health, natural antioxidants received much attention and are being extensively studied. A wide range of antioxidants have been isolated from plant materials including cereal crops, herbs, spices, vegetables, fruits, oil seeds, leaves and roots (Ramarathnam *et al*., 1995).

2.3.3 Mushroom as source of antioxidant

Since decades, mushrooms have been recognized for its profound nutritional and pharmaceutical value (Wani *et al*., 2010). They are considered as functional food largely due to their chemical constitution (Elmastas *et al*., 2007). Mushrooms supply wide range of secondary metabolites which include phenolics, polyketides, steroids and terpenes (Cheung *et al*., 2003). Phenolic compounds such as phenolic acids and flavonoids are the main constituents of mushroom antioxidants, followed by tocopherols, ascorbic acid and carotenoids (Ferreira *et al*., 2009). Cheung *et al*. (2003) showed that total phenolic content of *Lentinus edodes* and *V. volvacea* are correlated positively with their antioxidant activities. Phenolic compounds as non-essential dietary substances also capable of inhibiting atherosclerosis and cancer (Teissedre *et al*., 1996; Williams & Iatropoulos, 1997).

There are many popular edible mushrooms such as *G. lucidum, H. erinaceus, P. sajor-caju* and *V. volvaceae* possessed antioxidant capacity (Noorlidah Abdullah, 2012). Antioxidant molecules have been isolated and quantified from mushrooms especially those from Taiwan, Korea, Finland, Portugal, India, and Turkey (Ferreira *et al*., 2009).

The pharmacologically active compounds maybe isolated from mycelia cultivated with solid substrates as well (Lindequist *et al*., 2005). Solid substrate fermentation takes shorter time to produce mycelia with comparable medicinal quality to fruiting bodies. Daker *et al*. (2008) investigated that methanol extract of maize fermented by *Marasmiellus* sp. had free radical scavenging activity and lipid peroxidation inhibitory effects. The lipid peroxidation inhibitory effects were better than catechin and BHA. Scientific studies related to antioxidants derived from *P. giganteus* are relatively rare. As *P. giganteus* is only recently available in the market, its medicinal properties are of market interest.

2.3.4 *In vitro* antioxidant assays

There is no single and universal method which able to elucidate the total antioxidant properties of a certain compound accurately as there were several mechanisms underlying antioxidant activity have been proposed including termination of chain reaction mediated by free radical, hydrogen donation, chelation of catalytic ions, and elimination of peroxides (Dorman *et al*., 2003).

Scavenging activity of DPPH free radicals is a rapid method popularly used to evaluate the antioxidant properties of potent compound (Elmastas *et al*., 2007). The DPPH free radical could readily be turned into a stable diamagnetic molecule by receiving a hydrogen radical or electron (Soares *et al*., 1997). It has advantage against other laboratory-generated free radicals including superoxide anion and hydroxyl radical as they are not affected by side reactions which include inhibition of enzyme and chelation of metal ion (Amarowicz *et al*., 2004).

Ferric reducing antioxidant power (FRAP) is one of the most accessible, easy and very rapid for regular analysis. Previous studies suggested that most ferric reducing agents contain antioxidant activity (Goh *et al*., 2010). The antioxidant activity based upon the production of Fe^{2+} *TPTZ* blue complex from the conversion of ferric ion to the ferrous ion, as measured at 593nm (Benzie & Strain, 1999).

Trolox equivalents antioxidant capacity (TEAC) assay is a method originally described by Miller *et al*. (1993) concerning on scavenging activity of long-lived ABTS radical (Scott *et al*., 1993). According to Re *et al*. (1999), TEAC is a suitable assay used to test on antioxidant properties of both hydrophilic and lipophilic antioxidants such as flavonoids, hydroxycinnamates, carotenoids, and plasma antioxidants. It is popularly used in the screening of compounds, food products, extracts and biological fluids such as plasma for their potent antioxidant capacity (Berg *et al*., 1999). The results for the TEAC assay are often expressed in comparison with the water-soluble α-tocopherol analogue, trolox.

The capacity of a compound to inhibit lipid peroxidation was often been evaluated. Lipid peroxidation is mainly due to autoxidation between molecular oxygen species and unsaturated lipid, which is initiated by enzymatic reaction of lipoxygenase, light, radiation, heat, metallo-protein catalysts and metal ions (Daker *et al*., 2008). Lipid peroxidation is a major concern to food industry as it caused food deterioration, rancidity and accumulation of harmful toxic compounds in foods (Gorelik *et al*., 2008). The toxic products from lipid peroxidation could damage biomolecules including DNA, too (Box & Maccubbin, 1997). The antioxidant effects on autoxidation rates are depend on the oxidation condition, structure and the nature of antioxidants (Shahidi & Wanasundra, 1992).

It is essential to determine the phenolic content in the extract and study its relationship with antioxidant properties. A powerful scavenging ability of phenolic compounds is done to their hydroxyl groups (Hatano *et al*., 1989). Phenolic compounds capable to prevent lipid peroxidation by donates hydrogen atom from the hydroxyl group(s) that attached to the benzene ring (Sawa *et al*., 1999). Foods with high phenolic content exhibit antioxidant properties to slow down the progression of atherosclerosis which thereby reduces the risk of heart disease (Meng *et al*., 2002).

2.4 Genoprotection

2.4.1 DNA damage and mushroom as genoprotective agent

Physiological activities or environmental factors which include UV light could result in DNA damage as much as 1,000 to 1,000,000 molecular lesions per cell per day (Lodish *et al*., 2004). Low levels of reactive oxygen species (ROS) have useful cellular functions in signal transduction and modulation of gene expression, but high levels of ROS may damage DNA and other biological macromolecules (Remacle *et al*., 1995; Halliwell & Gutteridge, 1999). Accumulation of ROS may be due to overproduction and failure of antioxidant defence system (Halliwell, 1999).

Oxidative stress exerted by free radicals could induce cancer, rheumatoid arthritis, liver cirrhosis, arteriosclerosis and degenerative diseases related with ageing (Barros *et al*., 2008). Occurrence of cancer is related to high mutation rate (Ferguson *et al*., 2005) and inappropriate diets based on epidemiological evidences (Angeli *et al*., 2009). Human being has high exposure to variety of chemicals including food and pharmaceutical products (Martins de Oliveira *et al*., 2002). According to Loeb *et al*. (2003), the reduction of mutation rate can delay the appearance of the neoplasms. Thus,

efforts to develop antimutagenic compounds as dietary supplements may offset the detrimental effects of ROS.

Ionising radiation cause DNA lesions resulted from intra or inter strand cross-linking, and breaking of single or double strand (Pillai *et al*., 2010). Chromosomal damage was demonstrated in the form of breaks or fragments which were obviously shown as micronuclei in the rapid growing cells (Hofer *et al*., 2000). Eukaryotic cells capable of identifying and repairing DNA damage within every phase of cell cycle (Craig & Alt, 2004). The repair of DNA may be defined as combination of processes when a cell identify and correct the damage to the DNA. However, the error in DNA repair may leads to genetic instability, alteration in cellular functions, cell death and neoplastic transformation in multicellular organisms (Pillai *et al*., 2010).

Several past studies have shown that mushrooms possessed ability to protect and/ or repair DNA. Aqueous extract of sun mushroom (*A. blazei*) that is native to south-eastern Brazil showed anti-genotoxic effects on V79 (Chinese hamster lung) cells *in vitro* (Martins de oliveira *et al*., 2002). Pillai *et al*. (2010) revealed that polysaccharides of *G. lucidum* capable of repairing DNA of human peripheral blood leukocytes after the DNA strands were induced damaged by radiation.

2.4.2 Comet assay

In general, there are a few methodologies used to evaluate DNA damage in the laboratory. The techniques frequently applied were unscheduled DNA synthesis (UDS) technique and alkaline elution assay. Unscheduled DNA synthesis (UDS) technique detects DNA repair synthesis while alkaline elution assay detects DNA single strand breaks (SSB) and alkaline labile sites (ALS) in pooled cell populations. However, UDS

method has limitation in sensitivity while alkaline elution assay neglects the significance of intercellular differences in DNA damage (Tice *et al*., 2000). Therefore, a more useful method called single cell gel electrophoresis or popularly known as comet assay which is able to detect DNA injury in all mammalian cells from any tissue has been developed (Anderson *et al*., 1998). Comet assay was first introduced by Ostling and Johanson in 1984 (Ostling & Johanson, 1984) and commonly used to evaluate the genotoxicity of various compounds (Rojas *et al*., 1999).

Generally, there are two versions of comet assay. One of the versions was developed by Singh *et al*. (1988) which used alkaline electrophoresis (pH 13) to analyze DNA after the DNA was treated with H_2O_2 or X-rays. This method can detect the breaks of single-strand DNA and alkali labile in individual cells. The second version was introduced by Olive and co-workers (Olive *et al*., 1990). This is a neutral technique of Ostling and Johanson that involved lysis in alkali condition followed by electrophoresis in either neutral or mild alkaline (pH 12.3) conditions to detect single strand breaks (Rojas *et al*., 1999). Although both techniques are similar in principle and working procedures, the alkaline version of comet assay has better sensitivity (Green *et al*., 1996; Tice, 1995). Alkaline version of comet assay has been extensively applied in the genotoxicity study of many compounds including pesticides, opiates, nitrosamines, antineoplastic drugs and metals. Multi range of normal and transformed cells which include human, animal and plant have been used in *in vitro* experiments (Rojas *et al*., 1999).

Comet assay holds several advantages against other genotoxicity assays in that the assay has high sensitivity to detect low level of DNA, small numbers of cells per sample are needed, flexible, low costs, simple and short time to perform (Tice *et al*.,

2000). Nowadays, comet assay has wide applications in various fields including DNA repair studies, genotoxicology, clinical area, human monitoring and environmental biomonitoring (Rojas *et al*., 1999).

All human cells were exposed to certain levels of H_2O_2 which mainly produced by mitochondria (Halliwell *et al*., 2000). According to Neill *et al*. (2002), the production of H_2O_2 increased in response to different stresses. In biological systems, H_2O_2 is produced by the dismutation of superoxide radical ($O2^{\cdot-}$) which maybe catalyzed by superoxide dismutase enzymes or non-enzymatically (Halliwell *et al*., 2000). Many studies revealed that ultraviolet light exposure or interaction with various transition metal ions particularly iron *in vivo* converted H_2O_2 to reactive hydrogen radical (Halliwell & Gutteridge, 1990; Ueda *et al*., 1996).

Hydrogen peroxide (H_2O_2) is often used to induce DNA damage in genotoxicity experiments. It is a pale blue liquid miscible with water and capable of crossing cell membranes although the actual mechanism has remained unclear (Halliwell & Gutteridge, 1999). Many studies have revealed the toxicity of high levels of H_2O_2 to various cells including animal, plant and bacteria. The EC_{50} (effective concentration, 50%) and cell death mode strongly dependent to types of cell, its physiological state, H_2O_2 exposure period, H_2O_2 concentration and culture media applied. As H_2O_2 regarded as highly toxic, thus it is important to eliminate it from the body by antioxidant defence enzymes including catalases, peroxidases, and thioredoxin-linked systems (Halliwell *et al*., 2000). Normally, H_2O_2 can be removed by cellular antioxidant system efficiently. However, in some circumstances when large amounts of H_2O_2 are being produced, any reduction in antioxidant status could bring damaging effects to the cellular tissues (Neill *et al*., 2002).

2.5 Liver

2.5.1 Liver diseases

Liver is an important organ actively involved in the detoxification of foreign substances, in bile secretion important for digestion, and in the metabolic functions of various nutrients (Saleem *et al*., 2010). Nowadays, liver diseases are the major global health problems (Asha & Pushpangadan, 1998). Acute and chronic liver diseases not only occur in adults but also in children with malnutrition accompanied the disease (Rigby & Schwarz, 2001). Chronic liver injury has serious aftermath, liver fibrosis as common chronic liver disease leads to end stage liver cirrhosis and liver cancer (Ao *et al*., 2009).

Excessive alcohol consumption and viral infections have been recognised as the most common causes for liver diseases in developed countries while environmental pollution, hepatic viruses, parasitic infections and chemotherapeutics are the main factors to cause hepatic damage in developing countries (Alshawsh *et al*., 2010). Most of the hepatotoxic chemicals damaged the cells via lipid peroxidation and oxidative stress (Dianzani *et al*., 1991). Besides that, some unknown factors related to gender may also involve in the occurrence of liver cirrhosis (Corrao *et al*., 1997).

Alcohol abuse causes a lot of life-threatening diseases including steatosis (fatty liver), hepatitis and cirrhosis (Pramyothin *et al*., 2005). Alcoholic liver disease is the main health problem associated with alcohol consumption and a primary factor of liver disease among Caucasians (Kono *et al*., 2000). According to Kulkarni *et al*. (2009), alcohol abusers displayed high levels of oxidative stress and low antioxidant levels. Further, alcohol oxidation over took oxidation of liver in the liver, this leads to lipid accumulation and caused fatty liver disease (Rigby & Schwarz, 2001). Previous

investigation has revealed that acetaldehyde formed due to oxidation of alcohol had hepatotoxic effects at high concentration (Lieber, 1993).

Cirrhosis is a worldwide health burden as ninth leading cause of death in the Western countries (Kim *et al*., 2002). It develops in 10-20% of chronic alcoholics and the symptoms are fatigue, anorexia, nausea, and sometimes hepatic encephalopathy and bleeding esophageal varices (Rigby & Schwarz, 2001). In North America, 75% of the cirrhotic patients were chronic alcoholics, viral hepatitis and other identified causes accounted for 15% of the patients, while the remaining 10% were patients with cryptogenic cirrhosis (cirrhosis due to unidentified causes). In Asia and Africa, cirrhosis is primarily associated with chronic viral hepatitis (Brown *et al*., 1997). Cirrhosis is the end stage of liver diseases and leads to liver failure, portal hypertension and hepatocarcinoma (Lv *et al*., 2006). It is represented by the fibrosis and normal hepatocellular alteration into structurally abnormal nodules (Pinzali *et al*., 2011). The fibrous scarring and hepatocellular regenerative nodules resulted in more hepatocytes exposed to different inflammatory or toxic cellular injuries leads to apoptosis (Park *et al*., 2001).

On the other hand, liver cancer has caused increasing mortality rates in the western countries and Japan (El-Serag, 2004; Kiyosawa *et al*., 2004). The common causes of liver cancer are alcohol consumption, viral infections and non-alcoholic fatty liver disease (NAFLD) (Muto *et al*., 2006). A lot of epidemiological and other studies have been performed to search for the risk factors of liver cancer due to chronic hepatic disorders which include severity of hepatic inflammation and fibrosis age, race and identifying sex (El-Serag, 2004; Kiyosawa *et al*., 2004).

Hepatotoxic agents such as acetaminophen (Rousar *et al*., 2009), carbon tetrachloride (Achliya *et al*., 2004), galactosamine (Kucera *et al*., 2006) and thioacetamide (TAA) (Alshawsh *et al*., 2011) have been used to induce experimental liver damages in both *in vivo* and *in vitro* study models. These hepatotoxic agents induced liver damage by reacting with basic cellular components (Stankova *et al*., 2010). Thioacetamide (TAA) as a thiono-sulfur containing compound has been widely used as organic solvent, fungicide, motor oil stabilizer and accelerator in the rubber vulcanization (Lee *et al*., 2003). Fitzhugh and Nelson (1948) were the first to report TAA as a hepatotoxic agent. Biochemically, it is metabolized by microsomal FAD monoxygenase (FADM) system to form reactive metabolites such as thioacetamide sulfoxide and thioacetamide-S,S-dioxide which then contribute to the toxicity effects of TAA (Chilakapati *et al*., 2007). Long term exposure to TAA may provoke hyperplastic liver nodules, liver cell adenomas and hepatocarcinomas (Yeh *et al*., 2004).

Besides that, generation of reactive oxygen species (ROS), mitochondrial dysfunction and antioxidant insufficiency have also been reported to advance the development of liver cirrhosis (Natarajan *et al*., 2006). In order to evaluate the effects of TAA on oxidative stress in rats, oxidative stress parameters such as serum MDA and urinary 8-OH-dG content which reflect oxidative damage to lipids and DNA respectively were often evaluated. Malondialdehyde (MDA) has been quantified since the sixties and is still widely used as biomarkers to detect lipid peroxidation due to their low cost and simplicity (Lykkesfeldt, 2007). Numerous reports have revealed that the measurement of TBARS was useful to study the pathological states in tissues of animal origin (Shi *et al*., 2008; Jayakumar *et al*., 2006).

The free 8-OH-dG assay is used to evaluate the DNA damage caused by oxidative stress. Free radicals may damage nucleic acids, cellular proteins and lipids at high concentrations (Valko *et al*., 2006). Although there are wide range of products resulted from oxidative damage of DNA, the main focus has been on nucleobase alterations especially the lesion of 8-OH-dG as it is produced *in vivo* and can be estimated after the DNA of the cells had been attacked by hydroxyl radicals (Lunec *et al*., 2002). Particularly, urinary 8-OH-dG content is the most commonly used due to the simplicity of the method and it is non invasive (Subash *et al*., 2010). Kasai and Nishimura (1984) were the first to report the usage of 8-OH-dG to analyse the DNA damage caused by hydroxylation at C8 position of the nucleoside guanosine. Since then, numerous studies and improvements have been made to the methods for the quantitation of 8-OH-dG in urine and plasma (Valavanidis *et al*., 2009).

2.5.2 Treatment of liver diseases

Liver cirrhosis and its complications have high mortality rate. In spite of medical advances, there is no reliable medicine for this pathology, yet conventional medicinal approach brings severe undesirable adverse effects and not cost-effective especially for third world countries (Stickel & Schuppan, 2007). Elimination of risk factors and alleviation of liver fibrosis are common approaches to prevent liver deterioration (Brenner *et al*., 2000). As for alcoholic cirrhosis, cirrhotic progression maybe limited by alcohol withdrawal. The complications derived from the disease such as variceal hemorrhage, ascites, and encephalopathy are prevented and treated (Sorensen *et al*., 2003).

According to Rigby and Schwarz (2001), nutritional intervention is important in the management of liver diseases. Protein-energy malnutrition is a frequent phenomenon occurred in cirrhotic patients and it worsens the prognosis of the patients (Merli *et al*., 1996). As the malnutrition closely related to disorders of protein metabolism and energy metabolism, thus improving the conditions of these metabolic disorders would likely to improve the condition of the patients (Kato & Moriwaki, 2004). Yamauchi *et al*. (2001) suggested that supplementation of branched chain amino acid (BCAA) could improve the protein catabolism and lypolysis in patients with liver cirrhosis.

Liver transplantation is the treatment of last resort for patients with end-stage cirrhosis (Francoz *et al*., 2007). Thus, there is an urgent need to search for harmless alternative medicine to treat this pathology, contrarily to conventional medicine. Herbal drugs have caught popularity due to their safety, efficacy and cost effective (Saleem *et al*., 2010). In this modern era, there has been a paradigm shift towards medicinal evaluation of herbal products in liver diseases model by means of evidence-based medicinal evaluation, standardization and randomized controlled clinical trials to confirm its clinical efficiency (Thyagarajan *et al*., 2002). Several reports showed that there were sharp increase in the botanical drug usage in United States and Europe with up to 65% of patients suffered from liver diseases took herbal formulations (De Smet, 2002; Strader *et al*., 2002). The expenses for silymarin reached $180 millions in Germany (Breevort, 1996). Overall, there are as much as 160 phytoconstituents originated from 101 plants claimed to contain hepatoprotective effects (Saleem *et al*., 2010). These may include silymarin used for antifibrotic treatment, glycyrrhizin to treat chronic viral hepatitis and *Phyllantus amarus* for chronic hepatitis B (Stickel & Schuppan, 2007).

Perhaps the most popular herbal drug used is silymarin extracted from the milk thistle *Silybum marianum* (L.) Gaertnc which was applied in the treatment of liver diseases for the past 2000 years (Gazak *et al*., 2007). Nowadays, silymarin is used as medication in both acute and chronic types of liver disorders (Wellington & Jarvis, 2001). Approximately, there were 10-15% of the patients in United States received treatments of silymarin-derivatives (Hoofnagle, 2005). *Sylibum marianum* has been suggested of comprising antioxidant, anti-lipid peroxidation, anti-inflammation, antifibrotic, immunomodulating properties besides of its role in aiding liver regeneration (Saleem *et al*., 2010). Studies reported that silymarin mainly make up of flavonoids silibinin, isosilibinin, silidianin and silichristin, of which silibinin is bioactive component commonly used for standardisation of pharmaceutical products (Wagner, 1976). Hepatoprotective effects of silymarin were well defined with *in vitro* and *in vivo* investigations (Stickel & Schuppan, 2007). In particular, many researchers revealed that actions of silymarin are mainly contributed by its capability to reduce lipid peroxidation (Rastogi *et al*., 2001; Comoglio *et al*., 1995).

Glycyrrhizin, a liquorice root extract (*Glycyrrhiza glabra*) is used as medication to relieve jaundice, gastritis and bronchitis (Stickel & Schuppan, 2007). Glycyrrhizin can reduce liver damage in animal models (Okamoto & Kanda, 1999; Okamoto 2000) and induced proliferation of hepatocyte in rats (Kimura *et al*., 2001). Furthermore, Kimura *et al*. (2008) studied that glycyrrhizin accelerated liver regeneration and lowered the activities of transaminase in rats. More than 100 million doses of glycyrrhizin have been administered to patients facing chronic hepatitis and allergy problem (Sato *et al*., 1996).

Plants of the genus *Phyllanthus* are widely distributed in tropical and subtropical regions and have long history as the medication for chronic liver diseases (Stickel & Schuppan, 2007). *Phyllanthus amarus* Schum. et Thonn. (*Bhuia amla*) is commonly used in ayurvedic medicine to treat secondary hepatitis and other illnesses for more than 2000 years (Naaz *et al*., 2007). The main ingredients of *Phyllanthus amarus* were reported to be phyllantins, hypophyllantins and several polyphenoles of which their chemical and medicinal properties have been reported (Calixto *et al*., 1998). Comprehensive research on *Phyllanthus amarus* showed that it contained anti-inflammation, immunomodulating, hepatoprotective, and anti-viral properties (hepatitis B and C viruses) (Thyagarajan *et al*., 2002). Although available data suggested the hepatoprotective effects of *Phyllanthus,* it needs further studies in well-designed experimental model with clear outcome (Stickel & Schuppan, 2007).

2.5.3 Mushroom as hepatoprotective agent

Mushrooms have protective effects due to their appreciable amounts of vitamins A, C and b-carotene (Murcia *et al*., 2002). A number of mushroom spp. were studied and reported to contain hepatoprotective effects. *G. lucidum* (Fr.) Karst. (*Ganodermataceae*) is commonly used to treat chronic hepatic diseases of various etiologies and no side effects have been reported (Gao *et al*., 2003). Many studies have been done on the bioactive compounds isolated from *G. lucidum*. Zhou *et al*. (2002) showed that polysaccharides of *G. lucidum* able to prevent ethanol induced liver damage in mice. Besides that, Shi *et al*. (2008) reported that peptides of *G. lucidum* had significant hepatoprotective effects against d-galactosamine (d-GalN)-induced hepatocellular injury.

Gao *et al*. (2003) showed that polysaccharides and triterpenoids of *G. lucidum* are the bioactive compounds responssible for the hepatoprotective effects against toxin-induced hepatic injury. According to Wang *et al*. (2000), the triterpenoids of *G. lucidum* had hepatoprotective effects against hepatic injury induced by CCl_4, d-GalN and Bacillus Calmette-Guerin (BCG) plus lipopolysaccharide (LPS) *in vivo.* Kim *et al.* (1999) showed that triterpenoid of *G. lucidum, Ganoderenic acid* A inhibited the activity of β-glucuronidase, an indicator of hepatic damage.

Mushroom *P. ostreatus* had potent antioxidant activity to reduce CCl_4-induced hepatotoxicity in rats. Treatment with an extract of *P. ostreatus* improved the antioxidant status during ageing and protects the liver, heart, kidneys and brain of aged Wistar rats. Further, it inhibited lipid peroxidation and enhanced the enzymatic and non-enzymatic antioxidant activities (Jayakumar *et al*., 2006). Administration of mushroom insoluble non-starch polysaccharides (MINSP) isolated from *P. ostreatus* mycelia also showed protective effects against CCl_4-induced acute hepatotoxicity in albino rats (Nada *et al*., 2010).

Recent scientific reports showed that *Antrodia camphorata* (Niuchangchih) can prevent chemical and biological liver injury, inhibits hepatoma cells and improved conditions of liver fibrosis (Ao *et al*., 2009). Lu *et al*. (2007) showed that mycelia of *A. camphorata* and *Armillariella tabescens* in liquid culture exhibited hepatoprotective effects against ethanol-induced hepatotoxicity in rats comparable to silymarin. To the best of our knowledge, there is no literature reported on the hepatoprotective effects of *Panus* spp. Therefore, the present study into the hepatoprotective properties of *P. giganteus* laid the basis to encourage the cultivation and consumption of this edible mushroom.

CHAPTER THREE: MATERIALS AND METHODS

3.1 Fungus

The test fungus, *P. giganteus* (KUM 60427) was obtained from fungal culture collection of Mushroom Research Centre, University of Malaya. The fungus was preserved as stock culture on potato dextrose agar (PDA) slants at 4 ± 1℃. Working cultures were maintained on the potato dextrose agar (PDA) plates at 25 ± 2℃ with periodic transfer.

3.2 Mushroom

The fruiting bodies of *P. giganteus* were purchased from local mushroom grower, NAS Agrofarm Sdn. Bhd. and freeze-dried in Christ freeze dryer Alpha-1-4 LD plus system. Subsequently, the dried mushrooms were ground in a Waring commercial blender into powder which was then ready for ethanol extraction.

3.3 Solid substrate fermentation

Solid substrate fermentation was performed by using wheat grains as substrate. The flasks inoculated with *P. giganteus* fungal plugs (one week old) were incubated in the dark for a week at 25 ± 2℃ for full colonization of the substrate (Figure 3.1). After a week, fermented and unfermented wheat grains as control were harvested. Further, the harvested materials were freeze-dried (Christ freeze dryer Alpha-1-4 LD plus system) and ground into powder ready for ethanol extraction.

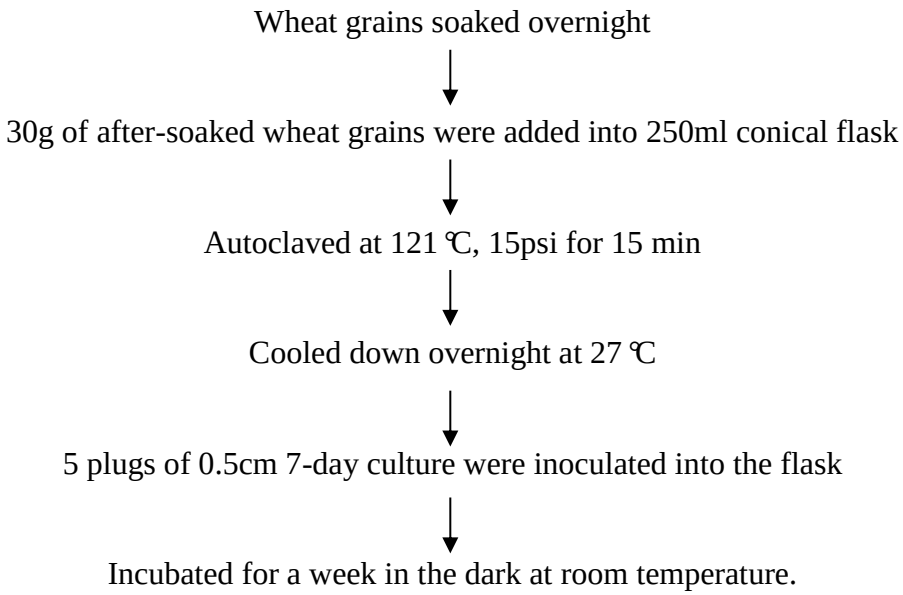


Figure 3.1: The process flow for solid substrate fermentation.

3.4 Nutritional composition

Two hundred grams powder of freeze-dried fruiting bodies, fermented and unfermented wheat grains were sent to Consolidated Laboratory (M) Sdn. Bhd. for analysis of nutritional composition (methods were based on AOAC).

3.5 Ethanol extraction

The ethanol extracts of freeze-dried powder of fruiting bodies, fermented and unfermented wheat grains were prepared (Lee *et al*., 2007). The samples (100g) were extracted with 1 Litre of 95% (v/v in dH_2O) ethanol. After shaking for three days in a shaking incubator, the contents of each flask were filtered through Whatman No.1 filter paper. Later, the solvent was evaporated to dryness by using a rotary evaporator (Thermo Scientific) at 40 ℃.

3.6 Assessment of antioxidant properties and total phenolic content

The ethanol extracts prepared were analyzed for their antioxidant properties. Different important *in-vitro* antioxidant models which included scavenging effects on 1,1-diphenyl-2-picrylhydrazyl (DPPH), ferric reducing antioxidant power (FRAP), trolox equivalent antioxidant capacity (TEAC) and inhibition of lipid peroxidation were performed. Total phenolic content of the extracts were also evaluated.

3.6.1 Chemicals

Gallic acid (3,4,5-trihydroxybenzoic acid), ferrous sulfate ($FeSO_4$), TPTZ (1,3,5-triazine, 2,4,6-tri-2-pyridinyl), trolox (6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic Acid) and DPPH (2,2-diphenyl-1-picrylhydrazyl) were purchased from Sigma-Aldrich Malaysia. Analar grade ascorbic acid was obtained from BDH chemicals while all other chemicals were of analytical grade and purchased from Merck Malaysia.

3.6.2 Scavenging effects on 1,1-diphenyl-2-picrylhydrazyl (DPPH)

The DPPH free radical scavenging activity was determined according to Gorinstein *et al*. (2003). L-ascorbic acid (0-25µM) was used as standard. Initially, 5µl extracts of various concentrations (4-40mg/ml) were loaded into microtiterplate and then followed by 195µl of DPPH reagent (40X dilution). The absorbance of the mixture was read at 517nm for three hrs with 20 min intervals at lowest temperature on the microtiterplate reader (Power Wave X 340, Bio-Tek Instruments, Inc.). The radical scavenging activity was calculated according to the equation: Radical scavenging activity (%)= (Abs Blank - Abs Sample) / Abs Blank x 100 of where Abs Sample is the absorbance of the sample whereas Abs Blank is the absorbance of the DPPH solution. The radical scavenging ability of the extracts was expressed in terms of IC_{50} value that

is concentration to quench 50% of available DPPH content. Butylated hydroxytoluene (BHT) was used as control in this assay.

3.6.3 Ferric reducing antioxidant power (FRAP)

Ferric reducing antioxidant power (FRAP) assay was measured according to Benzie and Strain (1999). The reagent was prepared by mixing 25ml of 300mmol/L acetate buffer pH 3.6, 2.5ml of 10mmol/L TPTZ in 40mM HCl and 2.5ml of 20mM $FeCl_3 \bullet 6H_2O$ (ratio 10:1:1). Various concentration of ethanol extracts (4-20mg/ml) with volume of 10µl were mixed with 300µl of working FRAP reagent. Subsequently, the absorbance was read at 593nm after four min using microtiterplate reader (Power Wave X 340, Bio-Tek Instruments, Inc.). Ferrous sulfate ($FeSO_4$) of concentrations 0-20mM was used as standard and BHT as control. The FRAP value was expressed as µM of $FeSO_4$ equivalents.

3.6.4 Trolox equivalent antioxidant capacity (TEAC)

The capacity of the samples to scavenge long-lived 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) or $ABTS^{\bullet *}$ molecules were determined according to Re *et al*. (1999). The radical cation ($ABTS^{\bullet *}$) reagent was prepared by mixing 5ml of 7mM $ABTS^{\bullet *}$ stock solution with 89µl of 140mM potassium persulfate and kept in the dark for 12-16 hrs at room temperature before use. Subsequently, the mixture was diluted with absolute ethanol to obtain absorbance of 0.7 (± 0.02) at 734nm. The sample extracts (10µl) of different concentrations (4-20mg/ml) were mixed with 100µl of the $ABTS^{\bullet *}$ reagent and absorbance reading was measured at 734nm by using microtiterplate reader (Power Wave X 340, Bio-Tek Instruments, Inc.) after one minute. The TEAC value was expressed as µM of Trolox equivalents and BHT as control in this assay.

3.6.5 Inhibition of lipid peroxidation

This method was performed based on thiobarbituric acid reaction method as reported by Kuppusamy *et al*. (2002). Initially, the yolk suspension was prepared by diluting egg yolk with phosphate saline buffer. After that, 1ml of ethanol extract with different concentrations (8-20mg/ml) was mixed with 0.5ml yolk suspension and 0.5ml $FeSO_4$. Subsequently, the mixture was incubated at 37℃ for an hr and then treated with 0.5ml of 20% Trichloroacetic acid (TCA) and 1ml of 0.8% Thiobarbituric acid (TBA). The mixture was then heated in a boiling water bath for 15 min and centrifuged at 3500rpm for 20 min (Jouan C312 centrifuge) in order to remove precipitated protein. Finally, 200µl of the supernatant was subjected to an absorbance reading at 532nm by using microtiterplate reader (Power Wave X 340, Bio-Tek Instruments, Inc.) to measure formation of thiobarbituric acid reactive substances (TBARS) complex. Result was expressed in 1,1,3,3,-tetraethoxypropane (TEP) equivalents based on the TEP standard calibration. Butylated hydroxytoluene (BHT) was used as control.

3.6.6 Total phenolic content

The methodology to determine total phenolics was performed according to the method of Slinkard and Singleton (1977). The assay was started with mixing of 50µl ethanol extracts of various concentrations (1-5mg/ml) with 50µl Folin–Ciocalteu phenol reagent. After three min, 100µl of sodium carbonate solution was added to the mixture. The mixture was left to stand in the dark for an hr. Consequently, the absorbance was read at 750nm by using microtiterplate reader (Power Wave X 340, Bio-Tek Instruments, Inc.). A standard calibration curve using gallic acid with concentrations 0-100µg/ml was made. Butylated hydroxytoluene (BHT) was used as control. The results were expressed as mg/ml of gallic acid equivalents.

3.7 Genoprotection studies

Comet assay was performed to evaluate the potential of *P. giganteus* fruiting bodies, fermented and unfermented wheat grains to prevent and/ or repair DNA damage induced by hydrogen peroxide (H_2O_2). Figure 3.2 illustrates the process flow for the experiments carried out.

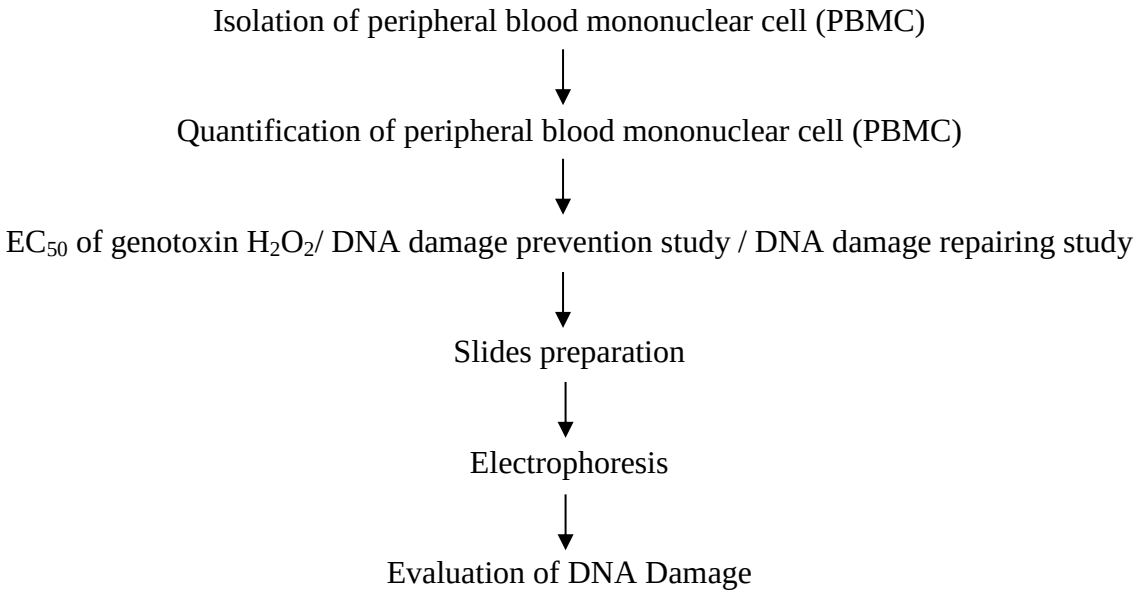


Figure 3.2: The process flow for comet assay.

3.7.1 Chemicals

Histopaque-1077 was a product from Sigma-Aldrich Malaysia. Fully frosted slides and all other chemicals were of analytical grade and purchased from Fisher Scientific Malaysia.

3.7.2 Isolation of peripheral blood mononuclear cell (PBMC)

The mononuclear cells were isolated as described by Boyum *et al.* (1968). This is demonstrated in Figure 3.3. Fresh blood (10ml) collected by venipuncture from a healthy donor was layered gently, added with Histopague-1077 (Sigma-Aldrich) and then centrifuged in order to separate the blood contents into layers. Subsequently, the second opaque layer which lies below PBS were aspirated slowly and mixed with phosphate buffered saline (PBS) in 1:1 ratio. After that, the mixture was centrifuged and the remaining pellet resuspended with ammonium chloride to lyse the red blood cells. Following that, the mixture was centrifuged, pellet added with PBS and then centrifuged again. Finally, the supernatant was discarded and packed lymphocytes were mixed with Roswell Park Memorial Institute-1640 (RPMI-1640) media which is commonly used for the culture of human lymphocytes.

3.7.3 Quantification of peripheral blood mononuclear cell (PBMC)

The concentration of mononuclear cell needs to be determined so that the cell concentration in agarose was known. It is an important criterion in ensuring good analysis as high cell densities may cause comets to overlap remarkably during high levels of DNA migration (Tice *et al.*, 2000). In this study, haemocytometer grid system was used. At first, 10µl of PBMC suspension was mixed with 10µl of Trypan blue (0.4%) to visualize the cells under microscope. Then, the mixture was mixed evenly and 10µl of the mixture loaded into haemocytometer. After that, the cells were observed under microscope at 40X and the cell number was determined based on the formula.

Cell concentration (cells/ml) = mean cell count x dilution factor x 10^4

= mean cell count x 2 x 10^4

Subsequently, the cells were mixed with RPMI-1640 media to the required concentration.

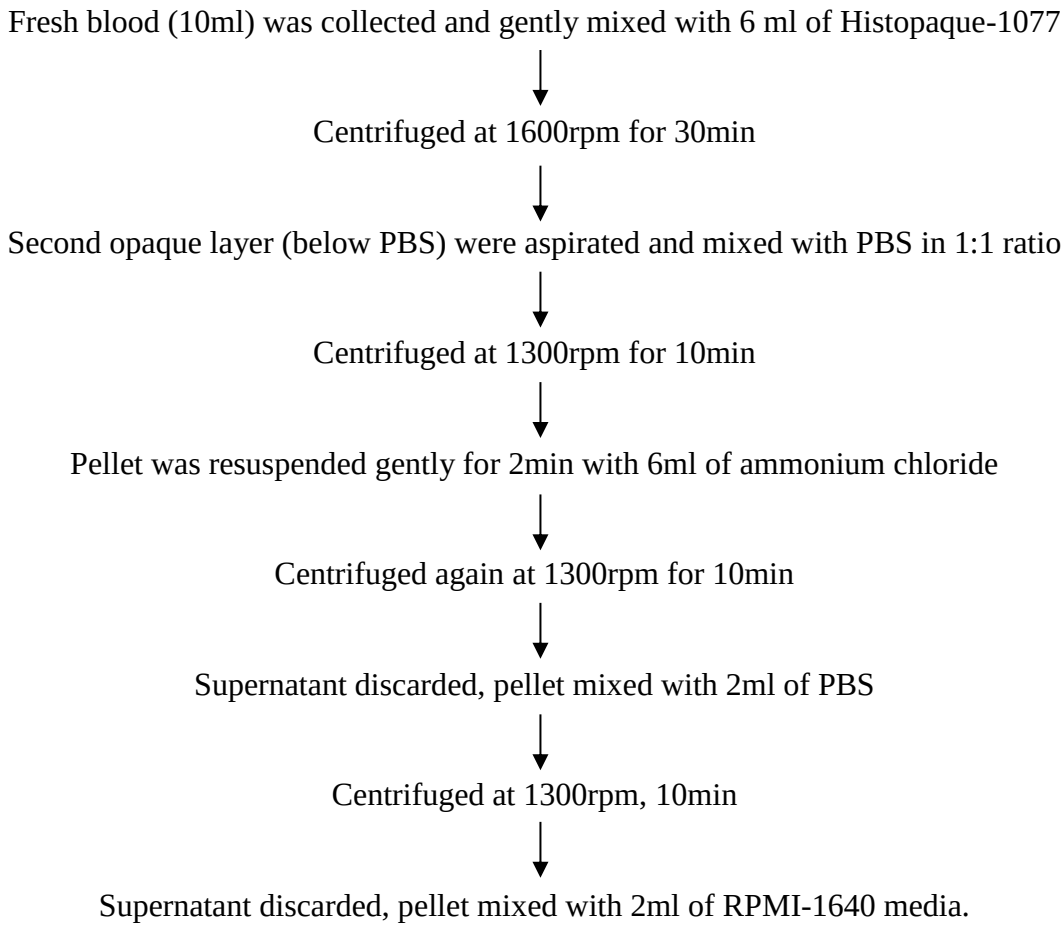


Figure 3.3: The process flow for the isolation of peripheral blood mononuclear cell (PBMC).

3.7.4 EC_{50} determination of genotoxin H_2O_2

The EC_{50} (effective concentration, 50%) that caused 50% DNA damage was first determined and subsequently used in the DNA damage prevention and repairing experiments. Figure 3.4 shows the flow chart of the methodology. Various concentrations of H_2O_2 (0-100 µM) were used. After the H_2O_2 was mixed with PBMC suspension, the mixture was incubated at 37 ℃ for 30min followed by centrifugation. Subsequently, the pellet was resuspended in PBS and centrifuged to wash the H_2O_2 away (performed twice). Finally, the supernatant was removed and pellet was resuspended in PBS ready for slides preparation.

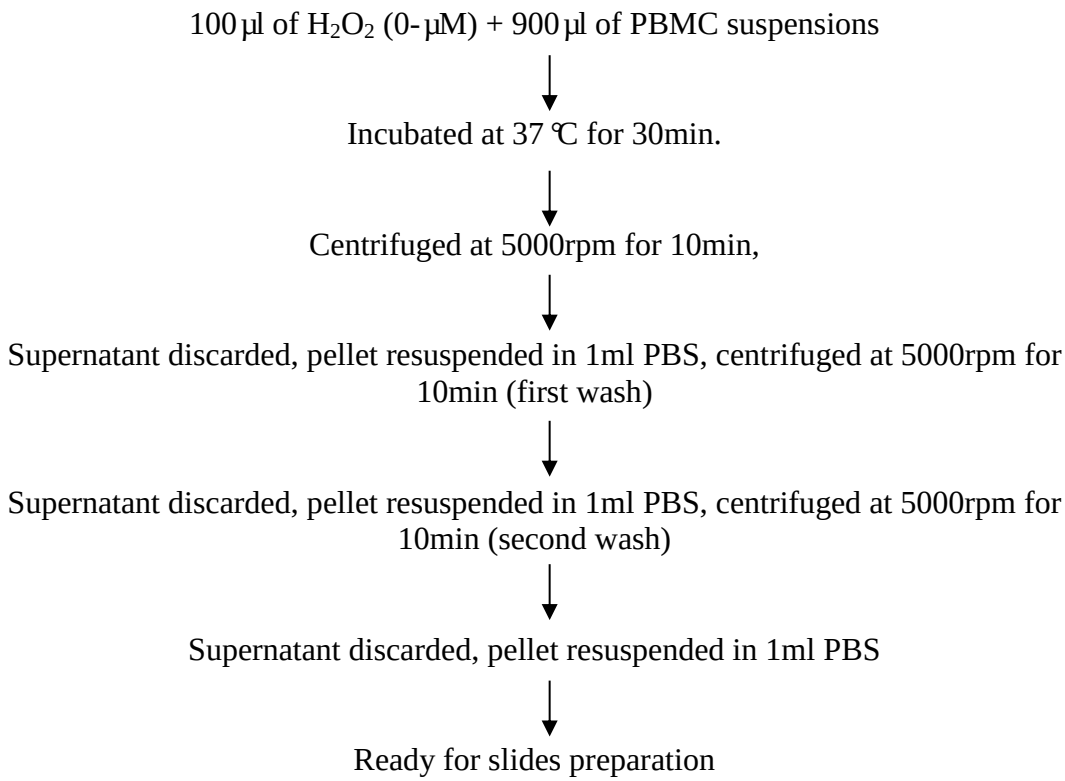


Figure 3.4: The process flow for the determination of EC_{50}.

3.7.5 Effects of ethanol extracts to prevent DNA damage in PBMC induced by H_2O_2

The PBMC suspension was pre-incubated with various ethanol extracts before being induced damage by H_2O_2. Ethanol extracts of *P. giganteus* fruiting bodies, fermented and unfermented wheat grains were used. The process flow is illustrated in Figure 3.5. This was initiated by incubating different concentrations of extracts (0-300 µg/ml) with PBMC suspension followed by centrifugation. The resulting pellet (supernatant discarded) was then resuspended with PBS and centrifuged (performed twice). Further, the remaining pellet resuspended in PBS again to form suspension and H_2O_2 was added to damage the cells. After centrifugation, PBS was used to wash the H_2O_2 away (Figure 3.5). Finally, the resulting pellet was resuspended in PBS and used to prepare slides.

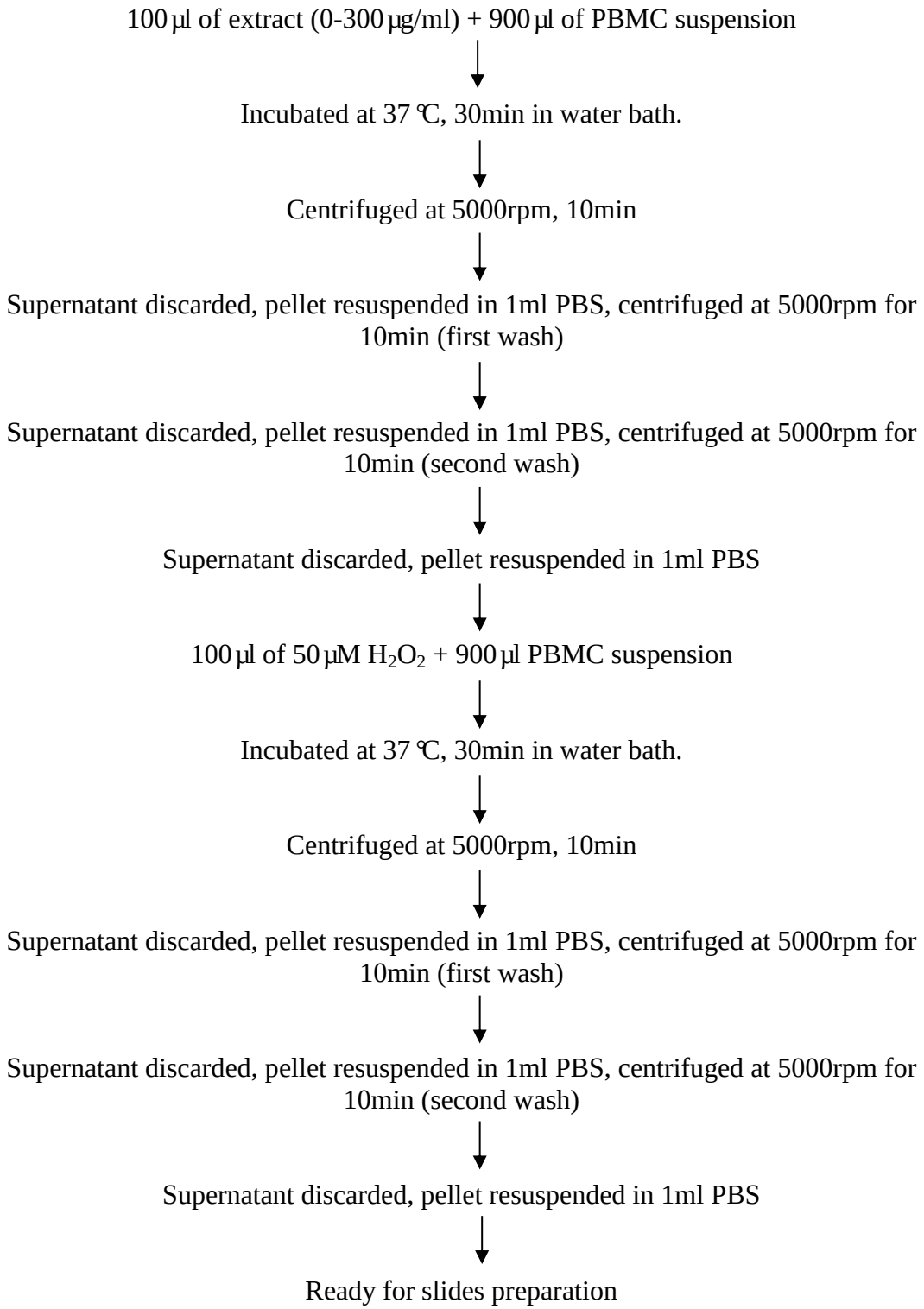


Figure 3.5: Schematic diagram of procedures to study the effects of ethanol extracts in prevention of DNA damage of PBMC induced by H_2O_2.

3.7.6 Effects of ethanol extracts to repair DNA of PBMC after H_2O_2-induced damage

This study aimed to investigate the potential of extracts to repair DNA of the cells after being induced damage by H_2O_2. The flow chart of the methodology is shown in Figure 3.6. The PBMC suspension was first incubated with H_2O_2 to induce DNA damage, followed by centrifugation. Then, the pellet formed was resuspended in PBS to get rid of the remaining H_2O_2. Subsequently, the remaining pellet after centrifuged was resuspended in PBS and the cell suspension treated with various concentrations of ethanol extracts (0-500 µg/ml). After the incubation and centrifugation steps, the remaining pellet was dissolved in PBS and then centrifuged to clean the pellet from any extract. Finally, the cell suspension was prepared by adding PBS and used for slides preparation.

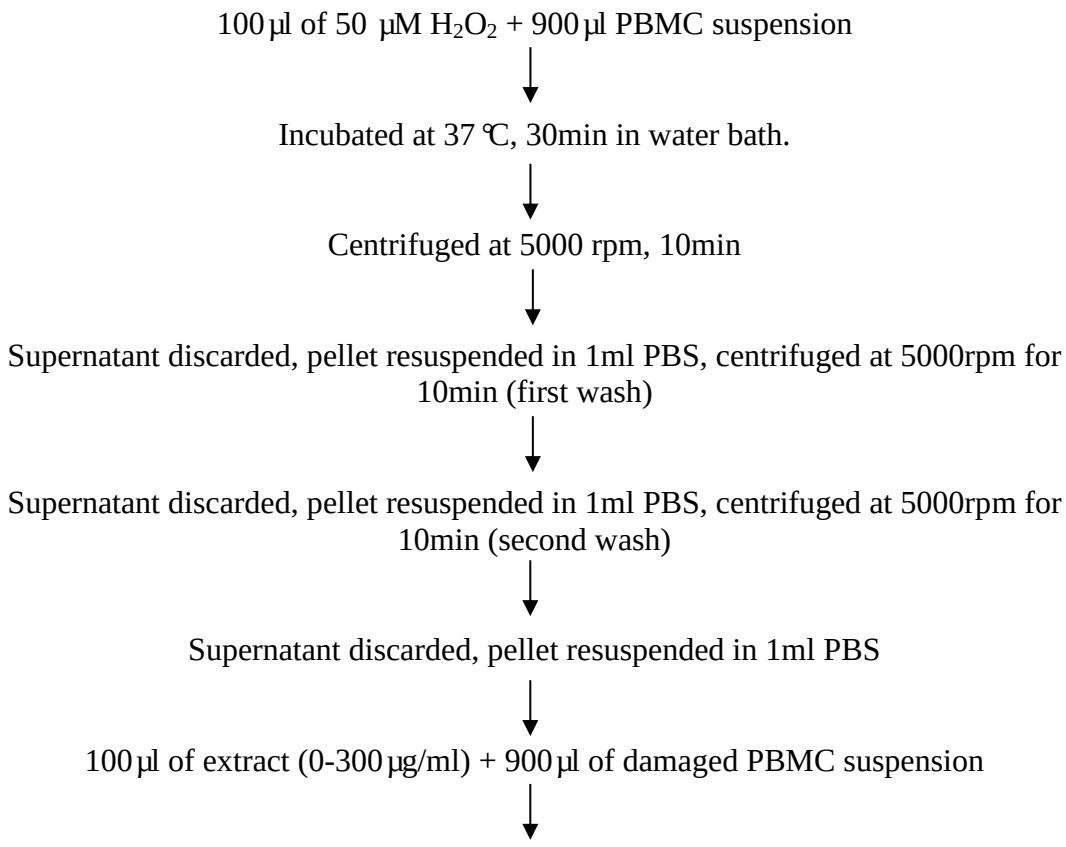


Figure 3.6: Schematic diagram of procedures to study the effects of ethanol extracts in repair of DNA damage of PBMC induced by H_2O_2.

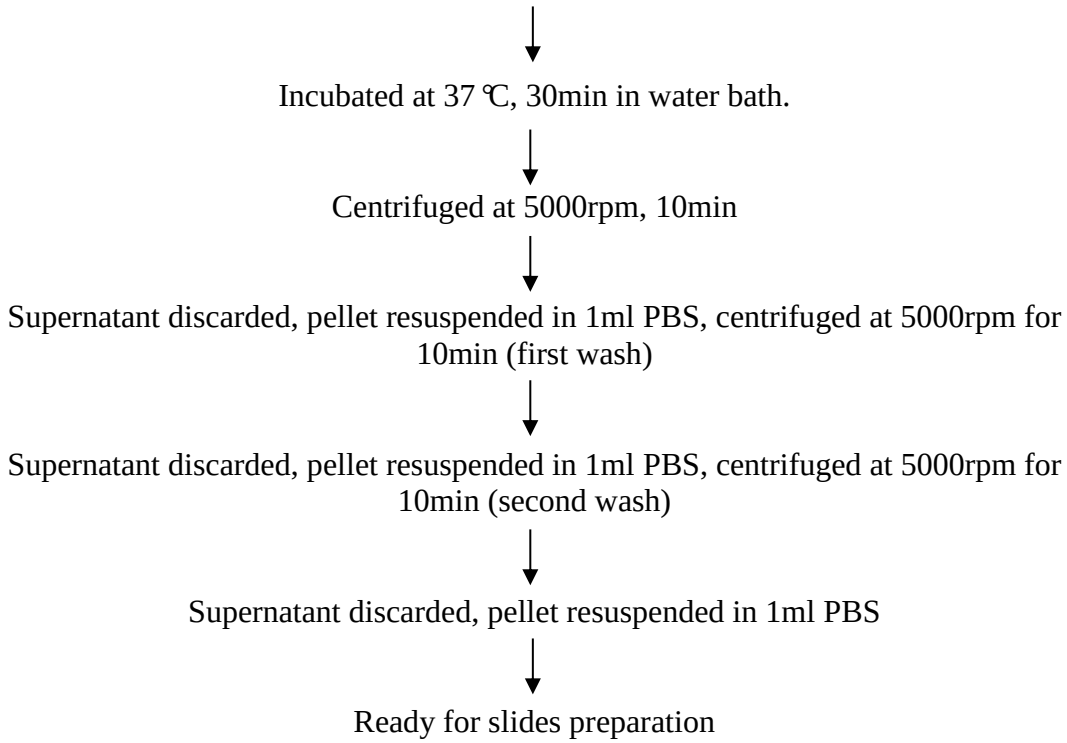


Figure 3.6, continued: Schematic diagram of procedures to study the effects of ethanol extracts in repair of DNA damage of PBMC induced by H_2O_2.

3.7.7 Slides preparation

The primary goal in the slide preparation was to have sufficient stable gels on the slides survived through the results analysis stage. The background noise which interrupts comets visualization should be minimized as much as possible (Tice *et al*., 2000). Fully frosted slides were used in this experiment. According to Tice *et al.* (2000), fully frosted slides are able to increase gel stability by increasing gel bonding. Figure 3.7 illustrates the steps involved in the slides preparation.

Initially, the normal melting point (NMP) agarose, 1% (w/v) in PBS was liquefied. Concentration of the agarose is important as it affects the extent of DNA migration (Tice *et al.*, 2000). After that, the NMP agarose was allocated on one side of the slide and covered with a cover slip. The first layer is a vital layer to promote firm and even attachment of the second layer (Singh *et al.*, 1988). The slide then chilled on a

metal tray full surrounded with ice for 10min to allow gelling of the agarose. After that, the previously treated PBMC suspension was mixed with low melting point (LMP) agarose, 1% (w/v) in PBS. The mixture then applied on the slide after the cover slip on the first solidified agarose layer was removed gently, new cover slip was applied on it. Again, all the slides were left on metal tray ice for 10min.

Finally, all the cover slips were removed and the slides were immersed into chilled lysine solution overnight for cell lysis. The solution was chilled 30min prior to use so that the stability of the agarose gel maintained (Tice *et al.*, 2000). After lysis, the slides were rinsed thrice in Tris Acetate EDTA (TAE) buffer in a coplin jar to remove salt and detergents. The both sides and underside of the slides were then dried by placing on paper towel gently.

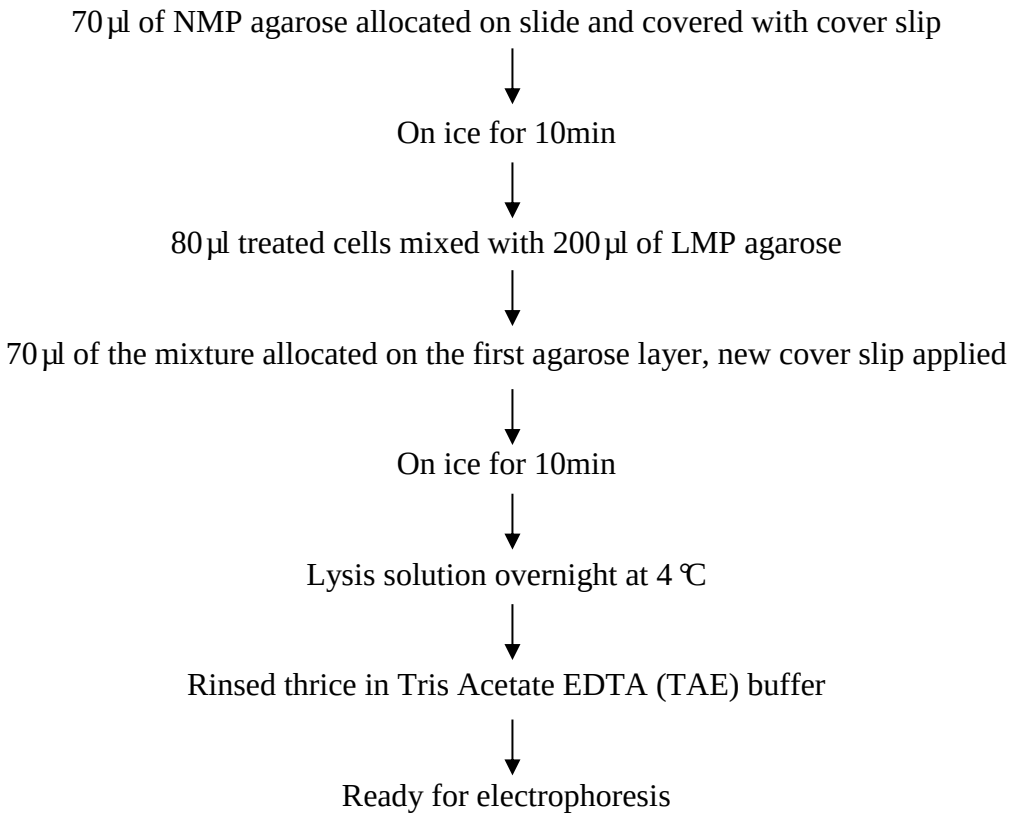


Figure 3.7: The process flow for the slides preparation of comet assay.

3.7.8 Electrophoresis

The procedure described here refer to electrophoresis under pH>13 alkaline conditions (Figure 3.8). First, the slides were put side by side on the electrophoresis tank near as close as possible to the anode. Then, the buffer reservoir was filled with freshly made pH>13 electrophoresis buffer until the liquid level completely covered the slides. The electrophoresis buffer was chilled 30min before use. The slides were soaked in electrophoresis buffer at 4℃ for 40min to produce single stranded DNA and to express alkaline labile sites (ALS) as single strand breaks (SSB). Subsequently, electrophoresis was performed with power supply of 300mA, 25V for 20min. The duration of electrophoresis mainly depends on the cell types and objectives of experiment (Rojas *et al.*, 1999). The tank was embedded on ice throughout the electrophoresis so that the gel layer not melted. After electrophoresis, the slides were taken out gently from the buffer and drained on a paper towel. Then, the slides were neutralized by neutralization buffer for 5min. Singh *et al.* (1988) suggested three washes for 5min each. The background intensity increases as the neutralisation time increases (Rojas *et al.*, 1999). After that, the slides were drained on a paper towel and soaked with 100% ethanol for 2 hrs. Finally, the slides were air-died and stored in a box.

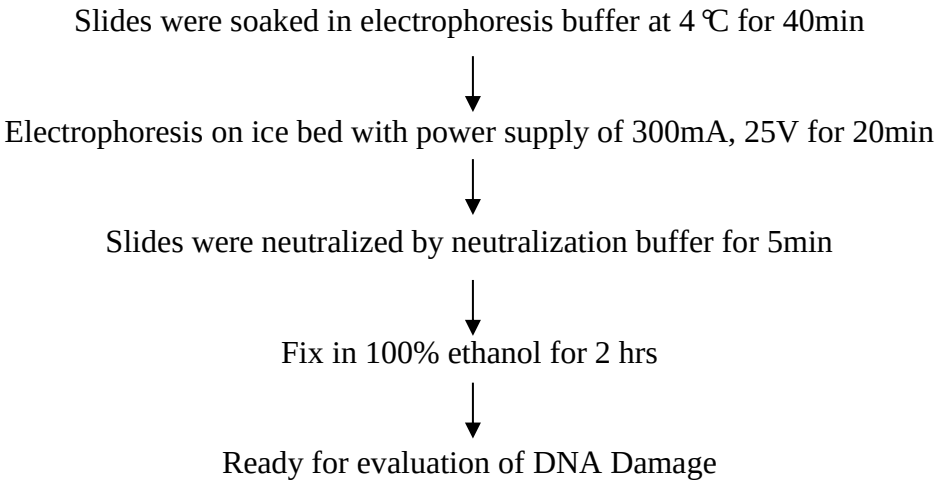


Figure 3.8: The process flow for the electrophoresis of comet assay.

3.7.9 Evaluation of DNA damage

Ethidium bromide was used to assess the DNA damage. The selection of fluorescent dye largely depends on constraints of the equipment and the way data was collected. The common dyes are ethidium bromide, 4',6-diamidino-2-phenylindole (DAPI) and propidium iodide (Rojas *et al.*, 1999). However, non fluorescent technique by using silver nitrate has been developed (Kizilian *et al.*, 1999). The slides with 10µl of 2% ethidium bromide added were covered with cover slip and examined at 200X magnification with a fluorescent microscope. According to Rojas *et al.* (1999), the magnification depends on the type of cells, migration range, constraints of the microscope and imaging system. Komet 5 image analysis software was used to analyse DNA damage, one hundred cells were scored for each concentration. The head and tail DNA percentage were the parameters measured.

3.8 Comparison of antioxidant and genoprotective activities between the extracts

The antioxidant properties and the effects of *P. giganteus* fruiting bodies to prevent DNA damage and/ or repair DNA were compared with fermented and unfermented wheat grains.

3.9 Animal studies

3.9.1 Mushroom samples and chemicals

The freeze-dried fruiting bodies in powder was mixed with dH_2O and administered orally to the rats. Silymarin was purchased from International Laboratory USA. According to Wills and Asha (2006), silymarin as a standard drug demonstrated excellent liver protection activity at a dose of 50mg/kg. Thus, a dose of 50mg/kg was selected in this experiment. Thioacetamide (TAA) and other chemicals were of analytical grade and mainly purchased from Fisher Scientific (M) Sdn. Bhd. and Sigma-Aldrich. Thioacetamide (TAA) was dissolved in sterile dH_2O and injected intraperitoneally to the rats at a concentration of 200mg/kg (Alshawsh *et al.*, 2011). The 8-hydroxy-2-deoxy Guanosine EIA detection kit was a product from Cayman Chemical (589320).

3.9.2 Experimental animals

The *Sprague Dawley* rats of both sexes (200g-250g) were purchased from Animal house, Faculty of Medicine, University of Malaya, Malaysia. They were housed in specially prepared cages at 25 ± 3 ℃, 12 hrs light-dark cycle and relative humidity of 50-60%. All the rats had free access to standard diet and water *ad libitum*. They were acclimatized for three days prior the experiment. The experimental protocol was approved by Animal Ethics Committee [Ethic No.: PM/28/08/2009/MAA (R)]. All experimental rats were handled appropriately in accordance with the criteria prepared by the National Academy of Sciences Malaysia as outlined in the "Guide for the Care and Use of Laboratory Animals".

3.9.3 Acute toxicity assay

Acute toxicity assay was performed to evaluate the toxicity of *P. giganteus* if any. The experimental flow is given in Figure 3.9. There were 36 *Sprague Dawley* rats (18 males, 18 females) used. Male and female *Sprague Dawley* rats were each divided into three different groups (n= 6) and assigned either as vehicle {sterile dH_2O, 5ml/ kg, oral feeding (po)}, low dose *P. giganteus* (2g/kg, po) and high dose *P. giganteus* (5g/kg, po). A minimum of six rats in each group is statistically valid and it achieved the scientific objectives of the study. The rats were not fed overnight prior to the treatments. After treatment, the rats were observed for toxicity symptoms and behavioural changes for a period of 48 hrs. The observations continued up to day 14. Then, the rats were sacrificed after fasting overnight on the 15th day. Livers and kidneys were excised for gross necropsy and histopathological examination.

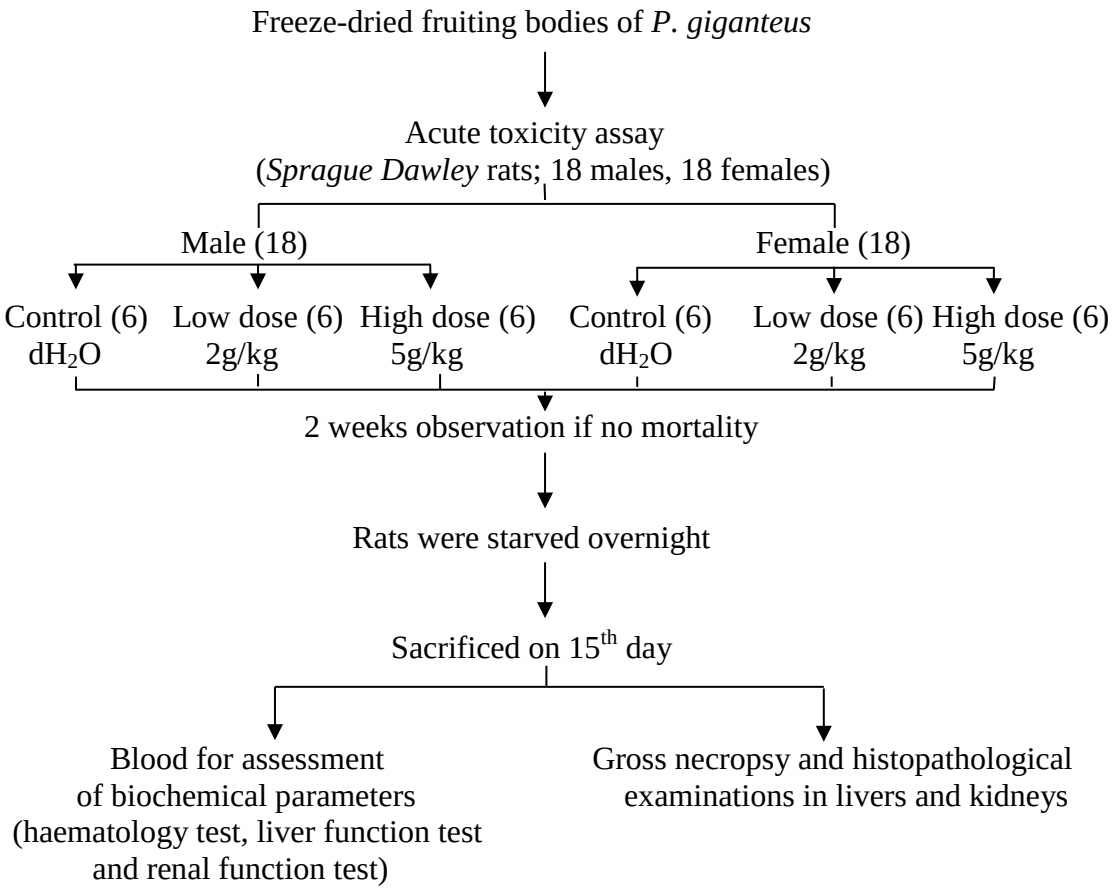


Figure 3.9: Experimental design of acute toxicity assay.

3.9.4 Effects of *P. giganteus* in the prevention of TAA-induced hepatotoxicity in rats

The potential of the *P. giganteus* to prevent hepatic damage was studied *in vivo* by using *Sprague-Dawl*ey rats as study model. *Sprague Dawley* rats of either sex were divided into six groups of six animals each and treated as given in Table 3.1 for two months. The experimental design is illustrated in Figure 3.10.

Table 3.1: Treatments of the rats in different groups during the two-month study.

No.	Group	Treatment
1	Control (dH_2O)	Sterile dH_2O (5ml/kg, po) daily; sterile dH_2O (5ml/kg, i.p) thrice weekly
2	Control (*P. giganteus*)	*Panus giganteus* (0.5g/kg, po) daily; sterile dH_2O (5ml/kg, i.p) thrice weekly
3	TAA control (200mg/kg)	Sterile dH_2O (5ml/kg, po) daily; TAA (200mg/kg, i.p) thrice weekly
4	Silymarin (50mg/kg) + TAA	Silymarin (50mg/kg, po) daily; TAA (200mg/kg, i.p) thrice weekly
5	*Panus giganteus* (0.5g/kg) + TAA	*Panus giganteus* (0.5g/kg, po) daily; TAA (200mg/kg, i.p) thrice weekly
6	*Panus giganteus* (1g/kg) + TAA	*Panus giganteus* (1g/kg, po) daily; TAA (200mg/kg, i.p) thrice weekly

po : Oral feeding
i.p : Intraperitoneal injection

The animals were weighed once a week and were observed for behavioural changes. At the end of the two-month treatment period, all rats were sacrificed under diethyl ether anesthesia after fasting overnight. Blood samples were collected and serum was isolated for biochemical assays. The livers were excised, rinsed in saline, blotted with filter paper and then weighed. Gross necropsy was performed to evaluate any abnormalities

of the livers. Subsequently, the livers were processed for histopathological examination (Alshawsh *et al*., 2011).

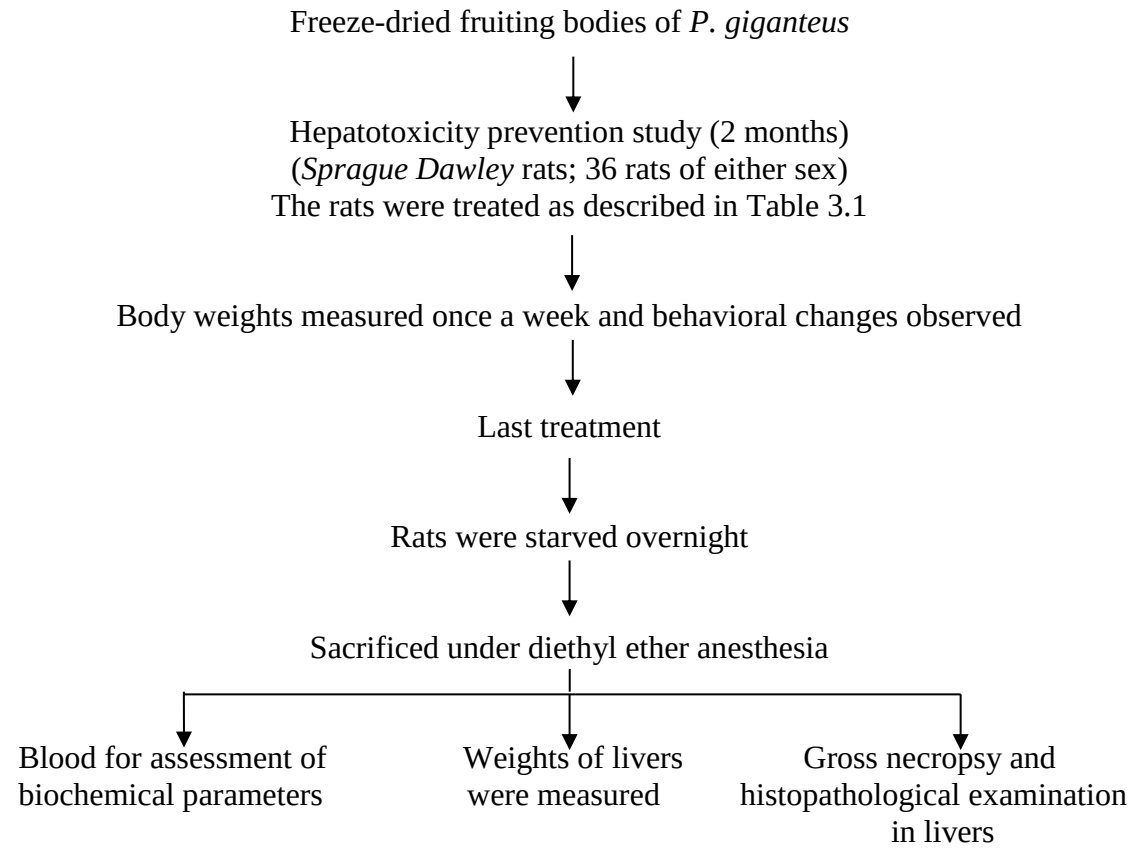


Figure 3.10: Experimental design to investigate the effects of *P. giganteus* in the prevention of TAA-induced hepatotoxicity in rats.

3.9.5 Effects of *P. giganteus* in the treatment of TAA-induced hepatotoxicity in rats

The study was to investigate the effects of *P. giganteus* fruiting bodies in the treatment of hepatotoxicity induced by thioacetamide (TAA). *Sprague Dawley* rats of either sex were divided into six groups of six animals each and treated as below for three months (Table 3.2). Figure 3.11 demonstrates the experimental design of the study. In the first two months, the rats were fed orally (po) with fruiting bodies or dH_2O and injected intraperitoneally with either TAA or dH_2O according to the group. After two months, the experimental rats were only fed orally according to the group without intraperitoneal injection.

Table 3.2: Treatments of the rats in different groups during the three-month study.

No.	Group	Treatment (First 2 months)	Treatment (Last month)
1	Control (dH_2O)	Sterile dH_2O (5ml/kg, po) daily; sterile dH_2O (5ml/kg, i.p) thrice weekly	Sterile dH_2O (5ml/kg, po) daily
2	Control (*P. giganteus*)	*Panus giganteus* (0.5g/kg, po) daily; sterile dH_2O (5ml/kg, i.p) thrice weekly	*Panus giganteus* (0.5g/kg, po) daily
3	TAA control (200mg/kg)	Sterile dH_2O (5ml/kg, po) daily; TAA (200mg/kg, i.p) thrice weekly	Sterile dH_2O (5ml/kg, po) daily
4	Silymarin (50mg/kg) + TAA	Silymarin (50mg/kg, po) daily; TAA (200mg/kg, i.p) thrice weekly	Silymarin (50mg/kg, po) daily
5	*Panus giganteus* (0.5g/kg) + TAA	*Panus giganteus* (0.5g/kg, po) daily; TAA (200mg/kg, i.p) thrice weekly	*Panus giganteus* (0.5g/kg, po) daily
6	*Panus giganteus* (1g/kg) + TAA	*Panus giganteus* (1g/kg, po) daily; TAA (200mg/kg, i.p) thrice weekly	*Panus giganteus* (1g/kg, po) daily

po : Oral feeding
i.p : Intraperitoneal injection

The behavioral changes of the experimental rats were observed and body weights were measured once a week. After the last treatment, the rats were starved overnight and sacrificed by using anesthetics diethyl ether. Blood was collected from the rats and subsequently serum isolated for biochemical analysis. The livers were excised from the animal's body, rinsed in saline buffer, blotted with filter paper and their weights were measured. Gross necropsy and histopathological examination were then performed.

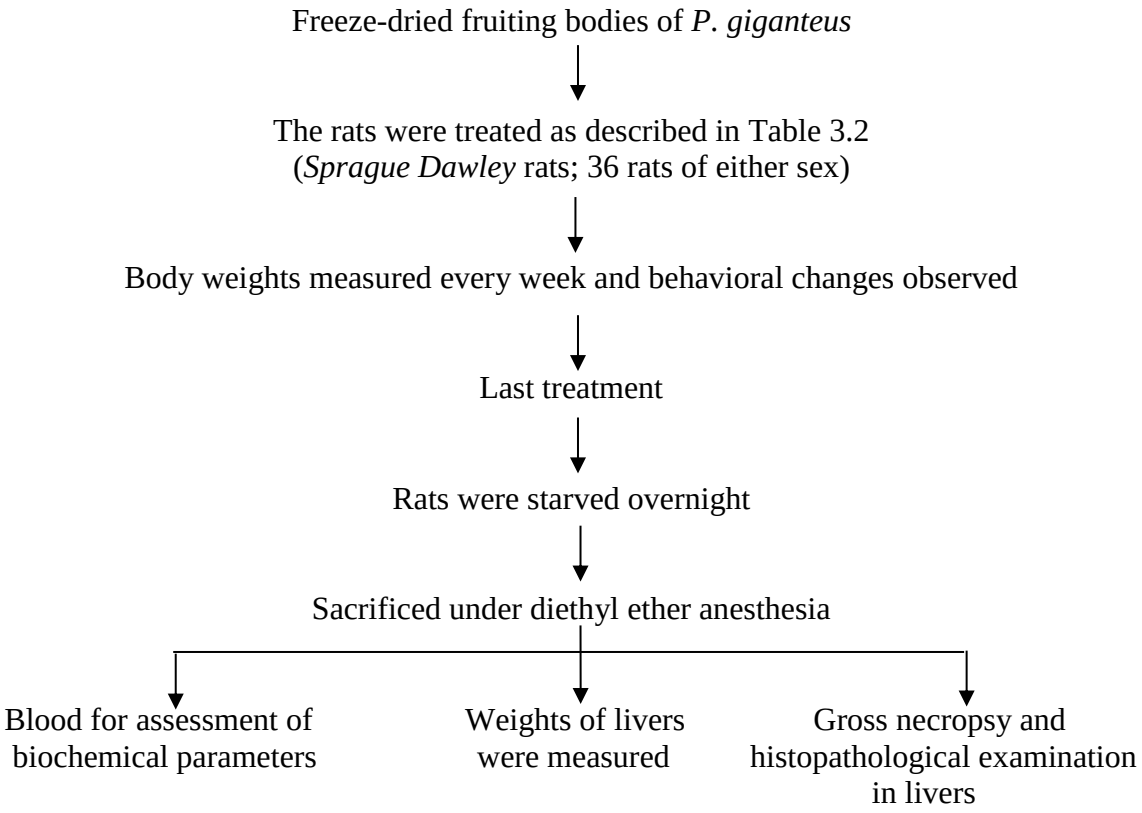


Figure 3.11: Experimental design to investigate the effects of *P. giganteus* in the treatment of TAA-induced hepatotoxicity in rats.

3.9.6 Assessment of biochemical parameters

The blood samples were centrifuged at 3500rpm (1534 x g) for 10min (Jouan C312 centrifuge). The resulting serum was then collected and sent to the Clinical Diagnostic Laboratory, University of Malaya Medical Centre for biochemical assessment. In regards to the acute toxicity assay, haematology test, liver function test and renal function test were performed while only liver function test was performed for the thioacetamide (TAA)-induced hepatotoxicity studies (prevention and treatment). In addition, serum MDA content and urinary free 8-OH-dG level were also determined for thioacetamide (TAA)-induced hepatotoxicity studies (prevention and treatment).

Determination of serum malondialdehyde (MDA) content was performed by thiobarbituric acid-reacting substances (TBARS) method as described by Daker *et al*. (2008) with minor modifications. The results were calculated as 1,1,3,3-tetraethoxypropane (TEP) equivalents based on the TEP standard calibration. Damage of DNA due to oxidative stress was determined by measuring the levels of free 8-OH-dG in urine according to the protocol of the manufacturer (Cayman Chemical- 589320). Urine was collected 24 hrs before the rats were sacrificed and kept in -80 ℃ freezer.

3.9.7 Gross necropsy and histopathological examination

Livers and kidneys in the animal studies were sliced and fixed in 10% (v/v) formalin immediately after collection for at least 24hrs. The sections were then processed in automated tissue processing machine, embedded in paraffin and cut into 5µm sections. After that, the sections were stained with haematoxylin-eosin dye and observed under microscope to evaluate histopathological changes.

3.10 Statistical analysis

All results were expressed as mean ± S.E.M. The data were analyzed by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test. The level of significance was set at $p < 0.05$ (Appendix C, Table C.1-C.63, pp. 123-171).

CHAPTER FOUR: RESULTS AND DISCUSSION

4.1 Nutritional Composition

The nutritional composition of the *P. giganteus* fruiting bodies, fermented and unfermented wheat grains are tabulated in Table 4.1-4.3. Fruiting bodies had higher protein, fat and dietary fibre content but lower carbohydrate and energy content when compared to fermented and unfermented wheat grains (Table 4.1). The recorded protein content in fruiting bodies was 15.4g/100g which was 36.28% and 46.67% higher than fermented and unfermented wheat grains, respectively. Furthermore, fruiting bodies had more than two folds higher fat and dietary fibre content than fermented and unfermented wheat grains. However, carbohydrate and energy content were lower in fruiting bodies when compared to fermented and unfermented wheat grains (Table 4.1). The results also showed that levels of cholesterol were less than 0.001mg/100g in all the three samples tested.

The fat composition of fruiting bodies, fermented and unfermented wheat grains are depicted in Table 4.2. Overall, the fat compositions of all the three samples were dominated by unsaturated fat (monounsaturated and polyunsaturated fat). Fruiting bodies had higher fat content than fermented and unfermented wheat grains. They contained more monounsaturated fat and saturated fat, but lower amounts of polyunsaturated fat than fermented and unfermented wheat grains. As shown in Table 4.2, fruiting bodies recorded 1.97g/100g of saturated fat which was several folds higher than fermented wheat grains (0.27g/100g) and unfermented wheat grains (0.33g/100g). Interestingly, levels of trans fat were less than 0.01g/100g in all the three samples.

Table 4.1: Nutritional composition of *P. giganteus* fruiting bodies, fermented and unfermented wheat grains.

Sample	Amount (per 100g)					
	Protein (g)	Fat (g)	Cholesterol (mg)	Carbohydrate (g)	Dietary fibre (g)	Energy (kcal)
P. giganteus fruiting bodies	15.4	3.7	(< 0.001)	67.2	33.3	364
Fermented wheat grains	11.3	1.5	(< 0.001)	83.4	12.6	392
Unfermented wheat grains	10.5	1.9	(< 0.001)	80	11.6	379
RDA for adults and children ≥ 4 years (g)	50	65	0.02	300	25	-

Table 4.2: Fat composition of *P. giganteus* fruiting bodies, fermented and unfermented wheat grains.

Sample	Amount (g/100g)			
	Monounsaturated fat	Polyunsaturated fat	Saturated fat	Trans fat
P. giganteus fruiting bodies	1.97	0.77	0.97	< 0.01
Fermented wheat grains	0.27	0.97	0.27	< 0.01
Unfermented wheat grains	0.33	1.17	0.35	< 0.01

Table 4.3: Mineral composition of *P. giganteus* fruiting bodies, fermented and unfermented wheat grains.

Sample	Amount (mg/100g)									
	Phosphorus	Potassium	Sodium	Zinc	Calcium	Copper	Iron	Magnesium	Manganese	Selenium
P. giganteus fruiting bodies	526.45	1345.7	5.7	2.68	5.78	0.59	1.85	67.64	0.41	< 0.002
Fermented wheat grains	274.43	267.1	< 0.01	1.94	33.27	3.74	2.92	114.22	3.59	< 0.002
Unfermented wheat grains	295.76	303.31	< 0.01	1.85	31.5	3.82	2.21	115.42	2.45	< 0.002
RDA for adults and children ≥ 4 years (g)	1000	3500	2400	15	1000	2	18	400	2	0.07

Fruiting bodies of *P. giganteus* had higher amounts of phosphorus, potassium, sodium and zinc but lower amounts of calcium, copper, iron, magnesium and manganese than fermented and unfermented wheat grains (Table 4.3). The potassium levels in fruiting bodies were 403.82% and 343.67% higher than fermented and unfermented wheat grains, respectively. However, selenium levels were less than 0.002 mg/100 g in all the three samples.

Protein as building block of our body plays important roles in wide range of metabolic functions and homeostasis. The protein content in mushrooms vary due to the type of mushroom selected, developmental stage, sampling part, quantity of nitrogen available and location (Flegg and Maw, 1977). The recommended daily allowance (RDA) for protein is 50g. The recorded protein content in the samples tested (10.5-15.4g/100g) can partially meet the standard daily protein requirement. When compared to other edible mushrooms, *P. giganteus* (15.4% of protein content) had higher protein content than other culinary mushrooms including *Agaricus arvensis* (2.87%), *Lactarius deliciosus* (2.96%), *Leucopaxillus giganteus* (3.40%), *Sarcodon imbricatus* (2.35%), and *Tricholoma portentosum* (2.12%) reported by Barros *et al*. (2007).

Fruiting bodies were also shown to have 164.29% and 187.07% higher fibre content than fermented and unfermented wheat grains, respectively. Dietary fibre is important to regulate functionalities of body digestive system. In regards to carbohydrate content, fruiting bodies recorded 24.11% and 19.05% lower carbohydrate content than fermented and unfermented wheat grains. Although fruiting bodies had higher amounts of fat and proteins which are also major energy sources than fermented and unfermented wheat grains, fruiting bodies recorded lower energy content probably due to their low carbohydrate content.

Lipids played vital roles in human body as hormones and energy storage. They also make up the phospholipid cell membrane, form the important component of myelin sheath, and act as thermal insulators (Burtis & Ashwood, 1997). The RDA for total fat is 65g, the low fat composition of the samples tested (1.9-3.5g/100g) were promising to be incorporated in daily diet. As depicted in Table 4.2, the fats in all the samples were dominated by unsaturated fat. This was reported by Barros *et al.* (2007) who also showed that unsaturated fatty acids predominated over saturated fatty acids in all mushroom species studied. Saturated fat increased the levels of low-density lipoprotein (LDL) cholesterol (Hu *et al.*, 1997) of which the risk of cardiovascular diseases was known to be positively associated with LDL cholesterol concentration but negatively associated with high-density lipoprotein (HDL) cholesterol concentration (Minamide & Hammond, 1985). Furthermore, all samples had very low amounts of trans fat and cholesterol. Trans fat was reported to increase the deposition of cholesterol into cellular plasma membranes and caused atherosclerosis (Chen *et al.*, 2011).

In conclusion, nutritional profile of fermented wheat grains is similar to that of unfermented wheat grains. *Panus giganteus* fruiting bodies, fermented and unfermented wheat grains are good sources of key nutrients including carbohydrate, protein, fibre and mineral with low fat content. The high nutritional quality encourages the incorporation of *P. giganteus* fruiting bodies or wheat grains fermented by *P. giganteus* in daily diet to maintain good health.

4.2 Extraction yield

The extraction yields of various ethanol extracts are shown in Table 4.4. Unfermented wheat grains had significantly ($p < 0.05$) higher yield (5.87%) than fruiting bodies (5.30%) and fermented wheat grains (4.90%).

4.3 Assessment of antioxidant properties and total phenolic content

4.3.1 Scavenging effects on 1,1-diphenyl-2-picrylhydrazyl (DPPH)

As shown in Figure 4.1, all extracts displayed DPPH free radical scavenging activities but to different extents. The scavenging activities were observed to be dose-dependent and no endpoint was observed for all the extracts. At 10mg/ml, the scavenging activities of all the extracts tested were less than 20%. The activities then gradually increased with increasing concentrations and were more than 30% at 30mg/ml. Unfermented wheat grains showed better scavenging effects than fruiting bodies and fermented wheat grains at all the concentrations studied. Further, unfermented wheat grains had significantly ($p < 0.05$) lower IC_{50} (21.03mg/ml) when compared to fermented wheat grains (40.76mg/ml) and fruiting bodies (42.46mg/ml) (Table 4.4). As a lower IC_{50} indicates a higher quenching activity, unfermented wheat grains were shown to be a better DPPH scavenger when compared to fruiting bodies and fermented wheat grains. Unfermented wheat grains may contain more hydrogen donating compounds as the DPPH scavenging activity was mainly attributed by hydrogen donating ability of bioactive molecules (Baumann *et al*., 1979). Besides that, BHT as synthetic antioxidant was a potent scavenger (IC_{50}: 0.85mg/ml) when compared to extracts in the present study (IC_{50}: 21.03-42.46mg/ml). This is due to BHT is a pure compound while in this study crude extracts were tested.

Table 4.4: Antioxidant properties of various ethanol extracts.

Extract	Extraction yield (%)	Total phenolic content (mg of GAEs/g extract)	DPPH scavenging activity *(IC_{50}) (mg/ml)	Ferric reducing antioxidant power (FRAP) (µmol of $FeSO_4.7H_2O$ equivalents/g extract)	Trolox equivalents antioxidant capacity (TEAC) (µmol of trolox equivalents/g extract)	Inhibition of lipid peroxidation at 10mg/ml (%)
Fruiting bodies	5.30 ± 0.06^{b}	6.59 ± 0.40^{ab}	42.46 ± 3.53^{b}	20.03 ± 1.04^{a}	11.10 ± 1.34^{a}	26.65 ± 1.96^{a}
Fermented wheat grains	4.90 ± 0.06^{a}	5.19 ± 0.06^{a}	40.76 ± 1.63^{b}	20.73 ± 1.00^{a}	10.94 ± 0.92^{a}	41.89 ± 2.14^{b}
Unfermented wheat grains	5.87 ± 0.03^{c}	7.60 ± 0.36^{b}	21.03 ± 0.67^{a}	82.33 ± 1.68^{b}	19.16 ± 0.74^{b}	39.95 ± 1.99^{b}

*IC_{50}: 50% radical scavenging activity**.** Each value is expressed as mean ± S.E.M (n=3)**.** Mean values in the same column with different letters are significantly different ($p < 0.05$).

The DPPH scavenging abilities of mushrooms extracted with 95% (v/v) ethanol have been reported in previous studies. In Portugal, wild mushrooms *Lepista inversa* and *Clitocybe alexandri* had scavenging activities at IC_{50} of 9.3mg/ml and 10.7mg/ml respectively (Vaz *et al.*, 2010). In addition, ethanol extract of *Pleurotus citrinopileatus* cultivated in Taiwan was more effective in scavenging activity (IC_{50}: 1.33mg/ml) when compared to the fruiting bodies in the present study. This may be due to the higher phenolic content in *P. citrinopileatus* than *P. giganteus*. *Pleurotus citrinopileatus* reported by Lee *et al.* (2007) had phenolic content of 8.62mg of GAEs/g compared to 6.59mg of GAEs /g for *P. giganteus*.

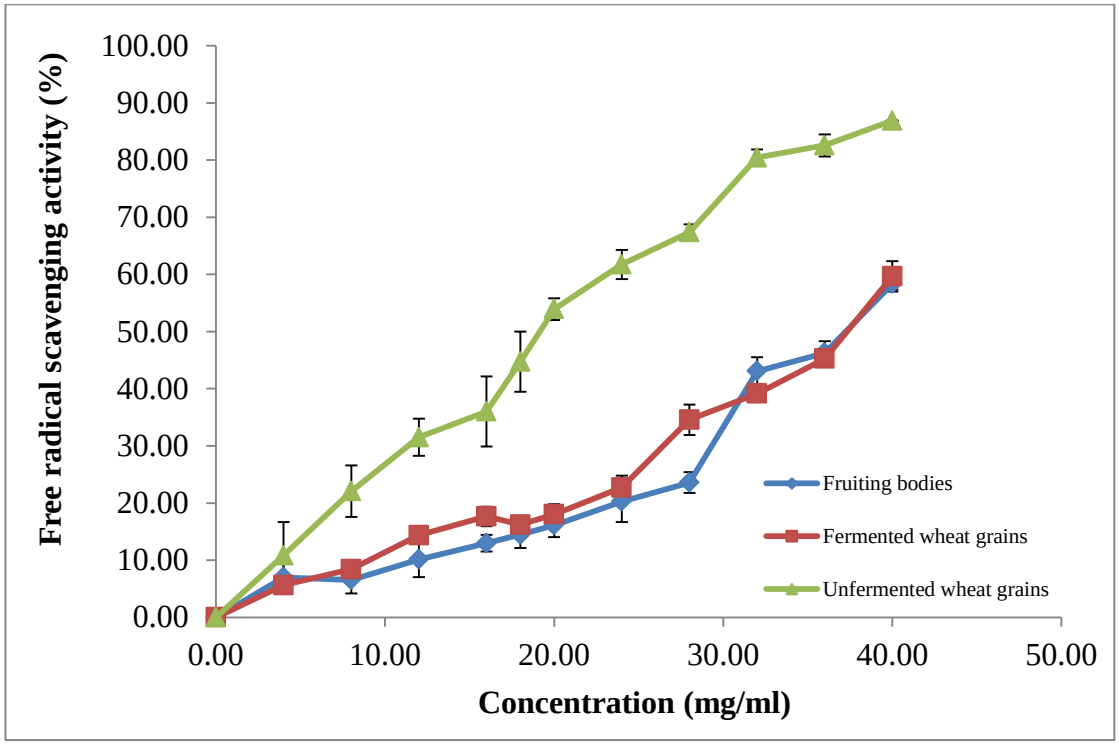


Figure 4.1: DPPH scavenging activities of ethanol extracts of *P. giganteus* fruiting bodies, fermented and unfermented wheat grains.

4.3.2 Ferric reducing antioxidant power (FRAP)

As shown in Table 4.4, unfermented wheat grains had the strongest reducing power as it attained significantly ($p < 0.05$) higher FRAP value (82.33 µmol of $FeSO_4.7H_2O$ equivalents/g) when compared to fruiting bodies (20.03 µmol of $FeSO_4.7H_2O$ equivalents/g) and fermented wheat grains (20.73 µmol of $FeSO_4.7H_2O$ equivalents/g). Furthermore, there were no significant differences in FRAP values between fruiting bodies and fermented wheat grains. The FRAP value of BHT was reported to be 1362.57 µmol of $FeSO_4.7H_2O$ equivalents/g.

When compared to local edible plants, the FRAP values of the extracts in the present study (20.03-82.33 µmol of $FeSO_4.7H_2O$ equivalents/g) were higher than 'Temu kunci' or *Boesenbergia rotunda* L (7.8 µmol of $FeSO_4.7H_2O$ equivalents/g) and cucumber (6.7 µmol of $FeSO_4.7H_2O$ equivalents/g) reported by Wan-Ibrahim *et al.* (2010).

4.3.3 Trolox equivalents antioxidant capacity (TEAC)

As shown in Table 4.4, all extracts displayed antioxidant capacities as they were able to scavenge ABTS radical cations. The TEAC values for fruiting bodies, fermented and unfermented wheat grains were 11.11, 10.94 and 19.16 µmol of trolox equivalents/g, respectively. Unfermented wheat grains displayed significantly ($p < 0.05$) higher ABTS' scavenging activity than fruiting bodies and fermented wheat grains. These may imply that unfermented wheat grains possessed greater antioxidant properties. Butylated hydroxytoluene (BHT) had TEAC value of 1332.78 µmol of trolox equivalents/g. The extracts in the present study were shown to have higher antioxidant capacity (11.10-19.16 µmol of trolox equivalents/g) than canola meal (3.62 µmol of trolox equivalents/g) reported by Hassas-Roudsari *et al.* (2009).

4.3.4 Inhibition of lipid peroxidation

The assay was performed to investigate the ability of the extracts to inhibit peroxidation of phospholipids in the egg yolk. At a concentration of 10mg/ml, fermented wheat grains (41.89%) and unfermented wheat grains (39.95%) recorded significantly ($p < 0.05$) higher percentage of inhibition of lipid peroxidation than fruiting bodies (26.66%) (Table 4.4). All extracts were comparable to BHT which recorded inhibition activity of 31.63% at 10mg/ml.

Noorlidah *et al.* (2012) reported that *L. edodes* and *Schizophyllum commune* had lipid peroxidation inhibition percentages of 38.29% and 36.24% at 10mg/ml. These were higher than 26.66% for *P. giganteus* fruiting bodies probably due to their higher phenolic content. *L. edodes* and *S. commune* had total phenolic content of 14.70mg GAEs/g and 16.47mg GAEs/g respectively, compared to the phenolic content of *P. giganteus* which was 6.59mg of GAEs/g.

4.3.5 Total phenolic content

In the present study, the total phenolic compounds in the extracts of unfermented wheat grains, fruiting bodies and fermented wheat grains were 7.60, 6.59 and 5.19 mg of GAEs/g respectively (Table 4.4). Unfermented wheat grains had significantly ($p < 0.05$) higher phenolic content when compared to fruiting bodies and fermented wheat grains. Butylated hydroxytoluene (BHT) had phenolic content of 634. 83mg of GAEs/g.

According to Slavin (2004), whole grains had high amount of antioxidant substances to inhibit oxidative stress in human. Ferulic and p-coumaric acids were the main phenolic acids in cereals (Mattila *et al*, 2005). Thus, it was not surprised that unfermented wheat grains recorded high phenolic content in the present study.

Furthermore, it was noted that the ethanol extract of fruiting bodies (6.59mg of GAEs/g) in the present study had higher phenolic content than ethanol extract of *C. alexandri* (6.30mg of GAEs /g), but lower than *L. inversa* (10.80mg of GAEs /g) (Vaz *et al.*, 2010), and *P. ostreatus* (54.90mg of GAEs /g) (Jayakumar *et al.*, 2009). A range of culinary mushrooms grown in Malaysia including *A. auricular-judae, H. erinaceus* and *L. edodes* and *P. sajor-caju* were also reported to contain phenolic compounds in the range of 6.19 – 17.70mg of GAEs/g hot water extract (Noorlidah *et al.*, 2012).

In conclusion, all extracts tested had appreciable amounts of phenolic compounds and possessed antioxidant properties by virtue of DPPH free radical scavenging activity, reducing power, antioxidant capacity and lipid peroxidation inhibition activity. The antioxidant activities may be attributed to phenolic compounds. Fermented wheat grains had antioxidant properties comparable to fruiting bodies. Although unfermented wheat grains had better antioxidant properties than *P. giganteus*, *P. giganteus* could still be regarded as reliable source of natural antioxidants as shorter time needed to grow it compared to wheat grains. *Panus giganteus* fruiting bodies or wheat grains fermented by *P. giganteus* may be formulated into functional food to prevent cellular injuries induced by oxidative stress.

4.4 Genoprotection studies

4.4.1 EC_{50} determination of genotoxin H_2O_2

Genotoxicity experiment was performed to find the EC_{50} of H_2O_2 that caused 50% DNA damage in PBMC. The cells were treated with various concentrations of H_2O_2 (0-100 µM). The DNA damage resulted were analysed by Komet 5 image analysis software. The EC_{50} (53.21 µM) obtained in this experiment was then applied in the genoprotection experiments (4.4.2 and 4.4.3) to induce 50% of DNA damage.

The percentages of tail DNA of PBMC are shown in Figure 4.2 while Plate 4.1 illustrates the comet images showing cell damage induced by H_2O_2. As shown in Figure 4.2, the percentage of tail DNA of PBMC in control group (incubated with zero concentration of H_2O_2) was close to zero. However, the percentage of tail DNA increased in a dose dependent manner from 1 to 75 µM of H_2O_2 concentrations. At the concentration of 75 µM, the percentage of tail DNA of PBMC was more than 70. The EC_{50} of H_2O_2 which caused 50% DNA damage was 53 µM.

The results showed that water as a universal solvent was not toxic to PBMC as the percentage of tail DNA of PBMC in control group was close to zero. On the other hand, H_2O_2 had toxicity effects to damage the DNA of PBMC as the percentage of tail DNA increased from 1 to 75 µM of H_2O_2 concentrations. Numerous studies had described the cytotoxic effects of high levels of H_2O_2 (more than 50 µM), and the toxicity depends on the period of exposure, concentration of H_2O_2, cell type and its culture media (Halliwell *et al.*, 2000).

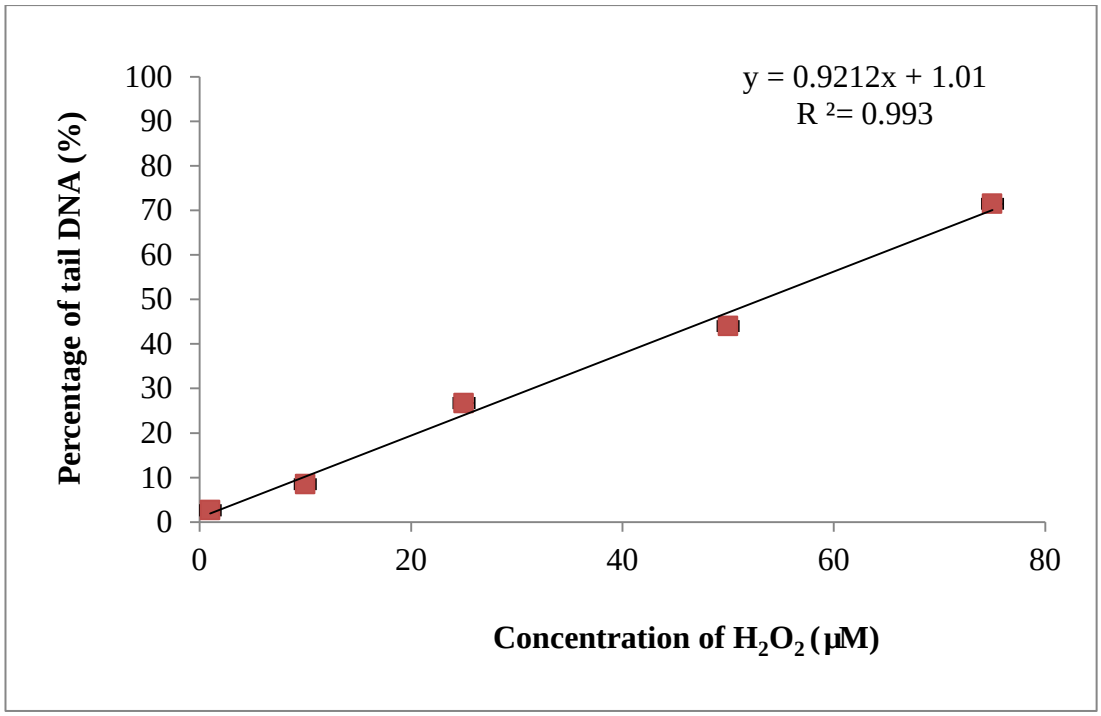


Figure 4.2: Percentage of tail DNA of PBMC (%) exposed to various concentrations of H_2O_2.

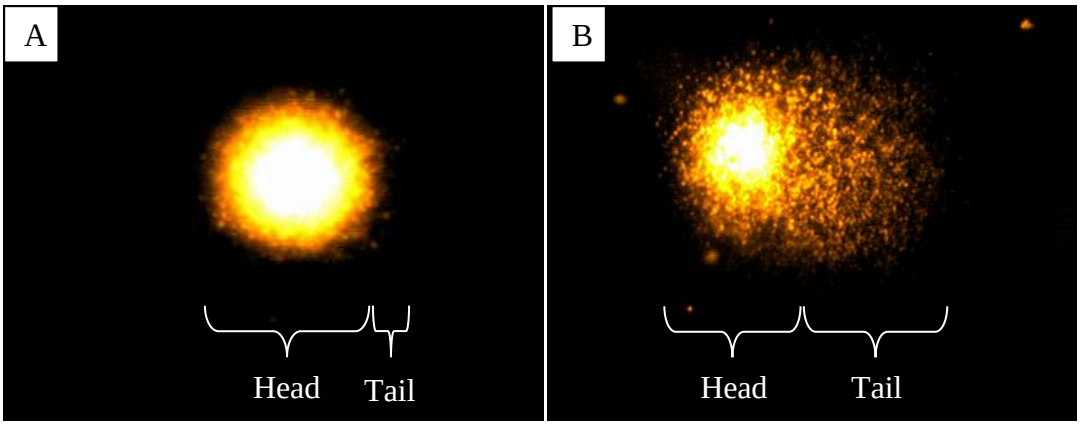
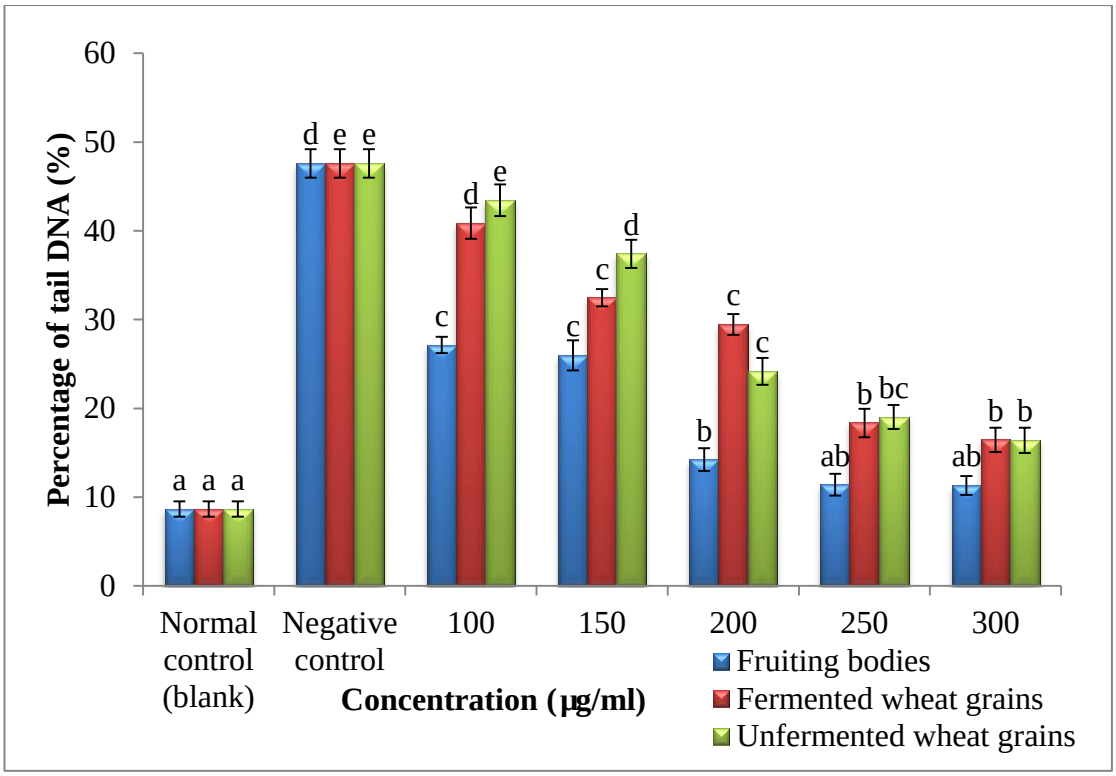


Plate 4.1: Representative comet images showing cell damage induced by H_2O_2. (A) Cell in control group (0-5% of tail DNA); (B) Cell damaged by 50 µM of H_2O_2 (40-50% of tail DNA).

4.4.2 Effects of ethanol extracts to prevent DNA damage in PBMC induced by H_2O_2

In the present study, the genoprotective effects of *P. giganteus* and wheat grains fermented by *P. giganteus* to protect the DNA of PBMC against damage induced by H_2O_2 were evaluated. Cells in the negative control group (blank) were not damaged by H_2O_2 possessed 8.67% of tail DNA. The lower percentage of tail DNA signified lower level of DNA damage occurred. The PBMC in the negative control group which incubated with dH_2O prior to the addition of H_2O_2 (53.21 μM) recorded tail DNA of 47.58%. Plate 4.2 illustrates the comet images depicting various degrees of DNA damage.

All the extracts showed DNA protection activity and decreased the tail DNA of PBMC to an extent comparable to normal control (Figure 4.3). The genoprotective effects were dose-dependent as the percentages of tail DNA decreased with increasing concentration. Fruiting bodies showed greatest genoprotective effects as PBMC treated with ethanol extract of *P. giganteus* fruiting bodies had lowest tail DNA than fermented and unfermented wheat grains at all the concentrations tested. There were no significant differences between fermented and unfermented wheat grains in the genoprotective effects. Genoprotective effects exhibited by the extracts could be associated with the antioxidant compounds in the extracts as H_2O_2 (free radical) induced DNA damage by causing oxidative stress.



Different alphabets are significantly different ($p < 0.05$).

Figure 4.3: Percentages of tail DNA of PBMC (%) treated with various concentrations of extracts in prevention of DNA damage of PBMC induced by H_2O_2.

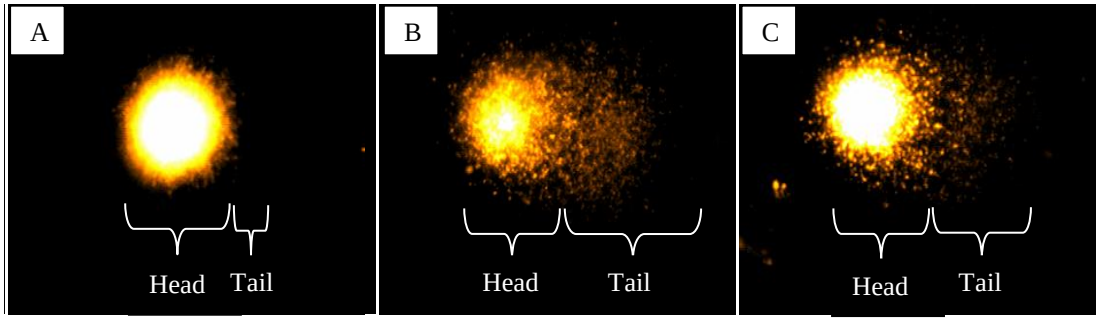


Plate 4.2: Representative comet images showing various degrees of damages in the study of the genoprotective effects of extracts against H_2O_2-induced DNA damage. (A) cell in blank group (5-10% tail DNA); (B) cell in control group (50-60% tail DNA); (C) cell treated with 150mg/ml of *P. giganteus* ethanol extract (20-30% tail DNA).

The antioxidants played vital roles in preventing oxidative stress and protect the DNA of PBMC. Gudkov *et al.* (2006) reported that guanosine and inosine displayed antioxidant activity, protected DNA *in vitro* from oxidative damage induced by free radicals. Flavonoids myricetin, quercetin, and rutin which exhibited antioxidant and free radical scavenging activities were also revealed to protect DNA against hydrogen peroxide-induced DNA damage in Caco-2 and Hep G2 cells (Aherne & O'Brien, 1999). Although unfermented wheat grains exhibited higher antioxidant activity than fruiting bodies and fermented wheat grains, genoprotection study showed that it exhibited lower DNA protection activity than fruiting bodies and equivalent to fermented wheat grains. The rationale was bioactive compounds other than antioxidants may have contributed to the genoprotective effects of fruiting bodies and fermented wheat grains. Therefore, investigation into the bioactive compounds involved in the genoprotective effects of fruiting bodies and fermented wheat grains need to be carried out.

There is growing interest to study edible mushrooms with genoprotective effects to prevent DNA damage. Several bioactive compounds with genoprotective effects have been isolated from mushrooms. Angeli *et al.* (2009) suggested the DNA protection effects of total polysaccharides and b-glucans extracted at different stages of fruiting body maturity (immature, mature stage with immature spores and mature stage with mature spores) of the mushroom *A. blazei.* All extracts possessed genoprotective effects against damage induced by H_2O_2, bleomycin and doxorubicin. Total polysaccharides demonstrated limited protective effects and not effective against doxorubicin. Further, Shi *et al.* (2002) isolated a heat labile protein, tyrosinase from the edible mushroom, *A. bisporus* which protected Raji cells (a human lymphoma cell line) against H_2O_2-induced DNA damage.

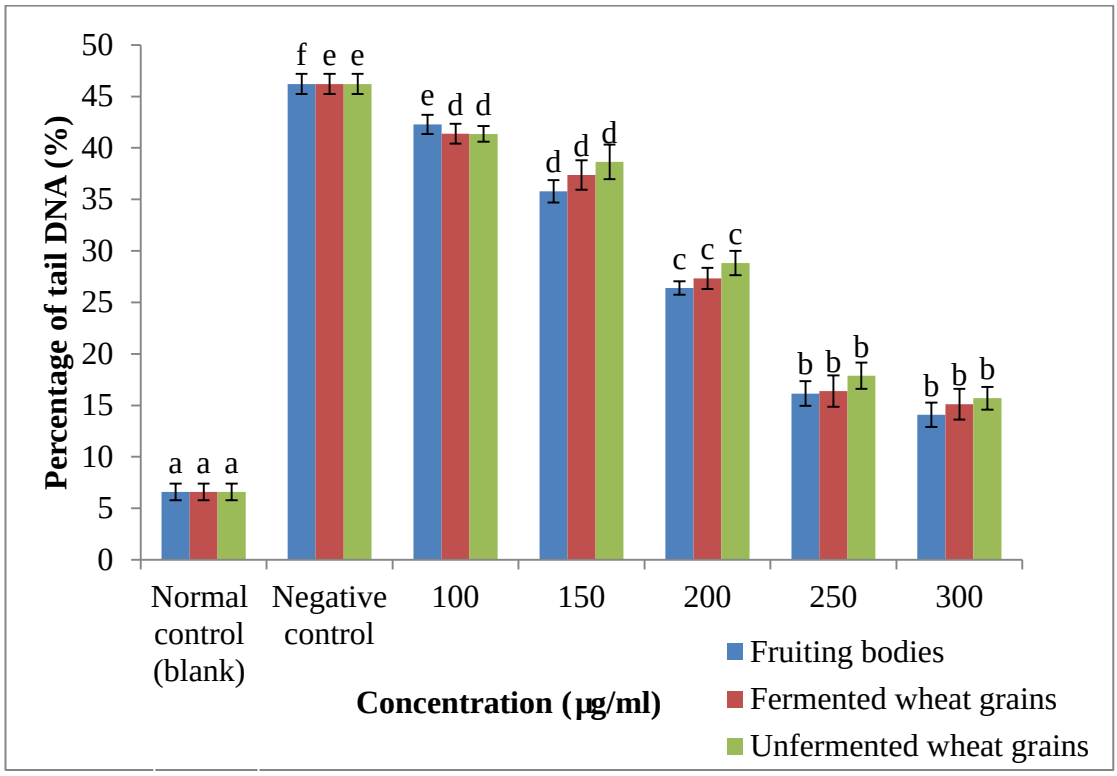
4.4.3 Effects of ethanol extracts to repair DNA of PBMC after H_2O_2-induced damage

The capacities of the extracts of fruiting bodies, fermented and unfermented wheat grains to repair the DNA of PBMC after H_2O_2-induced damage were evaluated. Normal control cells (blank) which were not damaged by H_2O_2 showed tail DNA of 6.6%. The cells in negative control group showed 46.21% of tail DNA which signified the cells experienced severe DNA damage.

The percentages of tail DNA (%) of PBMC treated with various extracts are shown in Figure 4.4 while Plate 4.3 illustrates the comet images showing various degrees of DNA damage. As shown in Figure 4.4, it was evident that all extracts were comparable in their abilities to repair the DNA of PBMC. When the concentration of the extracts increased, percentage of tail DNA decreased. Although fruiting bodies were less effective than fermented wheat grains in its antioxidant activity, it showed comparable DNA repair activities than fermented and unfermented wheat grains. Bioactive compounds other than antioxidants could have involved in the DNA repair mechanism which need further study.

Chromosomal damage is demonstrated as breaks and fragments which look like micronuclei in rapid growing cells (Hofer *et al.*, 2000). According to Craig and Alt. (2004), eukaryotic cells are capable of identifying and repairing DNA damage within each phase of cell cycle. In order to minimize any detrimental consequences, DNA repair mechanisms must respond to DNA damage rapidly. Edible mushrooms potential to enhance the DNA repair as another study performed by Pillai *et al.* (2010) suggested that polysaccharides isolated from *G. lucidum* enhanced the DNA repairing process assayed by comet assay in human peripheral blood leukocytes.

As fruiting bodies exhibited better genoprotective effects when compared to fermented and unfermented wheat grains, thus fruiting bodies were selected for further investigation of hepatoprotective effects.



Different alphabets are significantly different ($p < 0.05$).

Figure 4.4: Percentages of tail DNA of PBMC (%) treated with various concentrations of extracts in repair of DNA damage of PBMC induced by H_2O_2.

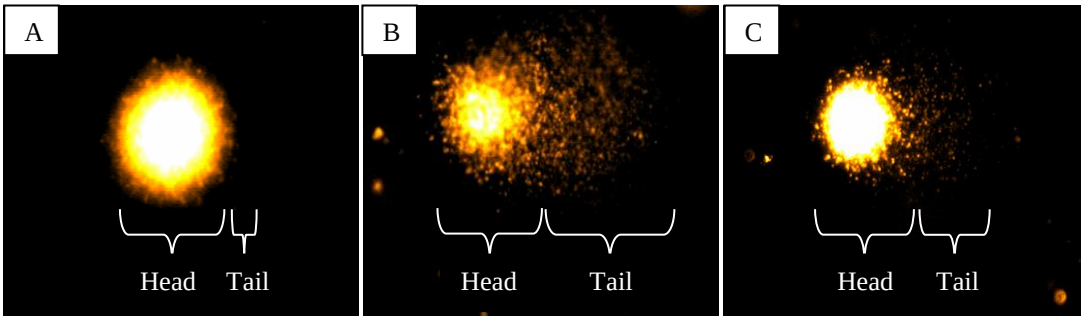


Plate 4.3: Representative comet images showing various degrees of damages in the study of the effects of extracts to repair DNA after H_2O_2-induced DNA damage. (A) cell in blank group (5-10% tail DNA); (B) cell in control group (50-60% tail DNA); (C) cell treated with 150mg/ml of *P. giganteus* ethanol extract (20-30% tail DNA).

4.5 Animal Studies

4.5.1 Acute toxicity assay

Throughout the study period, no morbidity and mortality was observed. Besides that, there were no significant differences in all the biochemical parameters monitored (haematology analysis, liver function test and renal function test) between the experimental rats in *P. giganteus* administered groups and control group as depicted in Table 4.5-4.10. Histopathological examination further confirmed the non-toxicity of *P. giganteus*. There were no significant differences in cellular structures of livers and kidneys of the rats in *P. giganteus* administered groups and control group. Plate 4.4 illustrates the histological sections of livers (1A, 1B and 1C) and kidneys (2A, 2B and 2C) in the acute toxicity study. Liver sections of rats administered with *P. giganteus* had regular hepatic architecture. Distinct hepatic cells and well-preserved cytoplasm were observed. The kidney tissues retained the tubular structure and the cellular outlines were similar to rats in the control group. Thus, it maybe concluded that *P. giganteus* did not have any adverse effects on experimental rats up to a high dose of 5g/kg (equivalent to 28.57g of fresh mushrooms) tested.

Table 4.5: Effects of *P.giganteus* on haematological parameters of female rats in acute toxicity assay.

Group	HGB (g/L)	HCT	RBC (10e12/L)	MCV (fl)	MCH (pg)	MCHC (g/L)	RDW (%)	WBC (10e9/L)	Platelet (10e9/L)
Control (dH_2O)	154.00 ± 1.55	0.46 ± 0.00	8.23 ± 0.13	56.33 ± 0.76	18.55 ± 0.40	340.00 ± 1.41	15.67 ± 0.32	5.62 ± 0.76	993.50 ± 41.90
Low dose *P. giganteus* (0.5g/kg)	149.00 ± 1.18	0.46 ± 0.00	8.40 ± 0.11	55.33 ± 0.61	17.67 ± 0.32	329.17 ± 5.00	16.92 ± 0.57	6.05 ± 0.28	960.67 ± 26.83
High dose *P. giganteus* (1g/kg)	150.67 ± 2.50	0.46 ± 0.00	8.58 ± 0.19	53.83 ± 0.87	17.48 ± 0.25	330.50 ± 5.81	17.13 ± 0.50	6.80 ± 0.28	1058.33 ± 28.46

All values are expressed as mean ± S.E.M, n=6. Mean values in the same column with different letters are significantly different ($p < 0.05$).

Table 4.6: Effects of *P.giganteus* on haematological parameters of male rats in acute toxicity assay.

Group	HGB (g/L)	HCT	RBC (10e12/L)	MCV (fl)	MCH (pg)	MCHC (g/L)	RDW (%)	WBC (10e9/L)	Platelet (10e9/L)
Control (dH_2O)	158.00 ± 0.26	0.47 ± 0.00	8.10 ± 0.15	56.17 ± 0.48	18.97 ± 0.06	334.50 ± 3.46	16.70 ± 0.39	9.97 ± 0.92	840.83 ± 50.03
Low dose *P. giganteus* (0.5g/kg)	151.17 ± 1.42	0.45 ± 0.00	7.84 ± 0.06	56.83 ± 0.48	18.70 ± 0.20	332.50 ± 2.51	16.78 ± 0.33	9.90 ± 1.11	925.50 ± 73.11
High dose *P. giganteus* (1g/kg)	151.50 ± 3.06	0.45 ± 0.00	7.93 ± 0.11	56.33 ± 0.80	18.88 ± 0.27	335.50 ± 3.13	17.55 ± 0.27	9.87 ± 0.92	905.50 ± 37.00

All values are expressed as mean ± S.E.M, n=6. Mean values in the same column with different letters are significantly different ($p < 0.05$).

Table 4.7: Effects of *P.giganteus* on liver function parameters of female rats in acute toxicity assay.

Group	ALP (IU/L)	ALT (IU/L)	AST (IU/L)	GGT (IU/L)	Bilirubin (µmol/L)	Total protein (g/L)	Albumin (g/L)
Control (dH_2O)	131.17 ± 15.64	43.17 ± 2.81	219.33 ± 19.45	2.33 ± 0.21	1.33 ± 0.21	70.00 ± 1.59	12.00 ± 0.58
Low dose *P. giganteus* (0.5g/kg)	120.33 ± 11.26	54.50 ± 4.57	263.00 ± 11.84	2.33 ± 0.21	2.17 ± 0.31	70.33 ± 0.88	13.17 ± 0.31
High dose *P. giganteus* (1g/kg)	115.83 ± 12.61	49.83 ± 7.88	226.67 ± 11.82	3.17 ± 0.31	1.33 ± 0.21	71.17 ± 1.19	11.50 ± 0.56

All values are expressed as mean ± S.E.M, n=6. Mean values in the same column with different letters are significantly different ($p < 0.05$).

Table 4.8: Effects of *P.giganteus* on liver function parameters of male rats in acute toxicity assay.

Group	ALP (IU/L)	ALT (IU/L)	AST (IU/L)	GGT (IU/L)	Bilirubin (µmol/L)	Total protein (g/L)	Albumin (g/L)
Control (dH_2O)	169.50 ±24.81	60.00 ±2.45	209.83 ±18.22	2.67 ±0.21	2.00 ±0.26	66.50 ±1.34	11.67 ±0.33
Low dose *P. giganteus* (0.5g/kg)	208.17 ±16.86	56.83 ±2.56	222.00 ±13.35	3.00 ±0.26	2.33 ±0.21	65.83 ±0.87	12.17 ±0.31
High dose *P. giganteus* (1g/kg)	190.50 ±10.93	61.50 ±5.95	233.60 ±18.84	3.17 ±0.17	2.33 ±0.21	65.67 ±1.05	11.83 ±0.70

All values are expressed as mean ±S.E.M, n=6. Mean values in the same column with different letters are significantly different ($p < 0.05$).

Table 4.9: Effects of *P.giganteus* on renal function parameters of female rats in acute toxicity assay.

Group	Sodium	Potassium	Chloride	Carbon dioxide	Anion Gap	Urea	Creatinine
Control (dH_2O)	141.33 ±1.87	4.30 ±0.07	104.50 ±1.28	19.58 ±0.31	21.17 ±0.40	5.92 ±0.22	27.50 ±0.56
Low dose *P. giganteus* (0.5g/kg)	138.50 ±0.56	4.88 ±0.11	101.83 ±0.31	18.97 ±0.34	22.83 ±0.40	7.53 ±0.84	26.00 ±3.86
High dose *P. giganteus* (1g/kg)	138.33 ±0.76	4.63 ±0.33	103.00 ±0.58	18.53 ±0.31	22.83 ±0.95	7.45 ±0.90	33.00 ±0.45

All values are expressed as mean ±S.E.M, n=6. Mean values in the same column with different letters are significantly different ($p < 0.05$).

Table 4.10: Effects of *P.giganteus* on renal function parameters of male rats in acute toxicity assay.

Group	Sodium	Potassium	Chloride	Carbon dioxide	Anion Gap	Urea	Creatinine
Control (dH_2O)	139.50 ±0.22	4.88 ±0.10	101.33 ±0.33	20.83 ±0.44	21.67 ±0.33	5.90 ±0.32	29.67 ±7.21
Low dose *P. giganteus* (0.5g/kg)	140.33 ±0.21	5.02 ±0.23	100.67 ±0.42	22.42 ±0.43	21.83 ±0.91	6.45 ±0.26	26.67 ±5.35
High dose *P. giganteus* (g/kg)	139.67 ±0.42	4.98 ±0.10	102.17 ±0.48	21.50 ±0.89	21.00 ±0.86	6.27 ±0.40	24.67 ±2.84

All values are expressed as mean ±S.E.M, n=6. Mean values in the same column with different letters are significantly different ($p < 0.05$).

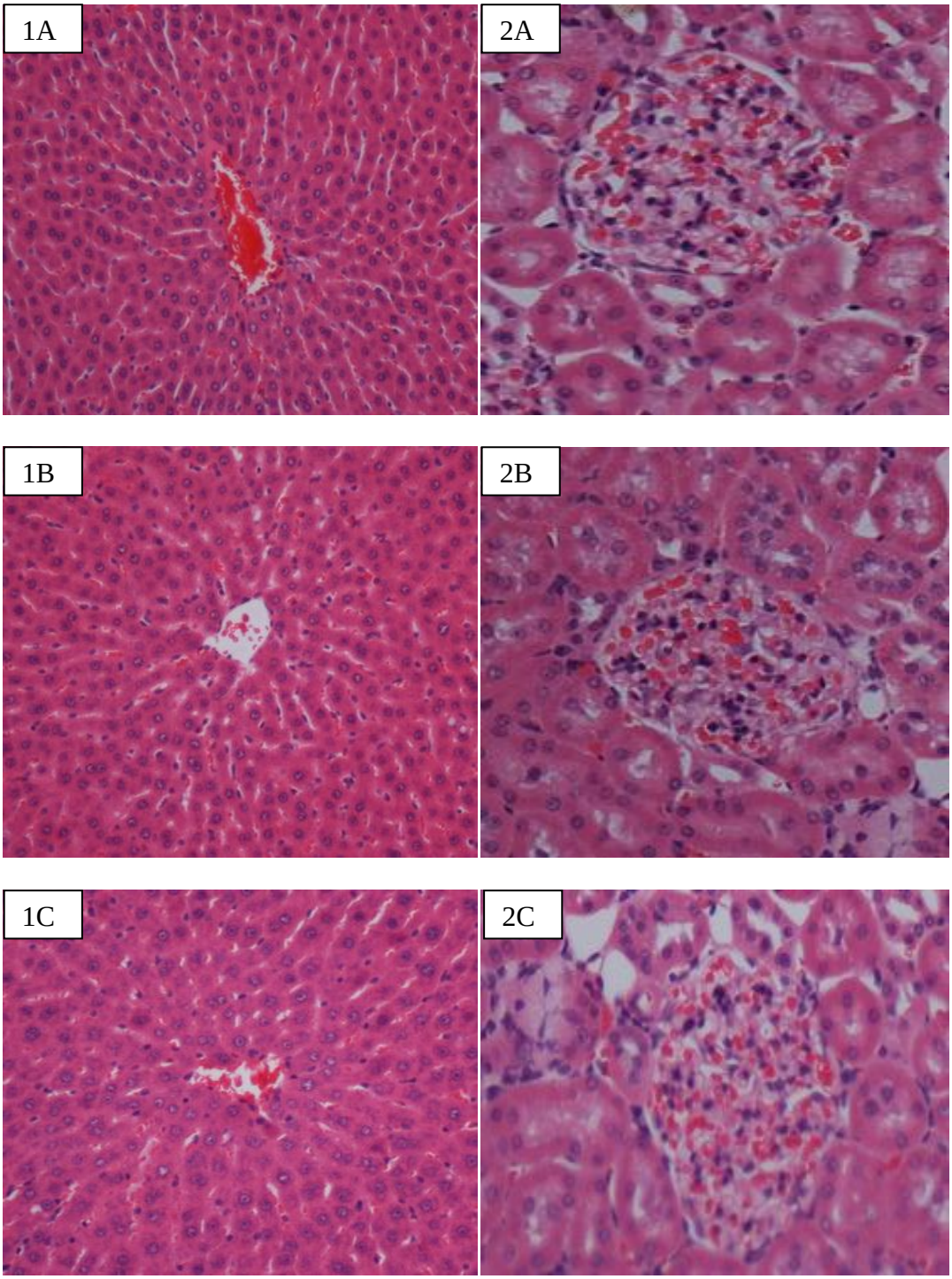


Plate 4.4: The photomicrography of liver and kidney sections of rats administered with *P. giganteus* at doses of 2g/kg, 5g/kg and dH_2O. (1A and 2A) liver and kidney sections of control rat; (1B and 2B) liver and kidney sections of rat administered with low dose of *P. giganteus* (2g/kg); (1C and 2C) liver and kidney sections of rat administered with high dose of *P. giganteus* (5g/kg). (H& E stain, original magnification: 20X).

4.5.2 Effects of *P. giganteus* in the prevention of TAA-induced liver injury

4.5.2.1 Effects of different treatments on body and liver weights of experimental rats

The body and liver weights of the rats after two months of different treatments are shown in Table 4.11. Overall, there were no significant differences in body weight and liver weight between the rats in the different experimental groups. However, the rats treated with TAA exhibited significantly ($p < 0.05$) higher liver body weight ratios when compared to rats in control groups. The highest liver body weight ratio observed in TAA control rats was 73.71% higher than the ratio in the control rats (dH_2O). Administration of *P. giganteus* (0.5g/kg and 1g/kg) lowered the liver body weight ratio and this was comparable to the effects observed in silymarin administered rats (Table 4.11).

Measurement of liver body weight ratio is a more accurate approach to determine the changes in liver size compared to measurement of liver weight alone as the liver weight largely depends on the size of the rat. The enlargement of livers in TAA treated rats signified hepatic lesions and liver injury associated with the toxicological effects of TAA. The enlargement of livers in TAA treated rats signified hepatic lesions and liver injury associated with the toxicological effects of TAA. However, the liver enlargement was significantly ($p < 0.05$) reduced in rats administered with *P. giganteus* and this was comparable to the effects of silymarin.

Table 4.11: Effects of different treatments on body and liver weights of experimental rats in the hepatotoxicity prevention study.

Group	Body Weight, BW (g)	Liver Weight, LW (g)	LW/BW (%)
Control (dH_2O)	384.50 ± 36.57^{b}	9.69 ± 0.99^{ab}	2.51 ± 0.04^{a}
Control (*P. giganteus*)	370.33 ± 41.87^{ab}	9.92 ± 1.10^{ab}	2.70 ± 0.08^{a}
TAA control (200mg/kg)	296.17 ± 18.47^{ab}	12.85 ± 0.87^{b}	4.36 ± 0.20^{c}
Silymarin (50mg/kg) + TAA	265.00 ± 15.53^{a}	8.55 ± 0.75^{a}	3.21 ± 0.13^{b}
Panus giganteus (0.5g/kg) + TAA	283.33 ± 17.06^{ab}	10.28 ± 0.54^{ab}	3.64 ± 0.09^{b}
Panus giganteus (1g/kg) + TAA	306.33 ± 18.79^{ab}	10.55 ± 0.63^{ab}	3.45 ± 0.09^{b}

Two-month treatment; food and water *ad libitum*. All values are expressed as mean ± S.E.M, n= 6. Mean values in the same column with different letters are significantly different ($p < 0.05$).

4.5.2.2 Effects of different treatments on biochemical parameters related to hepatoprotection

The changes in serum liver biomarkers are shown in Table 4.12. Rats in both control groups had similar biochemical indices. Particularly, TAA control rats exhibited the highest levels of ALP, ALT, AST, GGT and bilirubin but lowest total protein and albumin content when compared to rats in other experimental groups. The serum ALP, ALT, AST, GGT and bilirubin were 210.91%, 40.49%, 21.14%, 153.40% and 198.75% higher when compared to serum levels in rats in the control group (dH_2O). Total protein and albumin content dropped by 7.93% and 17.26% respectively when compared to rats in the control group (dH_2O) (Table 4.12). When a low dose of *P. giganteus* (0.5g/kg) was administered, the levels of ALP, ALT, GGT and bilirubin were significantly ($p < 0.05$) reduced while total protein and albumin content were significantly ($p < 0.05$) elevated. There were no significant differences in all the serum liver biomarkers of rats in the low dose (0.5g/kg) or high dose (1g/kg) treatment groups (Table 3). The rats administered with *P. giganteus* had significantly ($p < 0.05$) lower levels of ALP but comparable levels of ALT, AST, GGT, bilirubin, total protein and albumin when compared to the rats administered with silymarin.

Table 4.12: Effects of different treatments on serum liver biomarkers of experimental rats in the hepatotoxicity prevention study.

Group	ALP (IU/L)	ALT (IU/L)	AST (IU/L)	GGT (IU/L)	Bilirubin (µmol/L)	Total protein (g/L)	Albumin (g/L)
Control (dH_2O)	70.17 ± 5.62^{a}	46.50 ± 3.04^{a}	164.00 ± 14.68^{a}	5.00 ± 0.26^{a}	2.40 ± 0.20^{a}	69.33 ± 0.92^{cd}	13.50 ± 0.34^{b}
Control (*P. giganteus*)	79.17 ± 6.87^{a}	46.00 ± 1.59^{a}	151.00 ± 3.42^{a}	5.20 ± 0.40^{a}	2.60 ± 0.20^{a}	70.83 ± 0.79^{d}	13.83 ± 0.17^{b}
TAA control (200mg/kg)	218.17 ± 5.47^{c}	65.33 ± 0.67^{c}	198.67 ± 0.21^{b}	12.67 ± 1.87^{c}	7.17 ± 0.83^{c}	63.83 ± 0.48^{a}	11.17 ± 0.40^{a}
Silymarin (50mg/kg) + TAA	207.00 ± 11.93^{c}	56.33 ± 2.19^{b}	182.00 ± 5.82^{ab}	10.00 ± 0.52^{bc}	5.20 ± 0.31^{b}	65.50 ± 0.56^{ab}	12.83 ± 0.48^{ab}
Panus giganteus (0. 5g/kg) + TAA	165.67 ± 5.67^{b}	55.17 ± 2.34^{b}	174.00 ± 8.80^{ab}	7.17 ± 0.91^{ab}	5.00 ± 0.26^{b}	67.00 ± 0.26^{bc}	14.00 ± 0.68^{b}
Panus giganteus (1g/kg) + TAA	166.67 ± 9.19^{b}	53.33 ± 0.80^{ab}	164.00 ± 4.37^{a}	6.83 ± 0.60^{ab}	4.80 ± 0.16^{b}	67.17 ± 0.60^{bc}	13.83 ± 0.40^{b}

Two-month treatment; food and water *ad libitum*. All values are expressed as mean ± S.E.M, n= 6. Mean values in the same column with different letters are significantly different ($p < 0.05$).
ALP: Alkaline phosphatase; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; GGT: G-Glutamyl transferase.

Table 4.13: Effects of different treatments on serum MDA and urinary 8-OH-dG content of experimental rat in the hepatotoxicity prevention study.

Group	Serum MDA (nM of TEP equivalents)	Urinary 8-OH-dG (pg/ml)
Control (dH_2O)	292.61 ± 6.69^{a}	2452.87 ± 120.89^{a}
Control (*P. giganteus*)	292.97 ± 13.94^{a}	2473.89 ± 37.14^{a}
TAA control (200mg/kg)	374.14 ± 6.47^{b}	2792.46 ± 6.45^{b}
Silymarin (50mg/kg) + TAA	286.17 ± 8.81^{a}	2541.84 ± 65.44^{ab}
Panus giganteus (0.5g/kg) + TAA	288.61 ± 10.65^{a}	2658.60 ± 39.74^{ab}
Panus giganteus (1g/kg) + TAA	291.06 ± 11.20^{a}	2483.65 ± 31.56^{a}

Two-month treatment; food and water *ad libitum*. All values are expressed as mean ± S.E.M, n= 6. Mean values in the same column with different letters are significantly different ($p < 0.05$).
MDA: malondialdehyde; 8-OH-dG: 8-hydroxydeoxyguanosine

The levels of oxidative stress parameters (serum MDA and urinary 8-OH-dG) of the experimental rats are given in Table 4.13. In general, rats in both control groups displayed similar levels of serum MDA and urinary 8-OH-dG. Notably, TAA control rats had significantly ($p < 0.05$) higher level of MDA when compared to rats in other experimental groups and significantly ($p < 0.05$) higher urinary 8-OH-dG content when compared to rats in control groups and rats administered with high dose *P. giganteus* (Table 4.13).

Serum liver biomarkers (ALP, ALT, AST, GGT, bilirubin, total protein and albumin) are important criteria for the evaluation of liver toxicity. The amounts of enzymes that flow into the blood indicate the severity of hepatic injury (Nkosi *et al.*, 2005). In the present study, the levels of serum liver biomarkers in rats administered with *P. giganteus* (0.5g/kg and 1g/kg) were comparable to the control rats. *Panus giganteus* may have effects to treat liver injury induced by TAA. Besides, the decreased level of albumin or hypoalbuminemia and total protein in TAA control rats could be due to malnutrition related to liver cirrhosis. The malnutrition problem was less severe in rats administered with *P. giganteus* as they recorded higher albumin and total protein content than TAA control rats.

Production of ROS, mitochondrial dysfunction and antioxidant insufficiency have been reported to advance the development of liver cirrhosis (Natarajan *et al.*, 2006). In order to evaluate the effects of TAA on oxidative stress in rats, the oxidative stress parameters such as serum MDA and urinary 8-OH-dG content which reflect the oxidative damage to lipids and DNA respectively were examined. Earlier studies suggested that hepatotoxins including TAA, induced liver damage by forming free radicals which then react with cellular lipids to promote lipid peroxidation (Fadhel and

Amran, 2002). In the present study, the higher MDA level in TAA control rats supports this assumption.

Treatments of *P. giganteus* reduced the serum MDA and urinary 8-OH-dG content. In addition, there were no significant differences in oxidative stress biomarkers between the rats administered with silymarin and *P. giganteus*. *Panus giganteus* may possess bioactive compounds that could prevent the oxidative stress induced by TAA and thus alleviate the liver injury.

4.5.2.3 Gross necropsy and histopathological examination

Gross necropsy and histopathological examination of liver tissues were positively correlated with the serum biochemical indices. Gross images of the livers are illustrated in Plate 4.5 (Al-F1), while Plate 4.5 (A2-F2) demonstrates the histological sections of the livers. The livers of rats in both control groups had smooth surfaces without any irregularities (Figure 4.5-A1 and B1). Histological observations of the liver sections showed typical cellular architecture with distinct hepatic cells, sinusoidal spaces and a central vein. The hepatic cells displayed prominent nuclei and uniform cytoplasm (Plate 4.5 -A2 and B2).

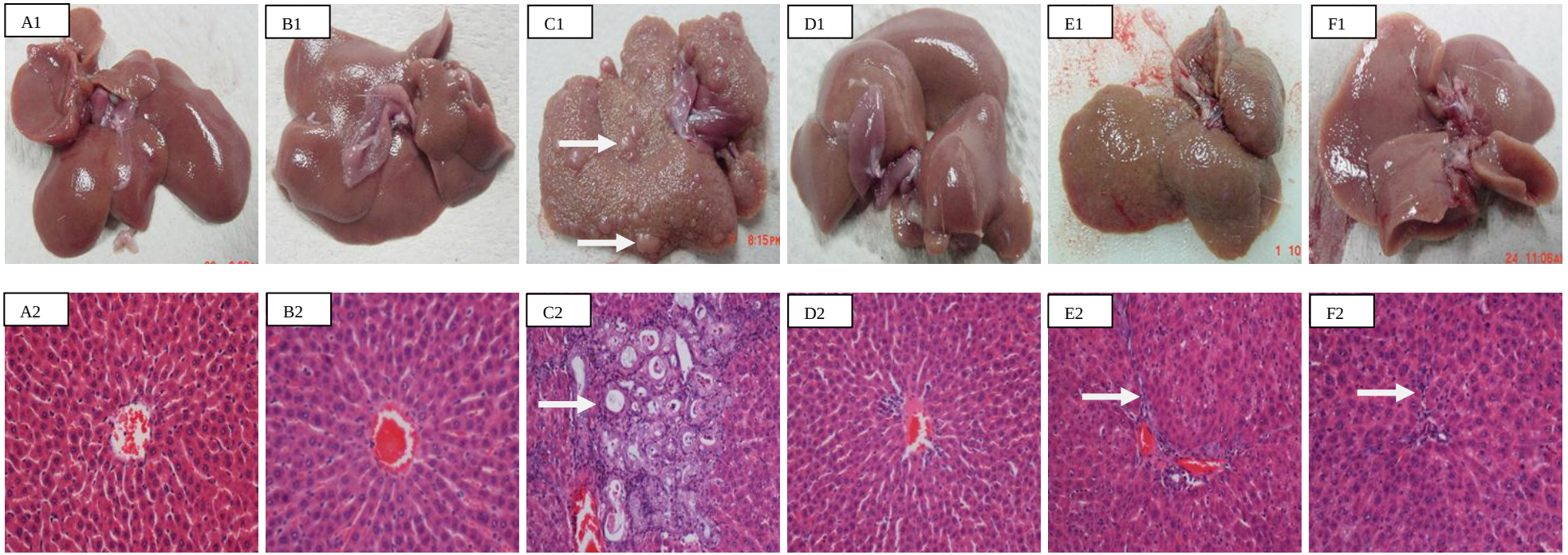


Plate 4.5: The gross liver morphology (A1-F1) and photomicrography of liver sections (A2-F2) of rats in the prevention of thioacetamide (TAA)-induced hepatotoxicity in rats. The rats in TAA, silymarin and *P. giganteus* treatment groups were injected with TAA via the intraperitoneal-route thrice a week. (A1 and A2) control rat (dH_2O); (B1 and B2) control rat (*P. giganteus*); (C1 and C2) TAA control rat; gross image shows many micro and macro-nodules in the liver (arrow), while light micrograph shows thick fibrotic septae with proliferation of bile duct (arrow); (D1 and D2) rat administered with silymarin. (E1 and E2) rat administered with low dose of *P. giganteus* (0.5g/kg): micro nodules were noted in the gross image (arrow), light micrograph shows narrow fibrotic septae (arrow); (F1 and F2) rat administered with high dose of *P. giganteus* (1g/kg): very minor fibrotic septae was observed in the light micrograph (arrow) (Plate A2-F2: H& E stain, original magnification: 20X).

On the other hand, the livers of the rats in TAA control group were enlarged with obvious inferior margins and contained many micro and macro-nodules (Plate 4.5-C1). The liver sections of the TAA control rats revealed extensive damage, characterized by severe necrosis, fatty degeneration, sinusoidal dilatation and congestion, centrilobular necrosis, proliferation of bile duct, presence of collagen bundles surrounding the lobules, which then leads to thick fibrotic septae that disrupted the cellular architecture (Plate 4.5-C2). However, liver recovery was present in rats administered with silymarin as the liver condition and hepatic architecture were similar to rats in control groups (Plate 4.5-D1 and D2).

Further, the liver enlargement and nodules were reduced in rats administered with low dose *P. giganteus* (0.5g/kg) (Plate 4.5-E1). The histology of the liver sections in rats administered with low dose *P. giganteus* showed significant improvement with less damage of liver tissue indicated by reduced level of necrosis, narrow fibrotic septae, remarkable increase in bile ductules, Kupffer cells and fat storing cells (Plate 4.5-E2). Excellent liver recovery was indicated in rats administered with high dose *P. giganteus* (1g/kg) with liver morphology comparable to the control rats (Plate 4.5-F1). There was minimal disruption of the hepatic cellular structure, very minor fibrotic septae and low degree of lymphocyte infiltration (Plate 4.5-F2).

Based on the body and liver weight analysis, biochemical indices, gross necropsy and histopathological examination, it may be concluded that *P. giganteus* had significant hepatoprotective effects against TAA-induced liver injury in rats. As there were no remarkable differences between high dose (1g/kg) (equivalent to 5.72 g of fresh mushrooms) and low dose (0.5g/kg) (equivalent to 2.86g of fresh mushrooms) treatments in their serum biochemical indices and histopathological evidences, it is

evident that low dose *P. giganteus* had hepatoprotective effects against TAA-induced liver deterioration. The hepatoprotective effects of *P. giganteus* were associated with its ability to prevent oxidative stress as several studies showed that TAA bind covalently to the cellular components and subsequently induced oxidative stress (Zaragoza *et al.*, 2000; Pallottini *et al.*, 2006). The antioxidant study (4.3) had proved that *P. giganteus* possessed antioxidant properties.

In the present study, the mushroom was administered at doses of 0.5g/kg (low dose) and 1g/kg (high dose) as previous related studies indicated that these doses would be effective in preventing liver injury. Dai *et al.* (2003) showed that oral administration of *A. camphorata* fruiting bodies in doses of 0.5g/kg and 1g/kg provided protection against ethanol-induced acute liver damage in *Sprague-Dawley* rats. Lu *et al.* (2007) also suggested the remarkable preventive effects of 0.5g/kg and 1g/kg *A. camphorata* mycelia in liquid culture against ethanol-induced hepatotoxicity in *Sprague–Dawley* rats.

In addition, other mushroom species including *G. lucidum* (Shi *et al.*, 2008) and *P. ostreatus* (Jayakumar *et al.*, 2006) had also been shown to exhibit hepatoprotective effects using rats as experimental model. Further investigation is required to identify for the bioactive compounds responsible for the hepatoprotective properties and to formulate functional food for the reduction of liver injury severity.

4.5.3 Effects of *P. giganteus* in the treatment of TAA-induced liver injury

4.5.3.1 Effects of different treatments on body and liver weights of experimental rats

The body and liver weights of the experimental rats after three-month treatment are depicted in Table 4.14. Generally, there were no significant differences in body and liver weight between different experimental groups. However, the rats treated with TAA exhibited significantly higher ($p < 0.05$) liver body weight ratios when compared to control rats. Thioacetamide (TAA) control rats displayed highest liver body weight ratio with 80% higher than rats in control groups. This may signify that TAA control rats experienced higher degree of liver injury than rats in other TAA treated groups. Treatment with *P. giganteus* had reduced the liver enlargement to a significant lower extent comparable to rats administered with silymarin (Table 4.14).

Table 4.14: Effects of different treatments on body and liver weights of experimental rats in the hepatotoxicity treatment study.

Group	Body Weight, BW (g)	Liver Weight, LW (g)	LW/BW
Control (dH_2O)	396.83 ± 44.23	10.01 ± 1.17	2.52 ± 0.04^{a}
Control (*P. giganteus*)	369.67 ± 42.76	9.20 ± 1.11	2.48 ± 0.04^{a}
TAA control (200mg/kg)	331.67 ± 29.89	11.60 ± 1.48	4.47 ± 0.15^{c}
Silymarin (50mg/kg) + TAA	293.83 ± 22.85	13.28 ± 1.44	3.44 ± 0.16^{b}
Panus giganteus (0.5g/kg) + TAA	322.17 ± 25.78	11.18 ± 0.86	3.48 ± 0.08^{b}
Panus giganteus (1g/kg) + TAA	315.17 ± 28.71	11.51 ± 0.71	3.70 ± 0.14^{b}

Three month treatment; food and water *ad libitum*. All values are expressed as mean ± S.E.M, n=6. Mean values in the same column with different letters are significantly different ($p < 0.05$).

4.5.3.2 Effects of different treatments on biochemical parameters related to treatment of TAA-induced liver injury

The changes in serum liver biomarkers are shown in Table 4.15. Thioacetamide (TAA) control rats showed remarkable highest levels of ALP, ALT, AST, GGT and bilirubin but lowest amounts of total protein and albumin. However, administration of low dose *P. giganteus* (0.5 g/kg) significantly ($p < 0.05$) reduced the levels of AST and GGT. Rats administered with high dose *P. giganteus* (1.0g/kg) recorded 32.68%, 10.05%, 13.85%, 57.47% and 40.99% decrease in ALP, ALT, AST, GGT and bilirubin level, but 15.60% and 44.18% increase in total protein and albumin when compared to TAA control rats.

Furthermore, as shown in Table 4.16, TAA control rats exhibited significantly ($p < 0.05$) higher MDA level and free 8-OH-dG content when compared to rats in other experimental groups. However, administration of low dose *P. giganteus* (0.5g/kg) lowered the MDA content by 28.25% and free 8-OH-dG content by 3.65% when compared to TAA control rats. Rats administered with high dose (1.0g/kg) *P. giganteus* significantly ($p < 0.05$) reduced the MDA and 8-OH-dG content comparable to control rats (Table 4.16).

Table 4.15: Effects of different treatments on serum liver biomarkers of experimental rats in the hepatotoxicity treatment study.

Group	ALP (IU/L)	ALT (IU/L)	AST (IU/L)	GGT (IU/L)	Bilirubin (µmol/L)	Total protein (g/L)	Albumin (g/L)
Control (dH_2O)	69.67 ± 4.02^{a}	48.67 ± 1.28^{abc}	153.17 ± 6.04^{a}	3.00 ± 0.26^{a}	1.50 ± 0.22^{a}	67.17 ± 3.00^{b}	12.67 ± 1.20^{b}
Control (*P. giganteus*)	68.67 ± 8.09^{a}	45.00 ± 0.77^{a}	148.17 ± 8.80^{a}	3.17 ± 0.17^{ab}	1.67 ± 0.21^{a}	66.67 ± 3.23^{b}	12.33 ± 1.05^{b}
TAA control (200mg/kg)	69.83 ± 8.89^{a}	45.67 ± 1.12^{ab}	158.33 ± 7.33^{a}	3.00 ± 0.45^{a}	2.00 ± 0.26^{ab}	60.67 ± 0.95^{ab}	12.17 ± 0.48^{b}
Silymarin (50mg/kg) + TAA	110.17 ± 3.24^{b}	56.33 ± 2.51^{d}	184.17 ± 2.01^{b}	7.83 ± 0.40^{c}	2.83 ± 0.17^{b}	54.50 ± 0.34^{a}	8.67 ± 0.21^{a}
Panus giganteus (0.5g/kg) + TAA	88.33 ± 7.82^{ab}	53.83 ± 0.48^{cd}	160.67 ± 1.31^{a}	5.00 ± 0.63^{b}	1.83 ± 0.17^{ab}	60.17 ± 0.31^{ab}	10.17 ± 0.17^{ab}
Panus giganteus (1g/kg) + TAA	74.17 ± 5.29^{a}	50.67 ± 0.56^{bc}	158.67 ± 2.36^{a}	3.33 ± 0.49^{ab}	1.67 ± 0.33^{a}	63.00 ± 0.89^{b}	12.50 ± 0.62^{b}

Three month treatment; food and water *ad libitum.* All values are expressed as mean ± S.E.M, n=6. Mean values in the same column with different letters are significantly different ($p < 0.05$).
ALP: Alkaline phosphatase; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; GGT: G-Glutamyl transferase.

Table 4.16: Effects of different treatments on serum MDA and urinary 8-OH-dG content of experimental rats in the hepatotoxicity treatment study.

Group	Serum MDA (nM of TEP equivalents)	8-OH-dG (pg/ml)
Control (dH_2O)	286.17 ± 14.00^{a}	2402.99 ± 33.62^{a}
Control (*P. giganteus*)	264.99 ± 2.42^{a}	2460.97 ± 15.54^{ab}
TAA control (200mg/kg)	511.82 ± 17.74^{c}	2752.29 ± 17.71^{e}
Silymarin (50mg/kg) + TAA	311.83 ± 6.11^{a}	2605.86 ± 22.72^{cd}
Panus giganteus (0.5g/kg) + TAA	367.22 ± 4.06^{b}	2651.41 ± 22.88^{d}
Panus giganteus (1g/kg) + TAA	304.90 ± 12.49^{a}	2533.79 ± 19.45^{bc}

Three month treatment; food and water *ad libitum.* All values are expressed as mean ± S.E.M, n=6. Mean values in the same column with different letters are significantly different ($p < 0.05$)..
MDA: malondialdehyde; 8-OH-dG: 8-hydroxydeoxyguanosine

Normally, the serum liver biomarker enzymes are cytoplasmic in nature, but eventually they will enter circulatory system due to changes in membrane permeability (Hu & Chen, 1992). Hepatic damages caused the alteration in transport function and membrane permeability of hepatocytes which in turn leads to leakage of marker enzymes from the cells (Zimmerman & Seef, 1970). Thioacetamide (TAA) control rats may have experienced serious hepatic damage as they recorded significant changes in serum liver biomarkers when compared to control rats.

The stabilization of the serum liver biomarker level in *P. giganteus* treatment groups signified the improvement of the liver functional status. There were no significant differences between high dose (1.0g/kg) and low dose (0.5g/kg) treatment groups in their serum liver biomarkers. The rats administered with high dose *P. giganteus* (1.0g/kg) were also shown to have similar biochemical index with rats administered with silymarin as depicted in Table 4.15. Our results indicated that there were no significant differences between the rats in both control groups in their serum liver biomarkers. Administrations of *P. giganteus* do not damage liver.

Increased levels of lipid peroxidation products have been linked with wide range of chronic diseases in human (Browne *et al.*, 1999). According to the results, experimental rats treated with TAA recorded higher level of MDA and free 8-OH-dG when compared to control rats. The oxidative stress could be contributed by TAA. However, *P. giganteus* reverted the oxidative stress induced by TAA and repaired the liver injury.

4.5.3.3 Gross necropsy and histopathological examination

Gross images of the livers are depicted in Plate 4.6 (Al-F1) while histological sections of the livers are shown in Plate 4.6 (A2-F2). As shown in Plate 4.6 (A1 and B1), there were no abnormalities or lesions in the livers of all control rats. The histology of the liver sections showed regular hepatic architecture and central vein. Prominent nuclei and well preserved cytoplasm were observed in the hepatic cells (Plate 4.6 –A2 and B2).

However, the livers of the TAA control rats exhibited extensive damage evidenced by its apparent enlargement, inferior margins and contained many micro and macro nodules (Plate 4.6–C1). The histopathological observations revealed destruction of the regular architecture, severe necrosis, massive fatty degeneration, sinusoidal dilatation and congestion, centrilobular necrosis, proliferation of bile duct, exhibited collagen bundles surrounding the lobules, which then leads to thick fibrotic septae (Plate 4.6-C2). Besides that, rats administered with silymarin showed prominent liver recovery as their liver gross appearance similar to the control rats (Plate 4.6–D1). Microscopic observations showed normal hepatic architecture (Plate 4.6-D2). Liver recovery was also shown in rats administered with low dose *P. giganteus* (0.5g/kg) with mild degree of lesions, low level of liver enlargement and lesser nodules observed (Plate 4.6-E1). The liver sections revealed remarkable improvement in cellular architecture, reduced level of necrosis, narrow fibrotic septae and low degree of lymphocyte infiltration (Plate 4.6–E2). Meanwhile, rats administered with high dose *P. giganteus* (1g/kg) demonstrated excellent liver recovery with liver appearance comparable to the control rats (Plate 4.6-F1). There was minor disruption of the hepatic architecture and narrow fibrotic septae (Plate 4.6-F2).

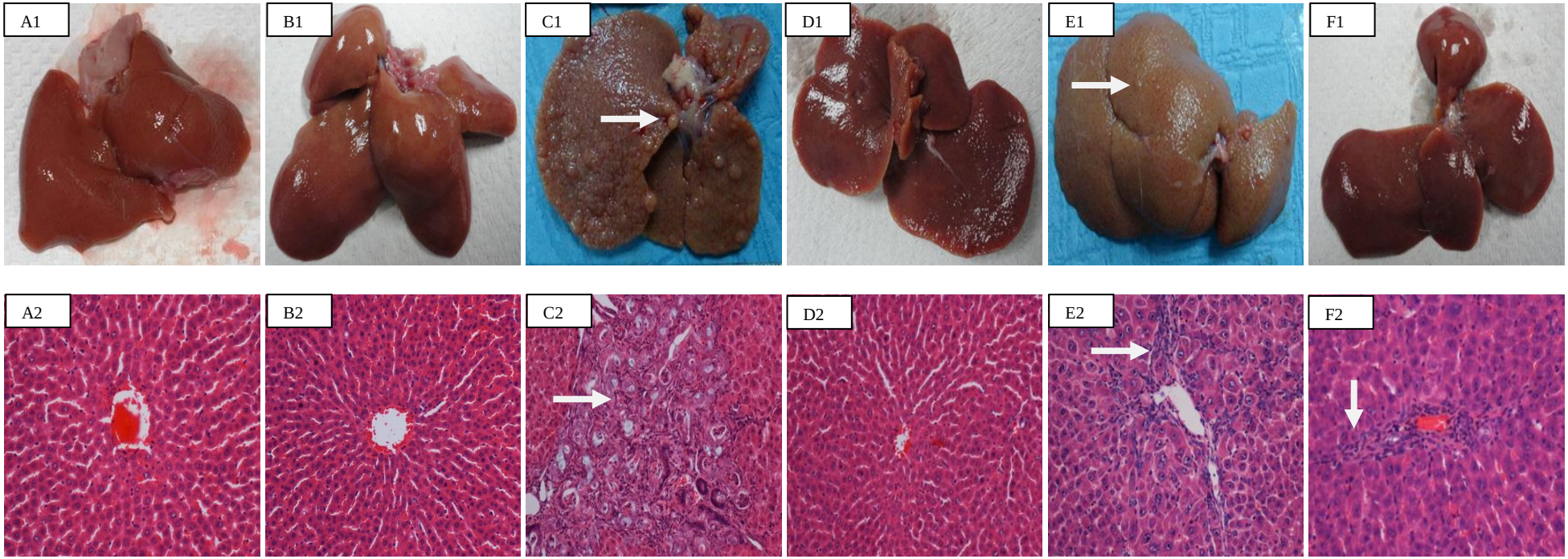


Plate 4.6: The gross liver morphology (A1-F1) and photomicrography (A2-F2) of the rats in the treatment of thioacetamide (TAA)-induced hepatotoxicity in rats. (A1and A2) control rat (dH_2O); (B1and B2) control rat (*P. giganteus*); (C1 and C2) TAA control rat: gross image shows numerous micro and macro-nodules in the liver (arrow), while light micrograph indicates thick fibrotic septae with severe necrosis (arrow); (D1and D2) rat administered with silymarin; (E1and E2) rat administered with low dose *P. giganteus* (0.5g/kg): micro nodules were observed in the gross image (arrow), light micrograph shows narrow fibrotic septae (arrow); (F1and F2) rat administered with high dose *P. giganteus* (1g/kg): very minor fibrotic septae was observed in the light micrograph (arrow). (Plate A2-F2: H& E stain, original magnification: 20X).

The present study aimed to evaluate the potential of *P. giganteus* to treat hepatic injury induced by TAA by using *Sprague Dawley* rats as experimental model. The TAA control rats, rats administered with silymarin and rats administered with *P. giganteus* were treated with TAA, a hepatotoxic agent for the first two months but ceased at the third month. Based on the results, *P. giganteus* had capabilities to treat TAA-induced liver injury. *Panus giganteus* reduced the hepatic toxic effects of TAA and restored the liver condition comparable to the standard drug, silymarin. There were relatively little studies concerned on the potential of bioactive compounds to treat hepatic damage. Most of the scientific investigations focus on the prevention effects of bioactive compounds against hepatic injury.

CHAPTER FIVE: GENERAL DISCUSSION, RECOMMENDATIONS FOR FUTURE STUDIES AND CONCLUSIONS

Conventional medicinal approaches often lack efficiency and may not provide efficient remedies to alleviate the disease conditions. Moreover, they bring side effects and are not cost-efficient especially for the third-world countries. Mushroom nutraceuticals are likely to provide good alternative to prevent diseases and improve healthy state. However, in Malaysia, there are relatively few studies on the locally grown mushrooms as source of nutraceuticals.

Mushrooms are popular in Asian countries as local cuisine especially among Malaysians. Although *P. giganteus* has been known to be consumed by selected indigenous communities in Malaysia, it was only recently that it caught the attention from local mushroom growers and consumers. *Panus giganteus* is well accepted by local consumers with good selling price, due to its delicate flavour. There is a paucity of scientific data pertaining to its medicinal and nutritional benefits with most of the reported literatures from China. To the best of our knowledge, there is no report available on the *P. giganteus* grown in Malaysia and its medicinal value is yet to be explored. The scientific studies on this species may help in large scale cultivation and consumer acceptance.

Panus giganteus fruiting bodies, fermented and unfermented wheat grains had good amounts of key nutrients which include carbohydrate, protein, fibre and mineral with low fat content. As *P. giganteus* fruiting bodies or wheat grain fermented by *P. giganteus* had promising nutritional benefits, it is suggested to market them in the form of tablets or capsules for easy consumptions of consumers.

Due to the toxicity issue related to the synthetic antioxidants including BHA, BHT and TBHQ, there was growing interest on the antioxidants isolated from plants and mushrooms. The antioxidant study showed that all extracts tested exhibited moderate antioxidant properties. The antioxidant properties of the extracts were postulated to be contributed by the phenolic compounds. Although unfermented wheat grains had better antioxidant properties than *P. giganteus* fruiting bodies, fruiting bodies may still be regarded as good source of natural antioxidants due to its lower cultivation period compared to wheat grains.

Oxidative stress exerted by free radicals was known to induce cancer and wide variety of degenerative diseases. As the extracts in the present study were shown to exhibit antioxidant properties, they may also carry potential to protect or repair the DNA. In the present study, fruiting bodies exhibited better DNA protection activity than fermented and unfermented wheat grains. All extracts displayed comparable DNA repair activities.

Genoprotective effects exhibited by the extracts could be associated with the antioxidant compounds in the extracts as H_2O_2 (free radical) induced DNA damage by causing oxidative stress. The antioxidants played vital roles in preventing oxidative stress and protect the DNA of PBMC. Flavonoids myricetin, quercetin, and rutin which exhibited antioxidant activities and free radical scavenging effects have been shown to protect DNA against H_2O_2-induced DNA damage in Caco-2 and human hepatoma Hep G2 cells (Aherne & O'Brien, 1999). Our on-going chemical profiling studies showed that hot water extracts of *P. giganteus* may possess several compounds including phenolics (benzoic acid and cinnamic acid), succinic acid and b-glucans (unpublished data). Lodovici *et al*. (2001) reported that cinnamic acid derivative, coumaric acid and

hydroxybenzoic acid derivatives, 2,3-diOH-BA and 3,4-diOH-BA inhibited oxidative DNA damage. Succinic acid could protect cells from etoposide-induced DNA damage and cytotoxicity (Wozniak *et al*., 1984). In addition, Angeli *et al*. (2009) suggested the DNA protection effects of total polysaccharides and b-glucans extracted at different stages of fruiting body maturity (immature, mature stage with immature spores and mature stage with mature spores) of the mushroom *A. blazei.* More detailed chemical studies should be done to identify the bioactive compounds of *P. giganteus* responsible for the genoprotective effects.

Fruiting bodies of *P. giganteus* that showed higher genoprotective and/or DNA repair activities were selected to evaluate for its hepatoprotective effects to prevent or treat the liver injury. In the present study, *P. giganteus* fruiting bodies had significant hepatoprotective effects against TAA-induced hepatic injury in rats. The fruiting bodies also exhibited potency to treat TAA-induced liver injury in the three-month study. Hepatoprotective effects demonstrated by *P. giganteus* fruiting bodies maybe related to its antioxidant properties to prevent or reduce oxidative stress. Preliminary chemical profiling of hot water extract of *P. giganteus* indicated the presence of ganoderic acid and silymarin flavonolignans (unpublished data). Li and Wang (2006) reported that ganoderic acid from *G. lucidum* protected the liver. Silymarin, a flavonolignan derived from the milk thistle plant, *Silybum marianum* has been used for centuries in the treatment of liver diseases (Stickel & Schuppan, 2007). This was the first comprehensive report on medicinal properties of *P. giganteus* fruiting bodies and wheat grains fermented by *P. giganteus*.

Future efforts should be focus on isolating pure bioactive compounds and validating the findings in the present study. Having established the antioxidant properties in the extracts, future works into the antioxidant compounds in *P. giganteus* fruiting bodies and fermented wheat grains are highly recommended. Since phenolic compounds were the most probable class of antioxidant in mushroom, purification and structural characterization of phenolic compounds can be undergone. So far, there was no study pertaining the characterization of pure antioxidant compounds isolated from *P. giganteus.* Besides, it is also encouraged to study the mechanisms underlying the antioxidant activity.

The extracts may also be fractionated by using column chromatography or HPLC/ anion exchange chromatography to obtain bioactive compounds which contribute to the genoprotective effects of *P. giganteus* fruiting bodies and/ or wheat grains fermented by *P. giganteus*. As *P. giganteus* had antioxidant properties and genoprotective effects, it may prevent cancer and tumour formation. Laboratory rats can be administered with *P. giganteus* in their diet and then tumour induced to evaluate the tumour prevention effects of *P. giganteus*.

Hepatoprotective effects of *P. giganteus* can be validated *in vitro* by using Hep-G2 cell line. As *P. giganteus* fruiting bodies had been shown to possess hepatoprotective effects, it was recommended to perform extraction on the mushroom powder and investigate the bioactive compounds contributed to the hepatoprotective effects of *P. giganteus*. Molecular studies should be done to investigate the gene expression and mechanisms underlying the hepatoprotective effects of *P. giganteus*.

Early studies done by Mushroom Research Centre (MRC), University of Malaya, Malaysia had show that *P. giganteus* can be easily cultivated in Malaysia. Optimization studies to cultivate *P. giganteus* should be performed. These may help to promote *P. giganteus* to public and enhance its large scale cultivation in Malaysia. Besides that, related promotions of the benefits of this mushroom should be organized to increase its popularities to a standard comparable to famous edible mushrooms such as *H. erinaceus* (lion's mane) and *L. edodes* (shitake).

In conclusion, our findings showed that fruiting bodies of *P. giganteus*, fermented and unfermented wheat grains

- contained good amounts of key nutrients including carbohydrate, protein, dietary fibre and minerals with low fat content.
- had phenolic compounds and antioxidant properties by virtue of DPPH free radical scavenging activity, reducing power, antioxidant capacity and inhibition of lipid peroxidation.
- exhibited genoprotective and/or DNA repair activity to protect and repair DNA of PBMC after H_2O_2-induced damage.

Fruiting bodies of *P. giganteus* also possessed hepatoprotective effects to prevent and treat TAA-induced liver injury in rats.

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APPENDIX

APPENDIX A: ANALYTICAL TECHNIQUES

1. Scavenging effects on 1,1-diphenyl-2-picrylhydrazyl (DPPH) (Gorinstein *et al.*, 2003)

Reagent
0.8% 2,2-diphenyl-1-picrylhydrazyl (DPPH) solution

Preparation of reagent
DPPH solution (0.8%)
The solution was prepared by dissolving 0.002g of DPPH in 50ml of absolute ethanol. It was then stirred until the DPPH was completely dissolved.

2. Ferric reducing antioxidant power (FRAP) (Benzie and Strain, 1999)

Reagents
10mmol/ L 2,4,6-tris(2-pyridyl)-1,3,5-triazine (TPTZ), 40mM hydrochloride acid (HCl), 20mM ferric chloride hexahydrate ($FeCl_3 \cdot 6H_2O$), sodium acetate trihydrate ($CH_3COONa.3H_2O$), glacial acetic acid and 2mM ferrous sulfate ($FeSO_4 \cdot 7H_2O$) stock solution.

Preparation of reagents
All reagents were prepared on the day of experiment.

FRAP reagent
The working FRAP reagent was prepared by mixing 10 volumes of 300mmol/ L acetate buffer pH 3.6, 10mmol/ L, with 1 volume of 10mmol/ L TPTZ in 40mM HCl and 1 volume of 20mM $FeCl_3 \cdot 6H_2O$.

300 mmol/ L Acetate buffer pH 3.6
The buffer was prepared by dissolving 0.0775g of AR grade sodium acetate trihydrate salt ($C_2H_3NaO_2 \cdot 3H_2O$) in 0.4ml of glacial acetic acid. The final volume was made to 25ml by using dH_2O and stirred until the salt was completely dissolved.

10mmol/ L TPTZ in 40 mM HCl
The solution was prepared by dissolving 0.0078g of TPTZ in 1ml of 1M HCl and mixed by vortex. The final volume was made to 2.5ml by using dH_2O and stirred for 10min.

20mM $FeCl_3 \cdot 6H_2O$
The solution was prepared by dissolving 0.0135g of AR grade $FeCl_3 \cdot 6H_2O$ salt in 2.5ml of dH_2O and stirred until the salt was completely dissolved.

2mM $FeSO_4 \cdot 7H_2O$ stock solution
It was prepared by dissolving 0.0056g of AR grade $FeSO_4 \cdot 7H_2O$ salt in 10ml dH_2O and stirred until the salt was completely dissolved.

Procedure for preparation of $FeSO_4 \cdot 7H_2O$ calibration plot

A calibration plot was constructed by using $FeSO_4 \cdot 7H_2O$ with concentrations ranging from 0-2000 µM. Serial dilutions were performed by using the stock solution (2mM $FeSO_4 \cdot 7H_2O$) according to the table below.

Table A.1: Volume of stock and dH_2O for serial dilution.

Concentration (µM)	Volume of Stock (µl)	Volume of dH_2O (µl)
0	0	1000
200	100	900
400	200	800
600	300	700
800	400	600
1000	500	500
1200	600	400
1400	700	300
1600	800	200
1800	900	100
2000	1000	0

Various concentration of $FeSO_4 \cdot 7H_2O$ (0-2000 µM) with volume of 10 µl were mixed with 300 µl of working FRAP reagent. For reagent blank, dH_2O instead of $FeSO_4 \cdot 7H_2O$ was mixed with the reagent. Subsequently, the absorbance was read at 593nm by using microtiterplate reader (Power Wave X 340, Bio-Tek Instruments, Inc.). All determinations were performed in triplicate. The $FeSO_4 \cdot 7H_2O$ calibration plot was obtained by plotting the change in absorbance against the concentration of the $FeSO_4 \cdot 7H_2O$ (µM).

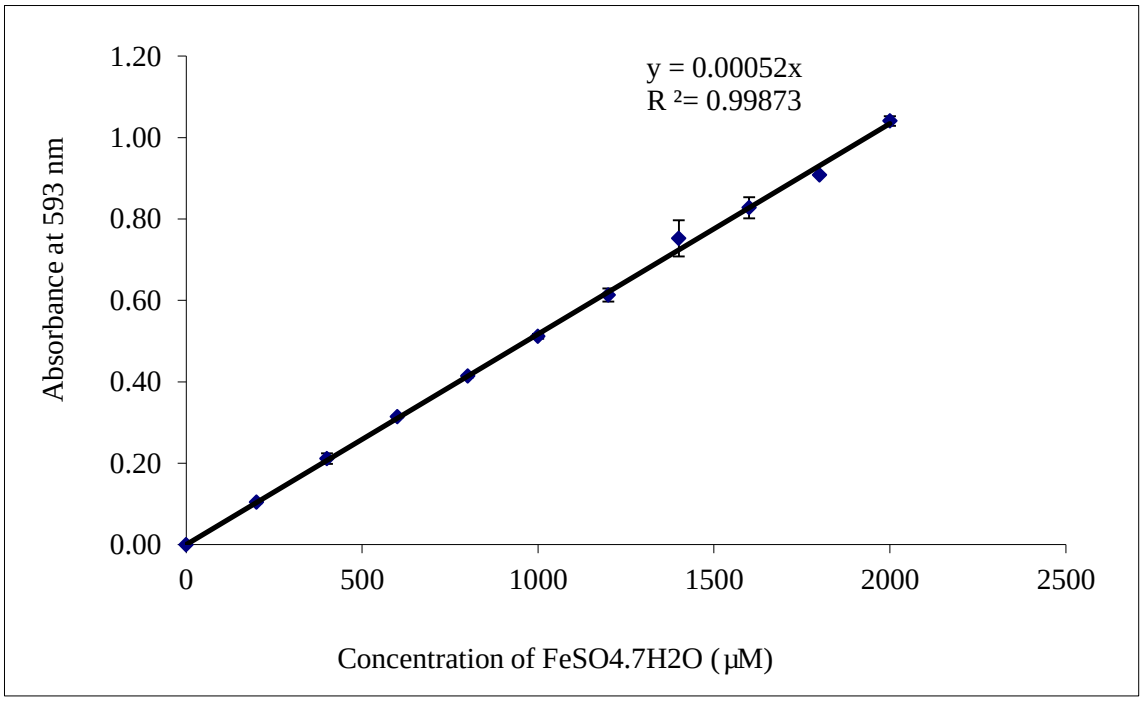

Figure A.1: $FeSO_4 \cdot 7H_2O$ calibration plot

FRAP assay of test extract and positive control

The change in absorbance of the test extracts and BHT after subtraction of blank was translated into FRAP value (µM of $FeSO_4 \cdot 7H_2O$ equivalents) using the $FeSO_4 \cdot 7H_2O$ calibration plot with the following formula:

$$\text{FRAP value (µM of } FeSO_4 \cdot 7H_2O \text{ equivalents)} = \frac{y}{0.00052}$$

3. Trolox equivalent antioxidant capacity (TEAC)

Reagents

7mM 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) $ABTS^{\bullet *}$, 104mM potasium persulfate ($K_2O_8S_2$) and 1mM 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (trolox) stock solution.

Preparation of reagents

$ABTS^{\bullet *}$ reagent

The working $ABTS^{\bullet *}$ reagent was prepared by mixing 5ml of 7mM $ABTS^{\bullet *}$ with 89µl of 140mM $K_2O_8S_2$. Then, it was left to stand in the dark for 12-16 hours. The $ABTS^{\bullet *}$ solution was further diluted with ethanol, to an absorbance of 0.70 (± 0.02) at 734nm and equilibrated at 30 ℃.

7mM $ABTS^{\bullet *}$

This is prepared by dissolving 0.0192g of AR grade 7mM $ABTS^{\bullet *}$ in 5ml dH_2O. The solution was stirred until the salt was completely dissolved.

140mM $K_2O_8S_2$

The solution was prepared by dissolving 0.0281g of AR grade $K_2O_8S_2$ salt in 1ml dH_2O and stirred until the salt was completely dissolved.

1mM trolox stock solution

The stock solution was prepared by dissolving 0.25g of AR grade trolox in 1ml dH_2O and stirred until the salt was completely dissolved.

Procedure for preparation of $FeSO_4 \cdot 7H_2O$ calibration plot

A calibration plot was constructed by using trolox with concentrations ranging from 0-1000 µM. Serial dilutions were performed by using stock solution (1mM) according to the table below.

Table A.2: Volume of stock and dH_2O for serial dilution.

Concentration (µM)	Volume of Stock (µl)	Volume of dH_2O (µl)
0	0	1000
200	200	800
400	400	600
600	600	400
800	800	200
1000	1000	0

Trolox (10µl) of different concentrations (0-1000µM) were mixed with 100µl of $ABTS^{\bullet *}$ reagent and absorbance read at 734nm by using microtiterplate reader (Power Wave X 340, Bio-Tek Instruments, Inc.) after 1 minute. All determinations were performed in triplicate. The trolox calibration plot was obtained by plotting the change in absorbance against the concentration of the trolox (µM).

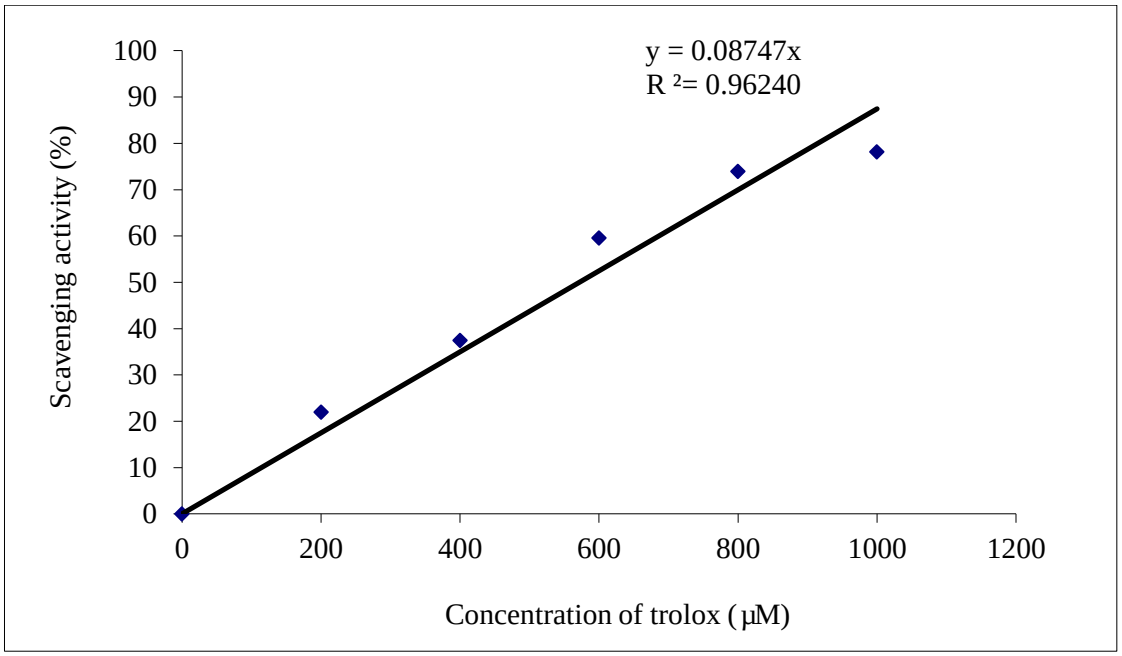

Figure A.2: Trolox calibration plot

TEAC assay of test extract and positive control

The change in absorbance of the test extracts and BHT after subtraction of blank was translated into TEAC value (µM of trolox equivalents) using the trolox calibration plot with the following formula:

$$\text{TEAC value (µM of trolox equivalents)} = \frac{y}{0.08747}$$

4. Inhibition of lipid peroxidation

Reagent

Phosphate saline buffer, trichloroacetic acid (TCA), thiobarbituric acid (TBA), ferrous sulfate ($FeSO_4$) and egg yolk suspension

Preparation of reagents

Phosphate saline buffer

The buffer was prepared by dissolving 1 tablet of AR grade PBS (Oxoid) in 10ml of dH_2O. It was then sterilized by autoclaved at 110 ℃ for 10 min.

TCA

The solution was prepared by dissolving 20g of AR grade TCA in 100ml of dH_2O and stirred until the salt was completely dissolved.

TBA
It was prepared by dissolving 0.8g of AR grade TBA in 100ml of dH_2O and stirred until the salt was completely dissolved.

$FeSO_4$
It was prepared by dissolving 0.334g of $FeSO_4$ in 50ml of dH_2O and stirred until the salt was completely dissolved.

Egg yolk suspension
Egg yolk suspension was prepared by mixing the egg yolk with PBS in 1:1 ratio. Then, it was stirred vigorously and diluted 40 times in PBS to become egg yolk suspension.

Inhibition of lipid peroxidation assay of test extract and positive control
The change in absorbance of the test extracts and BHT was translated percentage of lipid peroxidation inhibition at 10mg/ml.

5. Total phenolic content (TPC) (Slinkard and Singleton, 1977)

Reagents
10% Folin-Ciocalteu, 10% sodium carbonate (Na_2CO_3) and 1mg/ml gallic acid (3,4,5-trihydroxybenzoic acid) stock solution.

Preparation of reagents
10% Na_2CO_3
In a 100ml volumetric flask, approximately 10g of AR grade anhydrous sodium carbonate salt was dissolved in 80ml of dH_2O. The solution was stirred and heated until the salt was completely dissolved. The final volume was made to 100ml with dH_2O and stirred continuously. Subsequently, the solution was filtered and stored in bottles at room temperature.

1mg/ml gallic acid stock solution
The gallic acid stock solution used was 1mg/ml. It started by mixing 2mg of gallic acid in 2 ml of absolute ethanol and stirred until the gallic acid was completely dissolved.

Procedure for preparation of gallic acid calibration plot
A calibration plot was prepared by using gallic acid of various concentrations ranging from 0-100 µg/ml. Serial dilutions were performed by using stock solution (1mg/ml) as shown below.

Table A.3: Volume of stock and dH_2O for serial dilution.

Concentration (µg/ml)	Volume of Stock (µl)	Volume of dH_2O (µl)
0	0	1000
20	200	800
40	400	600
60	600	400
80	800	200
100	1000	0

Then, the reaction was initiated by mixing 50µl of gallic acid of various concentrations (0-100µg/ml) with 50µl of Folin–Ciocalteu phenol reagent. The blank contained absolute ethanol instead of gallic acid. After three min, 100µl of Na_2CO_3 solution was added to the mixture. The mixture was then left to stand in the dark for an hour. Subsequently, the absorbance was read at 750nm by using microtiterplate reader (Power Wave X 340, Bio-Tek Instruments, Inc.). All determinations were performed in triplicate. The gallic acid calibration plot was obtained by plotting the change in absorbance against concentration of the gallic acid (µg/ml).

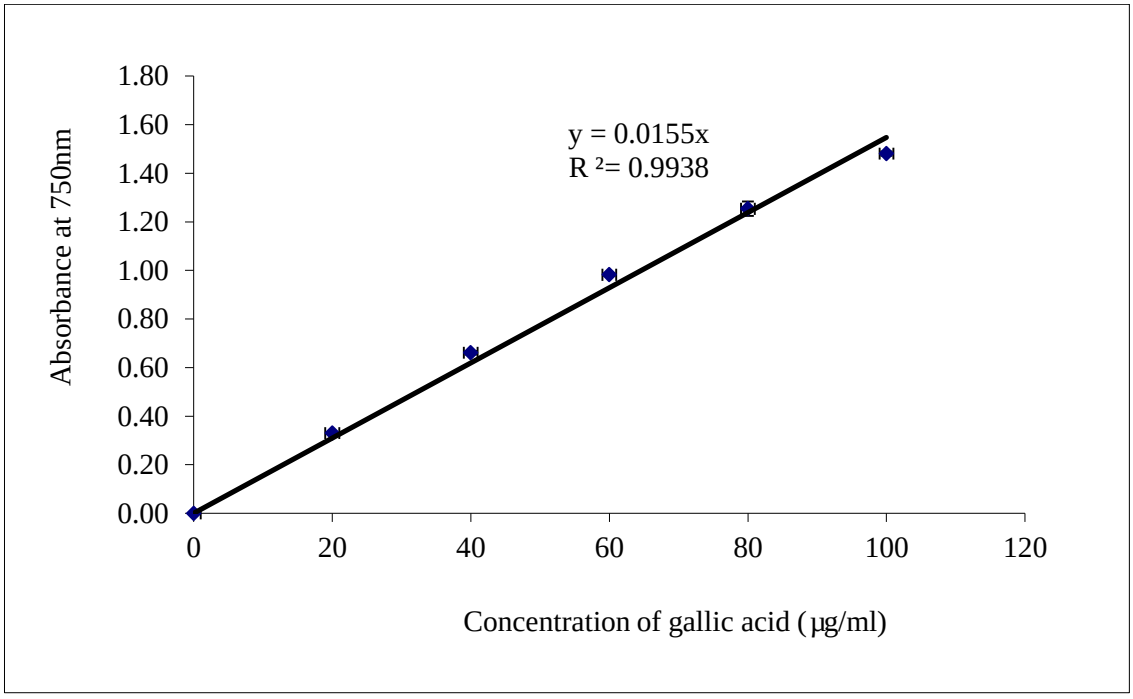

Figure A.3: Gallic acid calibration plot

Determination of total phenolic content in test extract and positive control

The absorbance value of the test extract and BHT after subtraction of blank was translated into total phenolic content [µg/ml of gallic acid equivalents (GAEs)] by using the gallic acid calibration plot based on the following formula:

$$\text{Total phenolic content (µg/ml of GAEs)} = \frac{(\ y\ )}{0.0155}$$

6. Comet assay

Reagents

Phosphate saline buffer, 1% normal melting agar, 1% low melting agar, sodium chloride (NaCl), disodium EDTA titriplex ($Na_2EDTA.2H_2O$), tris base [$(HOCH_2)_3CNH_2$], sodium hydroxide (NaOH), hydrochloric acid (HCl), triton X-100, sodium dodecyl sulfate (SDS), sodium EDTA ($Na_2EDTA.2H_2O$), glacial acetic acid and ethidium bromide (EtBr) stock solution

Preparation of reagents

Phosphate saline buffer

The buffer was prepared by dissolving 1 tablet of AR grade PBS (Oxoid) in 10ml of dH_2O. It was then sterilized by autoclaved at 110 ℃ for 10 min.

Lysine stock solution

Lysine stock solution was prepared by mixing 146.4g of NaCl (2.5M), 37.2g of $Na_2EDTA.2H_2O$ (100mM) and 1.2g of tris base (10mM) with 700ml of dH_2O. The solution was then stirred, added with 8g of NaOH and stirred again for 20 min. The pH of the solution was adjusted to 10 by using HCl or NaOH. Subsequently, the final volume was made to 890ml by using dH_2O and filtered.

Lysine working solution

The working solution was prepared by mixing 890ml of stock solution with 10ml triton X-100 (1%) and 10g of SDS (1%). The solution was then stirred until the salt was completely dissolved. Then, it was kept at room temperature.

Tris Acetate EDTA (TAE) buffer

The buffer was prepared by dissolving 4.85g of tris base (40mM) and 0.37g of $Na_2EDTA.2H_2O$ in 700ml of dH_2O. The pH was adjusted to 8 by using glacial acetic acid. Then, the final volume was made to 1 litre and filtered. It was kept at room temperature.

Electrophoresis buffer

The buffer was prepared by mixing 30ml of NaOH (0.3M) with 5 ml of EDTA (1mM) and stirred. The solution was then adjusted to pH>13 by using NaOH. After that, the final volume was made to 1 litre by using dH_2O.

NaOH stock solution

The buffer was prepared by dissolving 200g of NaOH pellet (10N) in 500ml of dH_2O and stirred until the salt was completely dissolved.

EDTA stock solution

This was prepared by dissolving 14.89 of electrophoresis grade EDTA (200mM) in 200ml of dH_2O. The pH was then adjusted to 10 by using NaOH pellet. After that, the final volume was made to 300ml with dH_2O and kept at room temperature.

Neutralization buffer

The buffer was prepared by dissolving 48.5g of tris base in 700ml of dH_2O. Then, it was adjusted to pH 7.5 with HCl and final volume made to 1 litre with dH_2O. It was kept at room temperature.

EtBr stock solution
The stock solution was prepared by dissolving 10mg of EtBr in 1ml of dH_2O.

EtBr working solution
It was prepared by mixing 2ml of stock solution with 0. 988ml of dH_2O.

6.1 EC_{50} of H_2O_2

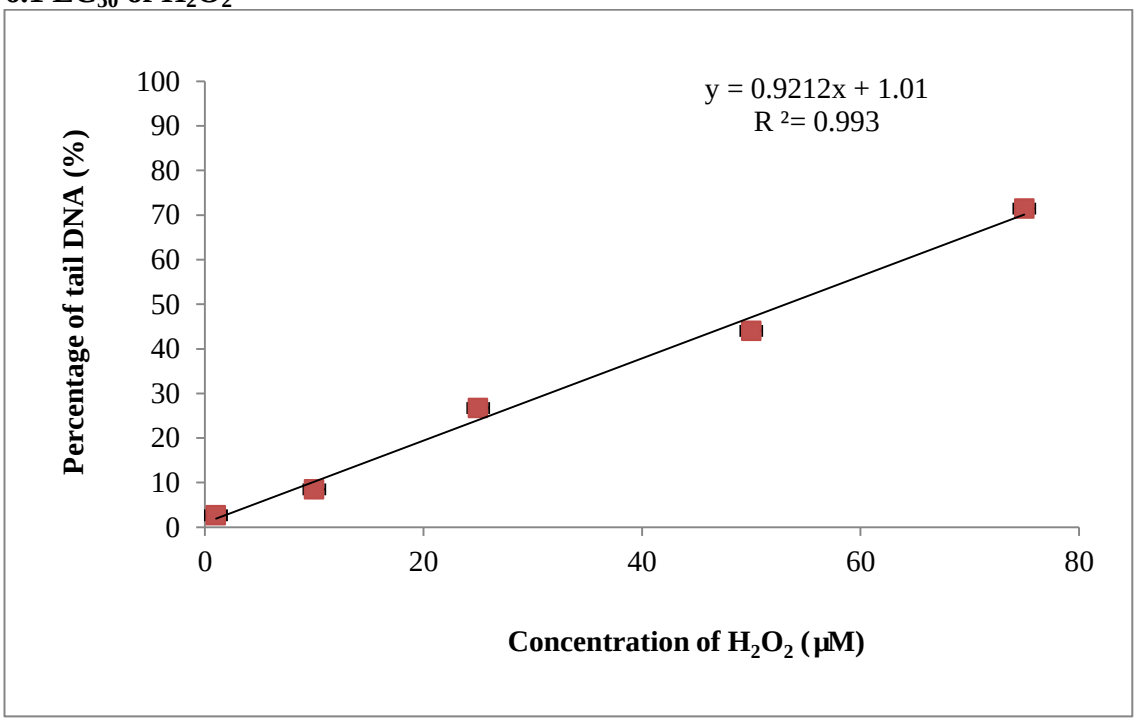

Figure A.4: Graph of tail DNA percentage against H_2O_2 concentration to obtain EC_{50}

$$LD_{50} = \left(\frac{y}{0.9396}\right)$$

6.2 Percentage of DNA protection:

$$\text{Percentage of DNA protection (\%)} = \frac{\text{Head DNA sample} - \text{Tail DNA sample}}{\text{Head DNA of blank}} \times 100\%$$

6.3 Percentage of DNA repair:

$$\text{Percentage of DNA repair (\%)} = \frac{\text{Head DNA sample} - \text{Tail DNA sample}}{\text{Head DNA of blank}} \times 100\%$$

7. Determination of serum MDA content

Reagent

Phosphate saline buffer, trichloroacetic acid (TCA), thiobarbituric acid (TBA), ferrous sulfate ($FeSO_4$) and 20 µM 1,1,3,3-tetra-ethoxy propane (TEP) stock solution.

Preparation of reagents

Phosphate saline buffer

The buffer was prepared by dissolving 1 tablet of AR grade PBS (Oxoid) in 10ml of dH_2O. It was then sterilized by autoclaved at 110 ℃ for 10 min.

TCA

The solution was prepared by dissolving 20g of AR grade TCA in 100ml of dH_2O and stirred until the salt was completely dissolved.

TBA

It was prepared by dissolving 0.8g of AR grade TBA in 100ml of dH_2O and stirred until the salt was completely dissolved.

$FeSO_4$

It was prepared by dissolving 0.334g of $FeSO_4$ in 50ml of dH_2O and stirred until the salt was completely dissolved.

20 µM TEP stock solution

The stock solution was prepared by mixing 12.5 µl of TEP with 50ml of dH_2O and vortex.

Procedure for preparation of $FeSO_4 \cdot 7H_2O$ calibration plot

A calibration plot was constructed by using TEP with concentrations ranging from 0-20 µM. These concentrations were obtained by performing serial dilutions on the stock solution (20 µM). It started by mixing 1ml of TEP of different concentrations (0-20 µM) with 0.5ml yolk suspension and 0.5ml $FeSO_4$. Subsequently, the mixture was incubated at 37 ℃ for an hour and then treated with 0.5ml of 20% TCA and 1ml of 0.8% TBA. The mixture then heated in boiling water bath for 15 min and centrifuged at 3500rpm for 20 min (Jouan C312 centrifuge) in order to remove precipitated protein. Finally, 200 µl of the supernatant was taken to read its absorbance at 532nm by using microtiterplate reader (Power Wave X 340, Bio-Tek Instruments, Inc.). This is to measure the formation of Thiobarbituric Acid Reactive Substances (TBARS) complex. All determinations were performed in triplicate. The TEP calibration plot was obtained by plotting the change in absorbance against the concentration of the TEP (µM).

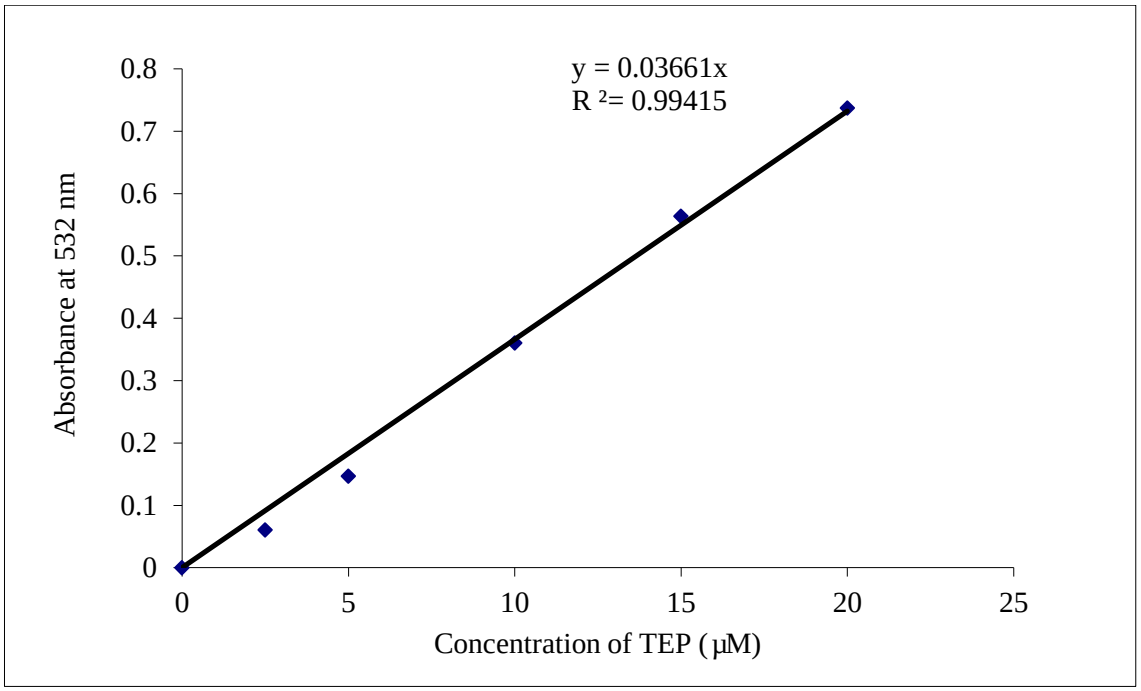

Figure A.5: TEP calibration plot

Determination of serum MDA content

The change in absorbance of the serum of rats in different experimental groups was translated into µM of TEP equivalents using the TEP calibration plot with the following formula:

$$\text{Serum MDA content} = \left(\frac{y}{0.0366}\right)$$

APPENDIX B: MEDIA, BUFFER AND POSITIVE CONTROL

1. Potato dextrose agar (PDA)

The medium was prepared by dissolving 11.7g of PDA in 300ml of dH_2O.

2. Glucose-Yeast-Malt-Peptone (GYMP) agar

The composition of GYMP was shown in the table below.

Table B.1: Composition of GYMP.

Nutrient	Weight (g)
NH_4Cl	0.3
$MgSO_4.7H_2O$	0.3
KH_2PO_4	0.3
K_2HPO_4	0.3
Glucose	4.5
Peptone	2.4
Yeast extract	2.4
Malt extract	2.4
Bacto agar	6
dH_2O	300 ml

Both media were autoclaved for 15 min at 15psi, 121 ℃. Subsequently, the media were left cooled to 45 -50 ℃ and dispensed into Petri dishes. Final pH should be 5.5 ± 0.2 at 25 ℃.

APPENDIX C: DATA AND STATISTICAL TABLES

Table C.1: One way analysis of variance (ANOVA): Extraction yield (between different extracts)

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1.416	2	.708	91.000	.000
Within Groups	.047	6	.008		
Total	1.462	8			

Table C.2: Multiple comparisons: Extraction yield (between different extracts)

Dependent variable: Extraction yield

	(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Tukey HSD	Fruiting bodies	Fermented wheat grains	.40000*	.07201	.003	.1791	.6209
		Unfermented wheat grains	-.56667*	.07201	.001	-.7876	-.3457
	Fermented wheat grains	Fruiting bodies	-.40000*	.07201	.003	-.6209	-.1791
		Unfermented wheat grains	-.96667*	.07201	.000	-1.1876	-.7457
	Unfermented wheat grains	Fruiting bodies	.56667*	.07201	.001	.3457	.7876
		Fermented wheat grains	.96667*	.07201	.000	.7457	1.1876

*. The mean difference is significant at the 0.05 level.

Table C.3: Multiple range tests: Extraction yield (between different extracts)

	Group	N	Subset for alpha = 0.05		
			1	2	3
Tukey HSD[a]	Fermented wheat grains	3	4.9000		
	Fruiting bodies	3		5.3000	
	Unfermented wheat grains	3			5.8667
	Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Table C.4: One way analysis of variance (ANOVA): Total phenolic content (between different extracts)

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	14.563	2	7.281	6.971	.010
Within Groups	12.534	12	1.044		
Total	27.097	14			

Table C.5: Multiple comparisons: Total phenolic content (between different extracts)

Dependent variable: Total phenolic content

	(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Tukey HSD	Fruiting bodies	Fermented wheat grains	1.39300	.64637	.120	-.3314	3.1174
		Unfermented wheat grains	-1.01040	.64637	.298	-2.7348	.7140
	Fermented wheat grains	Fruiting bodies	-1.39300	.64637	.120	-3.1174	.3314
		Unfermented wheat grains	-2.40340*	.64637	.008	-4.1278	-.6790
	Unfermented wheat grains	Fruiting bodies	1.01040	.64637	.298	-.7140	2.7348
		Fermented wheat grains	2.40340*	.64637	.008	.6790	4.1278

*. The mean difference is significant at the 0.05 level.

Table C.6: Multiple range tests: Total phenolic content (between different extracts)

	Group	N	Subset for alpha = 0.05	
			1	2
Tukey HSD[a]	Fermented wheat grains	5	5.1928	
	Fruiting bodies	5	6.5858	6.5858
	Unfermented wheat grains	5		7.5962
	Sig.		.120	.298

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 5.000.

Table C.7: One way analysis of variance (ANOVA): DPPH scavenging activity (between different extracts)

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	851.957	2	425.978	27.334	.001
Within Groups	93.506	6	15.584		
Total	945.462	8			

Table C.8: Multiple comparisons: DPPH scavenging activity (between different extracts)

Dependent variable: DPPH scavenging activity

	(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Tukey HSD	Fruiting bodies	Fermented wheat grains	1.70000	3.22328	.861	-8.1899	11.5899
		Unfermented wheat grains	21.43667*	3.22328	.001	11.5468	31.3266
	Fermented wheat grains	Fruiting bodies	-1.70000	3.22328	.861	-11.5899	8.1899
		Unfermented wheat grains	19.73667*	3.22328	.002	9.8468	29.6266
	Unfermented wheat grains	Fruiting bodies	-21.43667*	3.22328	.001	-31.3266	-11.5468
		Fermented wheat grains	-19.73667*	3.22328	.002	-29.6266	-9.8468

*. The mean difference is significant at the 0.05 level.

Table C.9: Multiple range tests: DPPH scavenging activity (between different extracts)

	Group	N	Subset for alpha = 0.05	
			1	2
Tukey HSD[a]	Unfermented wheat grains	3	21.0267	
	Fermented wheat grains	3		40.7633
	Fruiting bodies	3		42.4633
	Sig.		1.000	.861

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Table C.10: One way analysis of variance (ANOVA): Ferric reducing antioxidant power (FRAP) (between different extracts)

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	17913.332	2	8956.666	516.490	.000
Within Groups	312.145	18	17.341		
Total	18225.477	20			

Table C.11: Multiple comparisons: Ferric reducing antioxidant power (FRAP) (between different extracts)

Dependent variable: Ferric reducing antioxidant power (FRAP)

	(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Tukey HSD	Fruiting bodies	Fermented wheat grains	-.70271	2.22591	.947	-6.3836	4.9782
		Unfermented wheat grains	-62.30457*	2.22591	.000	-67.9855	-56.6237
	Fermented wheat grains	Fruiting bodies	.70271	2.22591	.947	-4.9782	6.3836

		Unfermented wheat grains	-61.60186*	2.22591	.000	-67.2828	-55.9210
	Unfermented wheat grains	Fruiting bodies	62.30457*	2.22591	.000	56.6237	67.9855
		Fermented wheat grains	61.60186*	2.22591	.000	55.9210	67.2828

*. The mean difference is significant at the 0.05 level.

Table C.12: Multiple range tests: Ferric reducing antioxidant power (FRAP) (between different extracts)

	Group	N	Subset for alpha = 0.05	
			1	2
Tukey HSD[a]	Fruiting bodies	7	20.0256	
	Fermented wheat grains	7	20.7283	
	Unfermented wheat grains	7		82.3301
	Sig.		.947	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 7.000.

Table C.13: One way analysis of variance (ANOVA): Trolox equivalents antioxidant capacity (TEAC) (between different extracts)

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	309.318	2	154.659	20.903	.000
Within Groups	133.180	18	7.399		
Total	442.497	20			

Table C.14: Multiple comparisons: Trolox equivalents antioxidant capacity (TEAC) (between different extracts)

Dependent variable: Trolox equivalents antioxidant capacity (TEAC)

	(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Tukey HSD	Fruiting bodies	Fermented wheat grains	.16043	1.45395	.993	-3.5503	3.8711
		Unfermented wheat grains	-8.06000*	1.45395	.000	-11.7707	-4.3493
	Fermented wheat grains	Fruiting bodies	-.16043	1.45395	.993	-3.8711	3.5503
		Unfermented wheat grains	-8.22043*	1.45395	.000	-11.9311	-4.5097
	Unfermented wheat grains	Fruiting bodies	8.06000*	1.45395	.000	4.3493	11.7707
		Fermented wheat grains	8.22043*	1.45395	.000	4.5097	11.9311

*. The mean difference is significant at the 0.05 level.

Table C.15: Multiple range tests: Trolox equivalents antioxidant capacity (TEAC) (between different extracts)

	Group	N	Subset for alpha = 0.05	
			1	2
Tukey HSD[a]	Fermented wheat grains	7	10.9400	
	Fruiting bodies	7	11.1004	
	Unfermented wheat grains	7		19.1604
	Sig.		.993	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 7.000.

Table C.16: One way analysis of variance (ANOVA): Inhibition of lipid peroxidation (between different extracts at 10 mg/ml)

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	412.866	2	206.433	16.677	.004
Within Groups	74.268	6	12.378		
Total	487.134	8			

Table C.17: Multiple comparisons: Inhibition of lipid peroxidation (between different extracts at 10 mg/ml)

Dependent variable: Inhibition of lipid peroxidation

(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Tukey HSD Fruiting bodies	Fermented wheat grains	-15.23879*	2.87263	.004	-24.0528	-6.4248
	Unfermented wheat grains	-13.29985*	2.87263	.009	-22.1139	-4.4858
Fermented wheat grains	Fruiting bodies	15.23879*	2.87263	.004	6.4248	24.0528
	Unfermented wheat grains	1.93894	2.87263	.786	-6.8751	10.7529
Unfermented wheat grains	Fruiting bodies	13.29985*	2.87263	.009	4.4858	22.1139
	Fermented wheat grains	-1.93894	2.87263	.786	-10.7529	6.8751

*. The mean difference is significant at the 0.05 level.

Table C.18: Multiple range tests: Inhibition of lipid peroxidation (between different extracts at 10 mg/ml)

	Group	N	Subset for alpha = 0.05	
			1	2
Tukey HSD[a]	Fruiting bodies	3	26.6532	
	Unfermented wheat grains	3		39.9531
	Fermented wheat grains	3		41.8920
	Sig.		1.000	.786

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Table C.19: One way analysis of variance (ANOVA): EC_{50} value of genotoxin H_2O_2- Head DNA percentage of PBMC (between different concentrations)

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	388019.347	5	77603.869	1273.541	.000
Within Groups	36195.681	594	60.935		
Total	424215.028	599			

Table C.20: Multiple comparisons: EC_{50} value of genotoxin H_2O_2- Head DNA percentage of PBMC (between different concentrations)

Dependent variable: Head DNA percentage

	(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Tukey HSD	Control	1 µm	1.91540	1.10395	.509	-1.2408	5.0716
		10 µm'	7.74750*	1.10395	.000	4.5913	10.9037
		25 µm	25.97870*	1.10395	.000	22.8225	29.1349
		50 µm	43.27880*	1.10395	.000	40.1226	46.4350
		75 µm	70.71210*	1.10395	.000	67.5559	73.8683
	1 µm	Control	-1.91540	1.10395	.509	-5.0716	1.2408
		10 µm'	5.83210*	1.10395	.000	2.6759	8.9883
		25 µm	24.06330*	1.10395	.000	20.9071	27.2195
		50 µm	41.36340*	1.10395	.000	38.2072	44.5196
		75 µm	68.79670*	1.10395	.000	65.6405	71.9529
	10 µm	Control	-7.74750*	1.10395	.000	-10.9037	-4.5913
		1 µm	-5.83210*	1.10395	.000	-8.9883	-2.6759
		25 µm	18.23120*	1.10395	.000	15.0750	21.3874
		50 µm	35.53130*	1.10395	.000	32.3751	38.6875

Table C.20, continued: Multiple comparisons: EC_{50} value of genotoxin H_2O_2- Head DNA percentage of PBMC (between different concentrations)

Dependent variable: Head DNA percentage

	75 µm	62.96460*	1.10395	.000	59.8084	66.1208
25 µm	Control	-25.97870*	1.10395	.000	-29.1349	-22.8225
	1 µm	-24.06330*	1.10395	.000	-27.2195	-20.9071
	10 µm'	-18.23120*	1.10395	.000	-21.3874	-15.0750
	50 µm	17.30010*	1.10395	.000	14.1439	20.4563
	75 µm	44.73340*	1.10395	.000	41.5772	47.8896
50 µm	Control	-43.27880*	1.10395	.000	-46.4350	-40.1226
	1 µm	-41.36340*	1.10395	.000	-44.5196	-38.2072
	10 µm'	-35.53130*	1.10395	.000	-38.6875	-32.3751
	25 µm	-17.30010*	1.10395	.000	-20.4563	-14.1439
	75 µm	27.43330*	1.10395	.000	24.2771	30.5895
75 µm	Control	-70.71210*	1.10395	.000	-73.8683	-67.5559
	1 µm	-68.79670*	1.10395	.000	-71.9529	-65.6405
	10 µm'	-62.96460*	1.10395	.000	-66.1208	-59.8084
	25 µm	-44.73340*	1.10395	.000	-47.8896	-41.5772
	50 µm	-27.43330*	1.10395	.000	-30.5895	-24.2771

*. The mean difference is significant at the 0.05 level.

Table C.21: Multiple range tests: EC_{50} value of genotoxin H_2O_2- Head DNA percentage of PBMC (between different concentrations)

			Subset for alpha = 0.05				
	Group	N	1	2	3	4	5
Tukey HSD[a]	75 µm	100	28.5402				
	50 µm	100		55.9735			
	25 µm	100			73.2736		
	10 µm	100				91.5048	
	1 µm	100					97.3369
	Control	100					99.2523
	Sig.		1.000	1.000	1.000	1.000	.509

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 100.000.

Table C.22: One way analysis of variance (ANOVA): Percentage of tail DNA in the assessment of genoprotective effects (between different extracts).

		Sum of Squares	df	Mean Square	F	Sig.
Fruiting bodies	Between Groups	115221.809	6	19203.635	120.484	.000
	Within Groups	110455.156	693	159.387		
	Total	225676.965	699			
Fermented wheat grains	Between Groups	117024.614	6	19504.102	105.276	.000
	Within Groups	128389.201	693	185.266		
	Total	245413.815	699			
Unfermented wheat grains	Between Groups	134643.689	6	22440.615	105.476	.000
	Within Groups	147439.129	693	212.755		
	Total	282082.818	699			

Table C.23: Multiple comparisons: Percentage of tail DNA in the assessment of genoprotective effects (between different extracts).

Dependent Variable	(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Fruiting bodies	blank	control	-38.91110*	1.78542	.000	-44.1906	-33.6316
		100 µg/ml	-18.47790*	1.78542	.000	-23.7574	-13.1984
		150 µg/ml	-17.30990*	1.78542	.000	-22.5894	-12.0304
		200 µg/ml	-5.57400*	1.78542	.031	-10.8535	-.2945
		250 µg/ml	-2.75360	1.78542	.719	-8.0331	2.5259
		300 µg/ml	-2.64780	1.78542	.755	-7.9273	2.6317
	control	blank	38.91110*	1.78542	.000	33.6316	44.1906
		100 µg/ml	20.43320*	1.78542	.000	15.1537	25.7127
		150 µg/ml	21.60120*	1.78542	.000	16.3217	26.8807
		200 µg/ml	33.33710*	1.78542	.000	28.0576	38.6166
		250 µg/ml	36.15750*	1.78542	.000	30.8780	41.4370
		300 µg/ml	36.26330*	1.78542	.000	30.9838	41.5428
	100 µg/ml	blank	18.47790*	1.78542	.000	13.1984	23.7574
		control	-20.43320*	1.78542	.000	-25.7127	-15.1537
		150 µg/ml	1.16800	1.78542	.995	-4.1115	6.4475
		200 µg/ml	12.90390*	1.78542	.000	7.6244	18.1834
		250 µg/ml	15.72430*	1.78542	.000	10.4448	21.0038
		300 µg/ml	15.83010*	1.78542	.000	10.5506	21.1096
	150 µg/ml	blank	17.30990*	1.78542	.000	12.0304	22.5894
		control	-21.60120*	1.78542	.000	-26.8807	-16.3217
		100 µg/ml	-1.16800	1.78542	.995	-6.4475	4.1115
		200 µg/ml	11.73590*	1.78542	.000	6.4564	17.0154
		250 µg/ml	14.55630*	1.78542	.000	9.2768	19.8358
		300 µg/ml	14.66210*	1.78542	.000	9.3826	19.9416
	200 µg/ml	blank	5.57400*	1.78542	.031	.2945	10.8535
		control	-33.33710*	1.78542	.000	-38.6166	-28.0576
		100 µg/ml	-12.90390*	1.78542	.000	-18.1834	-7.6244
		150 µg/ml	-11.73590*	1.78542	.000	-17.0154	-6.4564
		250 µg/ml	2.82040	1.78542	.695	-2.4591	8.0999
		300 µg/ml	2.92620	1.78542	.657	-2.3533	8.2057
	250 µg/ml	blank	2.75360	1.78542	.719	-2.5259	8.0331
		control	-36.15750*	1.78542	.000	-41.4370	-30.8780
		100 µg/ml	-15.72430*	1.78542	.000	-21.0038	-10.4448
		150 µg/ml	-14.55630*	1.78542	.000	-19.8358	-9.2768
		200 µg/ml	-2.82040	1.78542	.695	-8.0999	2.4591
		300 µg/ml	.10580	1.78542	1.000	-5.1737	5.3853
	300 µg/ml	blank	2.64780	1.78542	.755	-2.6317	7.9273
		control	-36.26330*	1.78542	.000	-41.5428	-30.9838
		100 µg/ml	-15.83010*	1.78542	.000	-21.1096	-10.5506
		150 µg/ml	-14.66210*	1.78542	.000	-19.9416	-9.3826
		200 µg/ml	-2.92620	1.78542	.657	-8.2057	2.3533
		250 µg/ml	-.10580	1.78542	1.000	-5.3853	5.1737
Fermented wheat grains	blank	control	-38.91110*	1.92492	.000	-44.6031	-33.2191
		100 µg/ml	-32.20190*	1.92492	.000	-37.8939	-26.5099
		150 µg/ml	-23.79420*	1.92492	.000	-29.4862	-18.1022
		200 µg/ml	-20.78480*	1.92492	.000	-26.4768	-15.0928
		250 µg/ml	-9.69190*	1.92492	.000	-15.3839	-3.9999

Table C.23, continued: Multiple comparisons: Percentage of tail DNA in the assessment of genoprotective effects (between different extracts).

		300 µg/ml	-7.78850*	1.92492	.001	-13.4805	-2.0965
	control	blank	38.91110*	1.92492	.000	33.2191	44.6031
		100 µg/ml	6.70920*	1.92492	.009	1.0172	12.4012
		150 µg/ml	15.11690*	1.92492	.000	9.4249	20.8089
		200 µg/ml	18.12630*	1.92492	.000	12.4343	23.8183
		250 µg/ml	29.21920*	1.92492	.000	23.5272	34.9112
		300 µg/ml	31.12260*	1.92492	.000	25.4306	36.8146
	100 µg/ml	blank	32.20190*	1.92492	.000	26.5099	37.8939
		control	-6.70920*	1.92492	.009	-12.4012	-1.0172
		150 µg/ml	8.40770*	1.92492	.000	2.7157	14.0997
		200 µg/ml	11.41710*	1.92492	.000	5.7251	17.1091
		250 µg/ml	22.51000*	1.92492	.000	16.8180	28.2020
		300 µg/ml	24.41340*	1.92492	.000	18.7214	30.1054
	150 µg/ml	blank	23.79420*	1.92492	.000	18.1022	29.4862
		control	-15.11690*	1.92492	.000	-20.8089	-9.4249
		100 µg/ml	-8.40770*	1.92492	.000	-14.0997	-2.7157
		200 µg/ml	3.00940	1.92492	.706	-2.6826	8.7014
		250 µg/ml	14.10230*	1.92492	.000	8.4103	19.7943
		300 µg/ml	16.00570*	1.92492	.000	10.3137	21.6977
	200 µg/ml	blank	20.78480*	1.92492	.000	15.0928	26.4768
		control	-18.12630*	1.92492	.000	-23.8183	-12.4343
		100 µg/ml	-11.41710*	1.92492	.000	-17.1091	-5.7251
		150 µg/ml	-3.00940	1.92492	.706	-8.7014	2.6826
		250 µg/ml	11.09290*	1.92492	.000	5.4009	16.7849
		300 µg/ml	12.99630*	1.92492	.000	7.3043	18.6883
	250 µg/ml	blank	9.69190*	1.92492	.000	3.9999	15.3839
		control	-29.21920*	1.92492	.000	-34.9112	-23.5272
		100 µg/ml	-22.51000*	1.92492	.000	-28.2020	-16.8180
		150 µg/ml	-14.10230*	1.92492	.000	-19.7943	-8.4103
		200 µg/ml	-11.09290*	1.92492	.000	-16.7849	-5.4009
		300 µg/ml	1.90340	1.92492	.956	-3.7886	7.5954
	300 µg/ml	blank	7.78850*	1.92492	.001	2.0965	13.4805
		control	-31.12260*	1.92492	.000	-36.8146	-25.4306
		100 µg/ml	-24.41340*	1.92492	.000	-30.1054	-18.7214
		150 µg/ml	-16.00570*	1.92492	.000	-21.6977	-10.3137
		200 µg/ml	-12.99630*	1.92492	.000	-18.6883	-7.3043
		250 µg/ml	-1.90340	1.92492	.956	-7.5954	3.7886
Unfermented wheat grains	blank	control	-38.91110*	2.06279	.000	-45.0108	-32.8114
		100 µg/ml	-35.81690*	2.06279	.000	-41.9166	-29.7172
		150 µg/ml	-28.75160*	2.06279	.000	-34.8513	-22.6519
		200 µg/ml	-15.49820*	2.06279	.000	-21.5979	-9.3985
		250 µg/ml	-10.37900*	2.06279	.000	-16.4787	-4.2793
		300 µg/ml	-7.73540*	2.06279	.004	-13.8351	-1.6357
	control	blank	38.91110*	2.06279	.000	32.8114	45.0108
		100 µg/ml	3.09420	2.06279	.745	-3.0055	9.1939
		150 µg/ml	10.15950*	2.06279	.000	4.0598	16.2592
		200 µg/ml	23.41290*	2.06279	.000	17.3132	29.5126
		250 µg/ml	28.53210*	2.06279	.000	22.4324	34.6318
		300 µg/ml	31.17570*	2.06279	.000	25.0760	37.2754
	100 µg/ml	blank	35.81690*	2.06279	.000	29.7172	41.9166
		control	-3.09420	2.06279	.745	-9.1939	3.0055

		150 µg/ml	7.06530*	2.06279	.012	.9656	13.1650

Table C.23, continued: Multiple comparisons: Percentage of tail DNA in the assessment of genoprotective effects (between different extracts).

		200 µg/ml	20.31870*	2.06279	.000	14.2190	26.4184
		250 µg/ml	25.43790*	2.06279	.000	19.3382	31.5376
		300 µg/ml	28.08150*	2.06279	.000	21.9818	34.1812
	150 µg/ml	blank	28.75160*	2.06279	.000	22.6519	34.8513
		control	-10.15950*	2.06279	.000	-16.2592	-4.0598
		100 µg/ml	-7.06530*	2.06279	.012	-13.1650	-.9656
		200 µg/ml	13.25340*	2.06279	.000	7.1537	19.3531
		250 µg/ml	18.37260*	2.06279	.000	12.2729	24.4723
		300 µg/ml	21.01620*	2.06279	.000	14.9165	27.1159
	200 µg/ml	blank	15.49820*	2.06279	.000	9.3985	21.5979
		control	-23.41290*	2.06279	.000	-29.5126	-17.3132
		100 µg/ml	-20.31870*	2.06279	.000	-26.4184	-14.2190
		150 µg/ml	-13.25340*	2.06279	.000	-19.3531	-7.1537
		250 µg/ml	5.11920	2.06279	.168	-.9805	11.2189
		300 µg/ml	7.76280*	2.06279	.003	1.6631	13.8625
	250 µg/ml	blank	10.37900*	2.06279	.000	4.2793	16.4787
		control	-28.53210*	2.06279	.000	-34.6318	-22.4324
		100 µg/ml	-25.43790*	2.06279	.000	-31.5376	-19.3382
		150 µg/ml	-18.37260*	2.06279	.000	-24.4723	-12.2729
		200 µg/ml	-5.11920	2.06279	.168	-11.2189	.9805
		300 µg/ml	2.64360	2.06279	.860	-3.4561	8.7433
	300 µg/ml	blank	7.73540*	2.06279	.004	1.6357	13.8351
		control	-31.17570*	2.06279	.000	-37.2754	-25.0760
		100 µg/ml	-28.08150*	2.06279	.000	-34.1812	-21.9818
		150 µg/ml	-21.01620*	2.06279	.000	-27.1159	-14.9165
		200 µg/ml	-7.76280*	2.06279	.003	-13.8625	-1.6631
		250 µg/ml	-2.64360	2.06279	.860	-8.7433	3.4561

*. The mean difference is significant at the 0.05 level.

Table C.24: Multiple range tests: Percentage of tail DNA in the assessment of genoprotective effects (between different extracts)

Fruiting bodies			Subset for alpha = 0.05			
Tukey HSD[a]	Group	N	1	2	3	4
	blank	100	8.6657			
	300 µg/ml	100	11.3135	11.3135		
	250 µg/ml	100	11.4193	11.4193		
	200 µg/ml	100		14.2397		
	150 µg/ml	100			25.9756	
	100 µg/ml	100			27.1436	
	control	100				47.5768
	Sig.		.719	.657	.995	1.000

Fermented wheat grains			Subset for alpha = 0.05				
Tukey HSD[a]	Group	N	1	2	3	4	5
	blank	100	8.6657				
	300 µg/ml	100		16.4542			
	250 µg/ml	100		18.3576			
	200 µg/ml	100			29.4505		
	150 µg/ml	100			32.4599		
	100 µg/ml	100				40.8676	
	control	100					47.5768
	Sig.		1.000	.956	.706	1.000	1.000

Table C.24, continued: Multiple range tests: Percentage of tail DNA in the assessment of genoprotective effects (between different extracts)

Unfermented wheat grains	Group	N	Subset for alpha = 0.05				
			1	2	3	4	5
Tukey HSD[a]	blank	100	8.6657				
	300 µg/ml	100		16.4011			
	250 µg/ml	100		19.0447	19.0447		
	200 µg/ml	100			24.1639		
	150 µg/ml	100				37.4173	
	100 µg/ml	100					44.4826
	control	100					47.5768
	Sig.		1.000	.860	.168	1.000	.745

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 100.000.

Table C.25: One way analysis of variance (ANOVA): Percentage of tail DNA in the assessment of DNA repair activity (between different extracts).

		Sum of Squares	df	Mean Square	F	Sig.
Fruiting bodies	Between Groups	140159.802	6	23359.967	235.781	.000
	Within Groups	68658.909	693	99.075		
	Total	208818.711	699			
Fermented wheat grains	Between Groups	137434.910	6	22905.818	156.787	.000
	Within Groups	101243.648	693	146.095		
	Total	238678.558	699			
Unfermented wheat grains	Between Groups	139877.258	6	23312.876	176.592	.000
	Within Groups	91486.647	693	132.015		
	Total	231363.905	699			

Table C.26: Multiple comparisons: Percentage of tail DNA in the assessment of DNA repair activity (between different extracts).

Dependent Variable	(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Fruiting bodies	blank	control	-40.16310*	1.40766	.000	-44.3256	-36.0006
		100 µg/ml	-35.67660*	1.40766	.000	-39.8391	-31.5141
		150 µg/ml	-29.17860*	1.40766	.000	-33.3411	-25.0161
		200 µg/ml	-19.81050*	1.40766	.000	-23.9730	-15.6480
		250 µg/ml	-9.55050*	1.40766	.000	-13.7130	-5.3880
		300 µg/ml	-7.49720*	1.40766	.000	-11.6597	-3.3347
	control	blank	40.16310*	1.40766	.000	36.0006	44.3256
		100 µg/ml	4.48650*	1.40766	.025	.3240	8.6490
		150 µg/ml	10.98450*	1.40766	.000	6.8220	15.1470
		200 µg/ml	20.35260*	1.40766	.000	16.1901	24.5151
		250 µg/ml	30.61260*	1.40766	.000	26.4501	34.7751
		300 µg/ml	32.66590*	1.40766	.000	28.5034	36.8284
	100 µg/ml	blank	35.67660*	1.40766	.000	31.5141	39.8391
		control	-4.48650*	1.40766	.025	-8.6490	-.3240
		150 µg/ml	6.49800*	1.40766	.000	2.3355	10.6605
		200 µg/ml	15.86610*	1.40766	.000	11.7036	20.0286
		250 µg/ml	26.12610*	1.40766	.000	21.9636	30.2886
		300 µg/ml	28.17940*	1.40766	.000	24.0169	32.3419
	150 µg/ml	blank	29.17860*	1.40766	.000	25.0161	33.3411
		control	-10.98450*	1.40766	.000	-15.1470	-6.8220
		100 µg/ml	-6.49800*	1.40766	.000	-10.6605	-2.3355
		200 µg/ml	9.36810*	1.40766	.000	5.2056	13.5306
		250 µg/ml	19.62810*	1.40766	.000	15.4656	23.7906

Table C.26, continued: Multiple comparisons: Percentage of tail DNA in the assessment of DNA repair activity (between different extracts).

		300 µg/ml	21.68140*	1.40766	.000	17.5189	25.8439
	200 µg/ml	blank	19.81050*	1.40766	.000	15.6480	23.9730
		control	-20.35260*	1.40766	.000	-24.5151	-16.1901
		100 µg/ml	-15.86610*	1.40766	.000	-20.0286	-11.7036
		150 µg/ml	-9.36810*	1.40766	.000	-13.5306	-5.2056
		250 µg/ml	10.26000*	1.40766	.000	6.0975	14.4225
		300 µg/ml	12.31330*	1.40766	.000	8.1508	16.4758
	250 µg/ml	blank	9.55050*	1.40766	.000	5.3880	13.7130
		control	-30.61260*	1.40766	.000	-34.7751	-26.4501
		100 µg/ml	-26.12610*	1.40766	.000	-30.2886	-21.9636
		150 µg/ml	-19.62810*	1.40766	.000	-23.7906	-15.4656
		200 µg/ml	-10.26000*	1.40766	.000	-14.4225	-6.0975
		300 µg/ml	2.05330	1.40766	.769	-2.1092	6.2158
	300 µg/ml	blank	7.49720*	1.40766	.000	3.3347	11.6597
		control	-32.66590*	1.40766	.000	-36.8284	-28.5034
		100 µg/ml	-28.17940*	1.40766	.000	-32.3419	-24.0169
		150 µg/ml	-21.68140*	1.40766	.000	-25.8439	-17.5189
		200 µg/ml	-12.31330*	1.40766	.000	-16.4758	-8.1508
		250 µg/ml	-2.05330	1.40766	.769	-6.2158	2.1092
Fermented wheat grains	blank	control	-40.16310*	1.70936	.000	-45.2177	-35.1085
		100 µg/ml	-34.77770*	1.70936	.000	-39.8323	-29.7231
		150 µg/ml	-30.77230*	1.70936	.000	-35.8269	-25.7177
		200 µg/ml	-20.71880*	1.70936	.000	-25.7734	-15.6642
		250 µg/ml	-9.79390*	1.70936	.000	-14.8485	-4.7393
		300 µg/ml	-8.51500*	1.70936	.000	-13.5696	-3.4604
	control	blank	40.16310*	1.70936	.000	35.1085	45.2177
		100 µg/ml	5.38540*	1.70936	.028	.3308	10.4400
		150 µg/ml	9.39080*	1.70936	.000	4.3362	14.4454
		200 µg/ml	19.44430*	1.70936	.000	14.3897	24.4989
		250 µg/ml	30.36920*	1.70936	.000	25.3146	35.4238
		300 µg/ml	31.64810*	1.70936	.000	26.5935	36.7027
	100 µg/ml	blank	34.77770*	1.70936	.000	29.7231	39.8323
		control	-5.38540*	1.70936	.028	-10.4400	-.3308
		150 µg/ml	4.00540	1.70936	.225	-1.0492	9.0600
		200 µg/ml	14.05890*	1.70936	.000	9.0043	19.1135
		250 µg/ml	24.98380*	1.70936	.000	19.9292	30.0384
		300 µg/ml	26.26270*	1.70936	.000	21.2081	31.3173
	150 µg/ml	blank	30.77230*	1.70936	.000	25.7177	35.8269
		control	-9.39080*	1.70936	.000	-14.4454	-4.3362
		100 µg/ml	-4.00540	1.70936	.225	-9.0600	1.0492
		200 µg/ml	10.05350*	1.70936	.000	4.9989	15.1081
		250 µg/ml	20.97840*	1.70936	.000	15.9238	26.0330
		300 µg/ml	22.25730*	1.70936	.000	17.2027	27.3119
	200 µg/ml	blank	20.71880*	1.70936	.000	15.6642	25.7734
		control	-19.44430*	1.70936	.000	-24.4989	-14.3897
		100 µg/ml	-14.05890*	1.70936	.000	-19.1135	-9.0043
		150 µg/ml	-10.05350*	1.70936	.000	-15.1081	-4.9989
		250 µg/ml	10.92490*	1.70936	.000	5.8703	15.9795
		300 µg/ml	12.20380*	1.70936	.000	7.1492	17.2584
	250 µg/ml	blank	9.79390*	1.70936	.000	4.7393	14.8485
		control	-30.36920*	1.70936	.000	-35.4238	-25.3146

Table C.26, continued: Multiple comparisons: Percentage of tail DNA in the assessment of DNA repair activity (between different extracts).

		100 µg/ml	-24.98380*	1.70936	.000	-30.0384	-19.9292
		150 µg/ml	-20.97840*	1.70936	.000	-26.0330	-15.9238
		200 µg/ml	-10.92490*	1.70936	.000	-15.9795	-5.8703
		300 µg/ml	1.27890	1.70936	.989	-3.7757	6.3335
	300 µg/ml	blank	8.51500*	1.70936	.000	3.4604	13.5696
		control	-31.64810*	1.70936	.000	-36.7027	-26.5935
		100 µg/ml	-26.26270*	1.70936	.000	-31.3173	-21.2081
		150 µg/ml	-22.25730*	1.70936	.000	-27.3119	-17.2027
		200 µg/ml	-12.20380*	1.70936	.000	-17.2584	-7.1492
		250 µg/ml	-1.27890	1.70936	.989	-6.3335	3.7757
Unfermented wheat grains	blank	control	-40.16310*	1.62490	.000	-44.9680	-35.3582
		100 µg/ml	-34.84140*	1.62490	.000	-39.6463	-30.0365
		150 µg/ml	-32.19630*	1.62490	.000	-37.0012	-27.3914
		200 µg/ml	-22.21940*	1.62490	.000	-27.0243	-17.4145
		250 µg/ml	-10.79060*	1.62490	.000	-15.5955	-5.9857
		300 µg/ml	-8.03430*	1.62490	.000	-12.8392	-3.2294
	control	blank	40.16310*	1.62490	.000	35.3582	44.9680
		100 µg/ml	5.32170*	1.62490	.019	.5168	10.1266
		150 µg/ml	7.96680*	1.62490	.000	3.1619	12.7717
		200 µg/ml	17.94370*	1.62490	.000	13.1388	22.7486
		250 µg/ml	29.37250*	1.62490	.000	24.5676	34.1774
		300 µg/ml	32.12880*	1.62490	.000	27.3239	36.9337
	100 µg/ml	blank	34.84140*	1.62490	.000	30.0365	39.6463
		control	-5.32170*	1.62490	.019	-10.1266	-.5168
		150 µg/ml	2.64510	1.62490	.664	-2.1598	7.4500
		200 µg/ml	12.62200*	1.62490	.000	7.8171	17.4269
		250 µg/ml	24.05080*	1.62490	.000	19.2459	28.8557
		300 µg/ml	26.80710*	1.62490	.000	22.0022	31.6120
	150 µg/ml	blank	32.19630*	1.62490	.000	27.3914	37.0012
		control	-7.96680*	1.62490	.000	-12.7717	-3.1619
		100 µg/ml	-2.64510	1.62490	.664	-7.4500	2.1598
		200 µg/ml	9.97690*	1.62490	.000	5.1720	14.7818
		250 µg/ml	21.40570*	1.62490	.000	16.6008	26.2106
		300 µg/ml	24.16200*	1.62490	.000	19.3571	28.9669
	200 µg/ml	blank	22.21940*	1.62490	.000	17.4145	27.0243
		control	-17.94370*	1.62490	.000	-22.7486	-13.1388
		100 µg/ml	-12.62200*	1.62490	.000	-17.4269	-7.8171
		150 µg/ml	-9.97690*	1.62490	.000	-14.7818	-5.1720
		250 µg/ml	11.42880*	1.62490	.000	6.6239	16.2337
		300 µg/ml	14.18510*	1.62490	.000	9.3802	18.9900
	250 µg/ml	blank	10.79060*	1.62490	.000	5.9857	15.5955
		control	-29.37250*	1.62490	.000	-34.1774	-24.5676
		100 µg/ml	-24.05080*	1.62490	.000	-28.8557	-19.2459
		150 µg/ml	-21.40570*	1.62490	.000	-26.2106	-16.6008
		200 µg/ml	-11.42880*	1.62490	.000	-16.2337	-6.6239
		300 µg/ml	2.75630	1.62490	.619	-2.0486	7.5612
	300 µg/ml	blank	8.03430*	1.62490	.000	3.2294	12.8392
		control	-32.12880*	1.62490	.000	-36.9337	-27.3239
		100 µg/ml	-26.80710*	1.62490	.000	-31.6120	-22.0022
		150 µg/ml	-24.16200*	1.62490	.000	-28.9669	-19.3571
		200 µg/ml	-14.18510*	1.62490	.000	-18.9900	-9.3802

	250 µg/ml	-2.75630	1.62490	.619	-7.5612	2.0486

Table C.27: Multiple range tests: Percentage of tail DNA in the assessment of DNA repair activity (between different extracts).

Fruiting bodies			Subset for alpha = 0.05					
Tukey HSD[a]	Group	N	1	2	3	4	5	6
	blank	100	6.5990					
	300 µg/ml	100		14.0962				
	250 µg/ml	100		16.1495				
	200 µg/ml	100			26.4095			
	150 µg/ml	100				35.7776		
	100 µg/ml	100					42.2756	
	control	100						46.7621
	Sig.		1.000	.769	1.000	1.000	1.000	1.000

Fermented wheat grains			Subset for alpha = 0.05				
	Group	N	1	2	3	4	5
	blank	100	6.5990				
Tukey HSD[a]	300 µg/ml	100		15.1140			
	250 µg/ml	100		16.3929			
	200 µg/ml	100			27.3178		
	150 µg/ml	100				37.3713	
	100 µg/ml	100				41.3767	
	control	100					46.7621
	Sig.		1.000	.989	1.000	.225	1.000

Unfermented wheat grains			Subset for alpha = 0.05				
	Group	N	1	2	3	4	5
	blank	100	6.5990				
Tukey HSD[a]	300 µg/ml	100		14.6333			
	250 µg/ml	100		17.3896			
	200 µg/ml	100			28.8184		
	150 µg/ml	100				38.7953	
	100 µg/ml	100				41.4404	
	control	100					46.7621
	Sig.		1.000	.619	1.000	.664	1.000

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 100.000.

Table C.28: One way analysis of variance (ANOVA): Acute toxicity assay- Haematology test for female rats (between different groups)

		Sum of Squares	df	Mean Square	F	Sig.
HGB	Between Groups	77.778	2	38.889	1.936	.179
	Within Groups	301.333	15	20.089		
	Total	379.111	17			
HCT	Between Groups	.000	2	.000	.046	.955
	Within Groups	.002	15	.000		
	Total	.002	17			
RBC	Between Groups	.368	2	.184	1.368	.285
	Within Groups	2.015	15	.134		
	Total	2.383	17			
MCV	Between Groups	19.000	2	9.500	2.767	.095
	Within Groups	51.500	15	3.433		
	Total	70.500	17			
MCH	Between Groups	3.903	2	1.952	3.001	.080
	Within Groups	9.757	15	.650		
	Total	13.660	17			
MCHC	Between Groups	418.778	2	209.389	1.737	.210
	Within Groups	1808.333	15	120.556		

	Total	2227.111	17			
RDW	Between Groups	7.521	2	3.761	2.779	.094

Table C.28, continued: One way analysis of variance (ANOVA): Acute toxicity assay- Haematology test for female rats (between different groups)

	Total	27.816	17			
WBC	Between Groups	4.301	2	2.151	1.457	.264
	Within Groups	22.143	15	1.476		
	Total	26.444	17			
Platelet	Between Groups	29640.333	2	14820.167	2.255	.139
	Within Groups	98566.167	15	6571.078		
	Total	128206.500	17			

Table C.29: Multiple comparisons: Acute toxicity assay- Haematology test for female rats (between different groups)

				Std.		95% Confidence Interval	
Dependent Variable	(I) Group	(J) Group	Mean Difference (I-J)	Error	Sig.	Lower Bound	Upper Bound
HGB Tukey HSD	Control	Low dose	5.00000	2.58772	.164	-1.7215	11.7215
		High dose	3.33333	2.58772	.423	-3.3882	10.0549
	Low dose	Control	-5.00000	2.58772	.164	-11.7215	1.7215
		High dose	-1.66667	2.58772	.798	-8.3882	5.0549
	High dose	Control	-3.33333	2.58772	.423	-10.0549	3.3882
		Low dose	1.66667	2.58772	.798	-5.0549	8.3882
HCT Tukey HSD	Control	Low dose	.00000	.00635	1.000	-.0165	.0165
		High dose	.00167	.00635	.963	-.0148	.0182
	Low dose	Control	.00000	.00635	1.000	-.0165	.0165
		High dose	.00167	.00635	.963	-.0148	.0182
	High dose	Control	-.00167	.00635	.963	-.0182	.0148
		Low dose	-.00167	.00635	.963	-.0182	.0148
RBC Tukey HSD	Control	Low dose	-.17000	.21163	.707	-.7197	.3797
		High dose	-.35000	.21163	.255	-.8997	.1997
	Low dose	Control	.17000	.21163	.707	-.3797	.7197
		High dose	-.18000	.21163	.678	-.7297	.3697
	High dose	Control	.35000	.21163	.255	-.1997	.8997
		Low dose	.18000	.21163	.678	-.3697	.7297
MCV Tukey HSD	Control	Low dose	1.00000	1.06979	.627	-1.7787	3.7787
		High dose	2.50000	1.06979	.081	-.2787	5.2787
	Low dose	Control	-1.00000	1.06979	.627	-3.7787	1.7787
		High dose	1.50000	1.06979	.365	-1.2787	4.2787
	High dose	Control	-2.50000	1.06979	.081	-5.2787	.2787
		Low dose	-1.50000	1.06979	.365	-4.2787	1.2787
MCH Tukey HSD	Control	Low dose	.88333	.46563	.174	-.3261	2.0928
		High dose	1.06667	.46563	.088	-.1428	2.2761
	Low dose	Control	-.88333	.46563	.174	-2.0928	.3261
		High dose	.18333	.46563	.919	-1.0261	1.3928
	High dose	Control	-1.06667	.46563	.088	-2.2761	.1428
		Low dose	-.18333	.46563	.919	-1.3928	1.0261
MCHC Tukey HSD	Control	Low dose	10.83333	6.33918	.234	-5.6325	27.2992
		High dose	9.50000	6.33918	.319	-6.9658	25.9658
	Low dose	Control	-10.83333	6.33918	.234	-27.2992	5.6325
		High dose	-1.33333	6.33918	.976	-17.7992	15.1325
	High dose	Control	-9.50000	6.33918	.319	-25.9658	6.9658
		Low dose	1.33333	6.33918	.976	-15.1325	17.7992
RDW Tukey HSD	Control	Low dose	-1.25000	.67157	.184	-2.9944	.4944
		High dose	-1.46667	.67157	.107	-3.2110	.2777
	Low dose	Control	1.25000	.67157	.184	-.4944	2.9944

		High dose	-.21667	.67157	.944	-1.9610	1.5277
	High dose	Control	1.46667	.67157	.107	-.2777	3.2110

Table C.29, continued: Multiple comparisons: Acute toxicity assay- Haematology test for female rats (between different groups)

			Low dose	.21667	.67157	.944	-1.5277	1.9610
WBC	Tukey HSD	Control	Low dose	-.43333	.70148	.813	-2.2554	1.3887
			High dose	-1.18333	.70148	.242	-3.0054	.6387
		Low dose	Control	.43333	.70148	.813	-1.3887	2.2554
			High dose	-.75000	.70148	.547	-2.5721	1.0721
		High dose	Control	1.18333	.70148	.242	-.6387	3.0054
			Low dose	.75000	.70148	.547	-1.0721	2.5721
Platelet	Tukey HSD	Control	Low dose	32.83333	46.80127	.766	-88.7316	154.3983
			High dose	-64.83333	46.80127	.373	-186.3983	56.7316
		Low dose	Control	-32.83333	46.80127	.766	-154.3983	88.7316
			High dose	-97.66667	46.80127	.126	-219.2316	23.8983
		High dose	Control	64.83333	46.80127	.373	-56.7316	186.3983
			Low dose	97.66667	46.80127	.126	-23.8983	219.2316

*. The mean difference is significant at the 0.05 level.

Table C.30: Multiple range tests: Acute toxicity assay- Haematology test for female rats (between different groups)

HGB			Subset for alpha = 0.05
	Group	N	1
Tukey HSD[a]	Low dose	6	149.0000
	High dose	6	150.6667
	Control	6	154.0000
	Sig.		.164
HCT			Subset for alpha = 0.05
	Group	N	1
Tukey HSD[a]	High dose	6	.4550
	Control	6	.4567
	Low dose	6	.4567
	Sig.		.963
RBC			Subset for alpha = 0.05
	Group	N	1
Tukey HSD[a]	Control	6	8.2267
	Low dose	6	8.3967
	High dose	6	8.5767
	Sig.		.255
MCV			Subset for alpha = 0.05
	Group	N	1
Tukey HSD[a]	High dose	6	53.8333
	Low dose	6	55.3333
	Control	6	56.3333
	Sig.		.081
MCH			Subset for alpha = 0.05
	Group	N	1
Tukey HSD[a]	High dose	6	17.4833
	Low dose	6	17.6667
	Control	6	18.5500
	Sig.		.088
MCHC			Subset for alpha = 0.05
	Group	N	1

Tukey HSD[a]	Low dose	6	329.1667
	High dose	6	330.5000
	Control	6	340.0000

Table C.30, continued: Multiple range tests: Acute toxicity assay- Haematology test for female rats (between different groups)

	Sig.		.234
RDW			Subset for alpha = 0.05
	Group	N	1
Tukey HSD[a]	Control	6	15.6667
	Low dose	6	16.9167
	High dose	6	17.1333
	Sig.		.107
WBC			Subset for alpha = 0.05
	Group	N	1
Tukey HSD[a]	Control	6	5.6167
	Low dose	6	6.0500
	High dose	6	6.8000
	Sig.		.242
Platelet			Subset for alpha = 0.05
	Group	N	1
Tukey HSD[a]	Low dose	6	960.6667
	Control	6	993.5000
	High dose	6	1058.3333
	Sig.		.126

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 6.000.

Table C.31: One way analysis of variance (ANOVA): Acute toxicity assay- Liver function test for female rats (between different groups)

		Sum of Squares	df	Mean Square	F	Sig.
ALP	Between Groups	745.444	2	372.722	.351	.709
	Within Groups	15911.000	15	1060.733		
	Total	16656.444	17			
ALT	Between Groups	389.333	2	194.667	1.071	.368
	Within Groups	2727.167	15	181.811		
	Total	3116.500	17			
AST	Between Groups	6561.333	2	3280.667	2.493	.116
	Within Groups	19740.667	15	1316.044		
	Total	26302.000	17			
GGT	Between Groups	2.778	2	1.389	3.788	.047
	Within Groups	5.500	15	.367		
	Total	8.278	17			
Total bilirudin	Between Groups	2.778	2	1.389	3.788	.047
	Within Groups	5.500	15	.367		
	Total	8.278	17			
Total protein	Between Groups	4.333	2	2.167	.229	.798
	Within Groups	142.167	15	9.478		
	Total	146.500	17			
Albumin	Between Groups	8.778	2	4.389	2.948	.083
	Within Groups	22.333	15	1.489		
	Total	31.111	17			

Table C.32: Multiple comparisons: Acute toxicity assay- Liver function test for female rats (between different groups)

Dependent Variable		(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
							Lower Bound	Upper Bound
ALP	Tukey HSD	Control	Low dose	10.83333	18.80366	.835	-38.0086	59.6753
			High dose	15.33333	18.80366	.699	-33.5086	64.1753
		Low dose	Control	-10.83333	18.80366	.835	-59.6753	38.0086
			High dose	4.50000	18.80366	.969	-44.3420	53.3420
		High dose	Control	-15.33333	18.80366	.699	-64.1753	33.5086
			Low dose	-4.50000	18.80366	.969	-53.3420	44.3420
ALT	Tukey HSD	Control	Low dose	-11.33333	7.78484	.339	-31.5542	8.8876
			High dose	-6.66667	7.78484	.675	-26.8876	13.5542
		Low dose	Control	11.33333	7.78484	.339	-8.8876	31.5542
			High dose	4.66667	7.78484	.823	-15.5542	24.8876
		High dose	Control	6.66667	7.78484	.675	-13.5542	26.8876
			Low dose	-4.66667	7.78484	.823	-24.8876	15.5542
AST	Tukey HSD	Control	Low dose	-43.66667	20.94472	.127	-98.0700	10.7366
			High dose	-7.33333	20.94472	.935	-61.7366	47.0700
		Low dose	Control	43.66667	20.94472	.127	-10.7366	98.0700
			High dose	36.33333	20.94472	.225	-18.0700	90.7366
		High dose	Control	7.33333	20.94472	.935	-47.0700	61.7366
			Low dose	-36.33333	20.94472	.225	-90.7366	18.0700
GGT	Tukey HSD	Control	Low dose	.00000	.34960	1.000	-.9081	.9081
			High dose	-.83333	.34960	.074	-1.7414	.0747
		Low dose	Control	.00000	.34960	1.000	-.9081	.9081
			High dose	-.83333	.34960	.074	-1.7414	.0747
		High dose	Control	.83333	.34960	.074	-.0747	1.7414
			Low dose	.83333	.34960	.074	-.0747	1.7414
Total bilirudin	Tukey HSD	Control	Low dose	-.83333	.34960	.074	-1.7414	.0747
			High dose	.00000	.34960	1.000	-.9081	.9081
		Low dose	Control	.83333	.34960	.074	-.0747	1.7414
			High dose	.83333	.34960	.074	-.0747	1.7414
		High dose	Control	.00000	.34960	1.000	-.9081	.9081
			Low dose	-.83333	.34960	.074	-1.7414	.0747
Total protein	Tukey HSD	Control	Low dose	-.33333	1.77743	.981	-4.9502	4.2835
			High dose	-1.16667	1.77743	.792	-5.7835	3.4502
		Low dose	Control	.33333	1.77743	.981	-4.2835	4.9502
			High dose	-.83333	1.77743	.887	-5.4502	3.7835
		High dose	Control	1.16667	1.77743	.792	-3.4502	5.7835
			Low dose	.83333	1.77743	.887	-3.7835	5.4502
Albumin	Tukey HSD	Control	Low dose	-1.16667	.70448	.254	-2.9965	.6632
			High dose	.50000	.70448	.762	-1.3299	2.3299
		Low dose	Control	1.16667	.70448	.254	-.6632	2.9965
			High dose	1.66667	.70448	.077	-.1632	3.4965
		High dose	Control	-.50000	.70448	.762	-2.3299	1.3299
			Low dose	-1.66667	.70448	.077	-3.4965	.1632

*. The mean difference is significant at the 0.05 level.

Table C.33: Multiple range tests: Acute toxicity assay- Liver function test for female rats (between different groups)

ALP			Subset for alpha = 0.05
	Group	N	1
Tukey HSD[a]	High dose	6	115.8333
	Low dose	6	120.3333
	Control	6	131.1667
	Sig.		.699
ALT			Subset for alpha = 0.05
	Group	N	1
Tukey HSD[a]	Control	6	43.1667
	High dose	6	49.8333
	Low dose	6	54.5000
	Sig.		.339
AST			Subset for alpha = 0.05
	Group	N	1
Tukey HSD[a]	Control	6	219.3333
	High dose	6	226.6667
	Low dose	6	263.0000
	Sig.		.127
GGT			Subset for alpha = 0.05
Tukey HSD[a]	Group	N	1
	Control	6	2.3333
	Low dose	6	2.3333
	High dose	6	3.1667
	Sig.		.074
Total bilirudin			Subset for alpha = 0.05
	Group	N	1
Tukey HSD[a]	Control	6	1.3333
	High dose	6	1.3333
	Low dose	6	2.1667
	Sig.		.074
Total protein			Subset for alpha = 0.05
	Group	N	1
Tukey HSD[a]	Control	6	70.0000
	Low dose	6	70.3333
	High dose	6	71.1667
	Sig.		.792
Albumin			Subset for alpha = 0.05
	Group	N	1
Tukey HSD[a]	High dose	6	11.5000
	Control	6	12.0000
	Low dose	6	13.1667
	Sig.		.077

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 6.000.

Table C.34: One way analysis of variance (ANOVA): Acute toxicity assay- Renal function test for female rats (between different groups)

		Sum of Squares	df	Mean Square	F	Sig.
Sodium	Between Groups	34.111	2	17.056	1.936	.179
	Within Groups	132.167	15	8.811		
	Total	166.278	17			
Potassium	Between Groups	1.028	2	.514	2.060	.162
	Within Groups	3.742	15	.249		
	Total	4.769	17			
Chloride	Between Groups	21.444	2	10.722	2.580	.109
	Within Groups	62.333	15	4.156		
	Total	83.778	17			
Carbon dioxide	Between Groups	3.341	2	1.671	2.767	.095
	Within Groups	9.055	15	.604		
	Total	12.396	17			
Anion gap	Between Groups	11.111	2	5.556	2.283	.136
	Within Groups	36.500	15	2.433		
	Total	47.611	17			
Urea	Between Groups	9.943	2	4.972	1.581	.238
	Within Groups	47.177	15	3.145		
	Total	57.120	17			
Creatinine	Between Groups	163.000	2	81.500	2.638	.104
	Within Groups	463.500	15	30.900		
	Total	626.500	17			

Table C.35: Multiple comparisons: Acute toxicity assay- Renal function test for female rats (between different groups)

Dependent Variable		(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
							Lower Bound	Upper Bound
Sodium	Tukey HSD	Control	Low dose	2.83333	1.71378	.255	-1.6182	7.2848
			High dose	3.00000	1.71378	.220	-1.4515	7.4515
		Low dose	Control	-2.83333	1.71378	.255	-7.2848	1.6182
			High dose	.16667	1.71378	.995	-4.2848	4.6182
		High dose	Control	-3.00000	1.71378	.220	-7.4515	1.4515
			Low dose	-.16667	1.71378	.995	-4.6182	4.2848
Potassium	Tukey HSD	Control	Low dose	-.58333	.28835	.141	-1.3323	.1657
			High dose	-.33333	.28835	.496	-1.0823	.4157
		Low dose	Control	.58333	.28835	.141	-.1657	1.3323
			High dose	.25000	.28835	.669	-.4990	.9990
		High dose	Control	.33333	.28835	.496	-.4157	1.0823
			Low dose	-.25000	.28835	.669	-.9990	.4990
Chloride	Tukey HSD	Control	Low dose	2.66667	1.17694	.092	-.3904	5.7237
			High dose	1.50000	1.17694	.430	-1.5571	4.5571
		Low dose	Control	-2.66667	1.17694	.092	-5.7237	.3904
			High dose	-1.16667	1.17694	.593	-4.2237	1.8904
		High dose	Control	-1.50000	1.17694	.430	-4.5571	1.5571
			Low dose	1.16667	1.17694	.593	-1.8904	4.2237
Carbon dioxide	Tukey HSD	Control	Low dose	.61667	.44858	.378	-.5485	1.7818
			High dose	1.05000	.44858	.081	-.1152	2.2152
		Low dose	Control	-.61667	.44858	.378	-1.7818	.5485
			High dose	.43333	.44858	.609	-.7318	1.5985
		High dose	Control	-1.05000	.44858	.081	-2.2152	.1152
			Low dose	-.43333	.44858	.609	-1.5985	.7318
Anion gap	Tukey HSD	Control	Low dose	-1.66667	.90062	.187	-4.0060	.6727

			High dose	-1.66667	.90062	.187	-4.0060	.6727

Table C.35, continued: Multiple comparisons: Acute toxicity assay- Renal function test for female rats (between different groups)

		Low dose	Control	1.66667	.90062	.187	-.6727	4.0060
			High dose	.00000	.90062	1.000	-2.3393	2.3393
		High dose	Control	1.66667	.90062	.187	-.6727	4.0060
			Low dose	.00000	.90062	1.000	-2.3393	2.3393
Urea	Tukey HSD	Control	Low dose	-1.61667	1.02390	.285	-4.2762	1.0429
			High dose	-1.53333	1.02390	.320	-4.1929	1.1262
		Low dose	Control	1.61667	1.02390	.285	-1.0429	4.2762
			High dose	.08333	1.02390	.996	-2.5762	2.7429
		High dose	Control	1.53333	1.02390	.320	-1.1262	4.1929
			Low dose	-.08333	1.02390	.996	-2.7429	2.5762
Creatinine	Tukey HSD	Control	Low dose	1.50000	3.20936	.887	-6.8362	9.8362
			High dose	-5.50000	3.20936	.233	-13.8362	2.8362
		Low dose	Control	-1.50000	3.20936	.887	-9.8362	6.8362
			High dose	-7.00000	3.20936	.107	-15.3362	1.3362
		High dose	Control	5.50000	3.20936	.233	-2.8362	13.8362
			Low dose	7.00000	3.20936	.107	-1.3362	15.3362

*. The mean difference is significant at the 0.05 level.

Table C.36: Multiple range tests: Acute toxicity assay- Renal function test for female rats (between different groups)

Sodium			Subset for alpha = 0.05
	Group	N	1
Tukey HSD[a]	High dose	6	138.3333
	Low dose	6	138.5000
	Control	6	141.3333
	Sig.		.220
Potassium			Subset for alpha = 0.05
	Group	N	1
Tukey HSD[a]	Control	6	4.3000
	High dose	6	4.6333
	Low dose	6	4.8833
	Sig.		.141
Chloride			Subset for alpha = 0.05
	Group	N	1
Tukey HSD[a]	Low dose	6	101.8333
	High dose	6	103.0000
	Control	6	104.5000
	Sig.		.092
Carbon dioxide			Subset for alpha = 0.05
	Group	N	1
Tukey HSD[a]	High dose	6	18.5333
	Low dose	6	18.9667
	Control	6	19.5833
	Sig.		.081
Anion gap			Subset for alpha = 0.05
	Group	N	1
Tukey HSD[a]	Control	6	21.1667
	Low dose	6	22.8333
	High dose	6	22.8333
	Sig.		.187
Urea	Group	N	Subset for alpha = 0.05

			1
Tukey HSD[a]	Control	6	5.9167

Table C.36, continued: Multiple range tests: Acute toxicity assay- Renal function test for female rats (between different groups)

	High dose	6	7.4500
	Low dose	6	7.5333
	Sig.		.285
Creatinine			Subset for alpha = 0.05
	Group	N	1
Tukey HSD[a]	Low dose	6	26.0000
	Control	6	27.5000
	High dose	6	33.0000
	Sig.		.107

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 6.000.

Table C.37: One way analysis of variance (ANOVA): Acute toxicity assay- Haematology test for male rats (between different groups)

		Sum of Squares	df	Mean Square	F	Sig.
HGB	Between Groups	178.111	2	89.056	3.879	.044
	Within Groups	344.333	15	22.956		
	Total	522.444	17			
HCT	Between Groups	.002	2	.001	3.478	.057
	Within Groups	.003	15	.000		
	Total	.005	17			
RBC	Between Groups	.203	2	.101	1.349	.289
	Within Groups	1.128	15	.075		
	Total	1.331	17			
MCV	Between Groups	1.444	2	.722	.328	.725
	Within Groups	33.000	15	2.200		
	Total	34.444	17			
MCH	Between Groups	.223	2	.112	.476	.631
	Within Groups	3.522	15	.235		
	Total	3.745	17			
MCHC	Between Groups	28.000	2	14.000	.249	.783
	Within Groups	842.500	15	56.167		
	Total	870.500	17			
RDW	Between Groups	2.634	2	1.317	1.975	.173
	Within Groups	10.003	15	.667		
	Total	12.638	17			
WBC	Between Groups	.031	2	.016	.003	.997
	Within Groups	87.727	15	5.848		
	Total	87.758	17			
Platelet	Between Groups	23500.444	2	11750.222	.637	.542
	Within Groups	276521.833	15	18434.789		
	Total	300022.278	17			

Table C.38: Multiple comparisons: Acute toxicity assay- Haematology test for male rats (between different groups)

Dependent Variable		(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
							Lower Bound	Upper Bound
HGB	Tukey HSD	Control	Low dose	6.83333	2.76620	.063	-.3518	14.0185
			High dose	6.50000	2.76620	.079	-.6851	13.6851
		Low dose	Control	-6.83333	2.76620	.063	-14.0185	.3518
			High dose	-.33333	2.76620	.992	-7.5185	6.8518
		High dose	Control	-6.50000	2.76620	.079	-13.6851	.6851
			Low dose	.33333	2.76620	.992	-6.8518	7.5185
HCT	Tukey HSD	Control	Low dose	.02000	.00876	.089	-.0027	.0427
			High dose	.02000	.00876	.089	-.0027	.0427
		Low dose	Control	-.02000	.00876	.089	-.0427	.0027
			High dose	.00000	.00876	1.000	-.0227	.0227
		High dose	Control	-.02000	.00876	.089	-.0427	.0027
			Low dose	.00000	.00876	1.000	-.0227	.0227
RBC	Tukey HSD	Control	Low dose	.25500	.15831	.272	-.1562	.6662
			High dose	.17167	.15831	.538	-.2395	.5829
		Low dose	Control	-.25500	.15831	.272	-.6662	.1562
			High dose	-.08333	.15831	.860	-.4945	.3279
		High dose	Control	-.17167	.15831	.538	-.5829	.2395
			Low dose	.08333	.15831	.860	-.3279	.4945
MCV	Tukey HSD	Control	Low dose	-.66667	.85635	.721	-2.8910	1.5577
			High dose	-.16667	.85635	.979	-2.3910	2.0577
		Low dose	Control	.66667	.85635	.721	-1.5577	2.8910
			High dose	.50000	.85635	.831	-1.7243	2.7243
		High dose	Control	.16667	.85635	.979	-2.0577	2.3910
			Low dose	-.50000	.85635	.831	-2.7243	1.7243
MCH	Tukey HSD	Control	Low dose	.26667	.27975	.616	-.4600	.9933
			High dose	.08333	.27975	.952	-.6433	.8100
		Low dose	Control	-.26667	.27975	.616	-.9933	.4600
			High dose	-.18333	.27975	.792	-.9100	.5433
		High dose	Control	-.08333	.27975	.952	-.8100	.6433
			Low dose	.18333	.27975	.792	-.5433	.9100
MCHC	Tukey HSD	Control	Low dose	2.00000	4.32692	.890	-9.2390	13.2390
			High dose	-1.00000	4.32692	.971	-12.2390	10.2390
		Low dose	Control	-2.00000	4.32692	.890	-13.2390	9.2390
			High dose	-3.00000	4.32692	.771	-14.2390	8.2390
		High dose	Control	1.00000	4.32692	.971	-10.2390	12.2390
			Low dose	3.00000	4.32692	.771	-8.2390	14.2390
RDW	Tukey HSD	Control	Low dose	-.08333	.47148	.983	-1.3080	1.1413
			High dose	-.85000	.47148	.202	-2.0747	.3747
		Low dose	Control	.08333	.47148	.983	-1.1413	1.3080
			High dose	-.76667	.47148	.266	-1.9913	.4580
		High dose	Control	.85000	.47148	.202	-.3747	2.0747
			Low dose	.76667	.47148	.266	-.4580	1.9913
WBC	Tukey HSD	Control	Low dose	.06667	1.39624	.999	-3.5600	3.6934
			High dose	.10000	1.39624	.997	-3.5267	3.7267
		Low dose	Control	-.06667	1.39624	.999	-3.6934	3.5600
			High dose	.03333	1.39624	1.000	-3.5934	3.6600
		High dose	Control	-.10000	1.39624	.997	-3.7267	3.5267
			Low dose	-.03333	1.39624	1.000	-3.6600	3.5934
Platelet	Tukey HSD	Control	Low dose	-84.66667	78.38960	.540	-288.2813	118.9480
			High dose	-64.66667	78.38960	.694	-268.2813	138.9480
		Low dose	Control	84.66667	78.38960	.540	-118.9480	288.2813

Table C.38, continued: Multiple comparisons: Acute toxicity assay- Haematology test for male rats (between different groups)

		High dose	20.00000	78.38960	.965	-183.6147	223.6147
	High dose	Control	64.66667	78.38960	.694	-138.9480	268.2813
		Low dose	-20.00000	78.38960	.965	-223.6147	183.6147

*. The mean difference is significant at the 0.05 level.

Table C.39: Multiple range tests: Acute toxicity assay- Haematology test for male rats (between different groups)

HGB			Subset for alpha = 0.05
	Group	N	1
Tukey HSD[a]	Low dose	6	151.1667
	High dose	6	151.5000
	Control	6	158.0000
	Sig.		.063
HCT			Subset for alpha = 0.05
	Group	N	1
Tukey HSD[a]	Low dose	6	.4450
	High dose	6	.4450
	Control	6	.4650
	Sig.		.089
RBC			Subset for alpha = 0.05
	Group	N	1
Tukey HSD[a]	Low dose	6	7.8417
	High dose	6	7.9250
	Control	6	8.0967
	Sig.		.272
MCV			Subset for alpha = 0.05
	Group	N	1
Tukey HSD[a]	Control	6	56.1667
	High dose	6	56.3333
	Low dose	6	56.8333
	Sig.		.721
MCH			Subset for alpha = 0.05
	Group	N	1
Tukey HSD[a]	Low dose	6	18.7000
	High dose	6	18.8833
	Control	6	18.9667
	Sig.		.616
MCHC			Subset for alpha = 0.05
	Group	N	1
Tukey HSD[a]	Low dose	6	332.5000
	Control	6	334.5000
	High dose	6	335.5000
	Sig.		.771
RDW			Subset for alpha = 0.05
	Group	N	1
Tukey HSD[a]	Control	6	16.7000
	Low dose	6	16.7833
	High dose	6	17.5500
	Sig.		.202
WBC			Subset for alpha = 0.05
	Group	N	1
Tukey HSD[a]	High dose	6	9.8667

Table C.39, continued: Multiple range tests: Acute toxicity assay- Haematology test for male rats (between different groups)

	Control	6	9.9667
	Sig.		.997
Platelet			Subset for alpha = 0.05
	Group	N	1
Tukey HSD[a]	Control	6	840.8333
	High dose	6	905.5000
	Low dose	6	925.5000
	Sig.		.540

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 6.000.

Table C.40: One way analysis of variance (ANOVA): Acute toxicity assay- Liver function test for male rats (between different groups)

		Sum of Squares	df	Mean Square	F	Sig.
ALP	Between Groups	4496.444	2	2248.222	1.103	.357
	Within Groups	30571.833	15	2038.122		
	Total	35068.278	17			
ALT	Between Groups	68.111	2	34.056	.355	.707
	Within Groups	1438.333	15	95.889		
	Total	1506.444	17			
AST	Between Groups	1704.333	2	852.167	.492	.621
	Within Groups	25962.167	15	1730.811		
	Total	27666.500	17			
GGT	Between Groups	.778	2	.389	1.400	.277
	Within Groups	4.167	15	.278		
	Total	4.944	17			
Total bilirudin	Between Groups	.444	2	.222	.714	.505
	Within Groups	4.667	15	.311		
	Total	5.111	17			
Total protein	Between Groups	2.333	2	1.167	.160	.854
	Within Groups	109.667	15	7.311		
	Total	112.000	17			
Albumin	Between Groups	.778	2	.389	.278	.761
	Within Groups	21.000	15	1.400		
	Total	21.778	17			

Table C.41: Multiple comparisons: Acute toxicity assay- Liver function test for male rats (between different groups)

Dependent Variable		(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
							Lower Bound	Upper Bound
ALP	Tukey HSD	Control	Low dose	-38.66667	26.06481	.326	-106.3692	29.0359
			High dose	-21.00000	26.06481	.705	-88.7026	46.7026
		Low dose	Control	38.66667	26.06481	.326	-29.0359	106.3692
			High dose	17.66667	26.06481	.780	-50.0359	85.3692
		High dose	Control	21.00000	26.06481	.705	-46.7026	88.7026
			Low dose	-17.66667	26.06481	.780	-85.3692	50.0359
ALT	Tukey HSD	Control	Low dose	3.16667	5.65358	.843	-11.5183	17.8517
			High dose	-1.50000	5.65358	.962	-16.1850	13.1850
		Low dose	Control	-3.16667	5.65358	.843	-17.8517	11.5183
			High dose	-4.66667	5.65358	.694	-19.3517	10.0183
		High dose	Control	1.50000	5.65358	.962	-13.1850	16.1850
			Low dose	4.66667	5.65358	.694	-10.0183	19.3517
AST	Tukey HSD	Control	Low dose	-12.16667	24.01951	.869	-74.5566	50.2233
			High dose	-23.83333	24.01951	.593	-86.2233	38.5566

Table C.41, continued: Multiple comparisons: Acute toxicity assay- Liver function test for male rats (between different groups)

		Low dose	Control	12.16667	24.01951	.869	-50.2233	74.5566
			High dose	-11.66667	24.01951	.879	-74.0566	50.7233
		High dose	Control	23.83333	24.01951	.593	-38.5566	86.2233
			Low dose	11.66667	24.01951	.879	-50.7233	74.0566
GGT	Tukey HSD	Control	Low dose	-.33333	.30429	.531	-1.1237	.4571
			High dose	-.50000	.30429	.259	-1.2904	.2904
		Low dose	Control	.33333	.30429	.531	-.4571	1.1237
			High dose	-.16667	.30429	.849	-.9571	.6237
		High dose	Control	.50000	.30429	.259	-.2904	1.2904
			Low dose	.16667	.30429	.849	-.6237	.9571
Total bilirudin	Tukey HSD	Control	Low dose	-.33333	.32203	.567	-1.1698	.5031
			High dose	-.33333	.32203	.567	-1.1698	.5031
		Low dose	Control	.33333	.32203	.567	-.5031	1.1698
			High dose	.00000	.32203	1.000	-.8365	.8365
		High dose	Control	.33333	.32203	.567	-.5031	1.1698
			Low dose	.00000	.32203	1.000	-.8365	.8365
Total protein	Tukey HSD	Control	Low dose	.66667	1.56110	.905	-3.3882	4.7216
			High dose	.83333	1.56110	.856	-3.2216	4.8882
		Low dose	Control	-.66667	1.56110	.905	-4.7216	3.3882
			High dose	.16667	1.56110	.994	-3.8882	4.2216
		High dose	Control	-.83333	1.56110	.856	-4.8882	3.2216
			Low dose	-.16667	1.56110	.994	-4.2216	3.8882
Albumin	Tukey HSD	Control	Low dose	-.50000	.68313	.749	-2.2744	1.2744
			High dose	-.16667	.68313	.968	-1.9411	1.6077
		Low dose	Control	.50000	.68313	.749	-1.2744	2.2744
			High dose	.33333	.68313	.878	-1.4411	2.1077
		High dose	Control	.16667	.68313	.968	-1.6077	1.9411
			Low dose	-.33333	.68313	.878	-2.1077	1.4411

Table C.42: Multiple range tests: Acute toxicity assay- Liver function test for male rats (between different groups)

ALP			Subset for alpha = 0.05
	Group	N	1
Tukey HSD[a]	Control	6	169.5000
	High dose	6	190.5000
	Low dose	6	208.1667
	Sig.		.326
ALT			Subset for alpha = 0.05
	Group	N	1
Tukey HSD[a]	Low dose	6	56.8333
	Control	6	60.0000
	High dose	6	61.5000
	Sig.		.694
AST			Subset for alpha = 0.05
	Group	N	1
Tukey HSD[a]	Control	6	209.8333
	Low dose	6	222.0000
	High dose	6	233.6667
	Sig.		.593
GGT			Subset for alpha = 0.05
	Group	N	1
Tukey HSD[a]	Control	6	2.6667
	Low dose	6	3.0000

Table C.42, continued: Multiple range tests: Acute toxicity assay- Liver function test for male rats (between different groups)

	High dose	6	3.1667
	Sig.		.259
Total bilirudin			Subset for alpha = 0.05
	Group	N	1
Tukey HSD[a]	Control	6	2.0000
	Low dose	6	2.3333
	High dose	6	2.3333
	Sig.		.567
Total protein			Subset for alpha = 0.05
	Group	N	1
Tukey HSD[a]	High dose	6	65.6667
	Low dose	6	65.8333
	Control	6	66.5000
	Sig.		.856
Albumin			Subset for alpha = 0.05
	Group	N	1
Tukey HSD[a]	Control	6	11.6667
	High dose	6	11.8333
	Low dose	6	12.1667
	Sig.		.749

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 6.000.

Table C.43: One way analysis of variance (ANOVA): Acute toxicity assay- Renal function test for male rats (between different groups)

		Sum of Squares	df	Mean Square	F	Sig.
Sodium	Between Groups	2.333	2	1.167	2.143	.152
	Within Groups	8.167	15	.544		
	Total	10.500	17			
Potassium	Between Groups	.058	2	.029	.200	.821
	Within Groups	2.165	15	.144		
	Total	2.223	17			
Chloride	Between Groups	6.778	2	3.389	3.280	.066
	Within Groups	15.500	15	1.033		
	Total	22.278	17			
Carbon dioxide	Between Groups	7.583	2	3.792	1.631	.229
	Within Groups	34.882	15	2.325		
	Total	42.465	17			
Anion gap	Between Groups	2.333	2	1.167	.349	.711
	Within Groups	50.167	15	3.344		
	Total	52.500	17			
Urea	Between Groups	.941	2	.471	.707	.509
	Within Groups	9.988	15	.666		
	Total	10.929	17			
Creatinine	Between Groups	76.000	2	38.000	.214	.810
	Within Groups	2660.000	15	177.333		
	Total	2736.000	17			

Table C.44: Multiple comparisons: Acute toxicity assay- Renal function test for male rats (between different groups)

Dependent Variable		(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
							Lower Bound	Upper Bound
Sodium	Tukey HSD	Control	Low dose	-.83333	.42601	.158	-1.9399	.2732
			High dose	-.16667	.42601	.920	-1.2732	.9399
		Low dose	Control	.83333	.42601	.158	-.2732	1.9399
			High dose	.66667	.42601	.291	-.4399	1.7732
		High dose	Control	.16667	.42601	.920	-.9399	1.2732
			Low dose	-.66667	.42601	.291	-1.7732	.4399
Potassium	Tukey HSD	Control	Low dose	-.13333	.21934	.818	-.7031	.4364
			High dose	-.10000	.21934	.893	-.6697	.4697
		Low dose	Control	.13333	.21934	.818	-.4364	.7031
			High dose	.03333	.21934	.987	-.5364	.6031
		High dose	Control	.10000	.21934	.893	-.4697	.6697
			Low dose	-.03333	.21934	.987	-.6031	.5364
Chloride	Tukey HSD	Control	Low dose	.66667	.58689	.508	-.8578	2.1911
			High dose	-.83333	.58689	.356	-2.3578	.6911
		Low dose	Control	-.66667	.58689	.508	-2.1911	.8578
			High dose	-1.50000	.58689	.054	-3.0244	.0244
		High dose	Control	.83333	.58689	.356	-.6911	2.3578
			Low dose	1.50000	.58689	.054	-.0244	3.0244
Carbon dioxide	Tukey HSD	Control	Low dose	-1.58333	.88042	.204	-3.8702	.7035
			High dose	-.66667	.88042	.734	-2.9535	1.6202
		Low dose	Control	1.58333	.88042	.204	-.7035	3.8702
			High dose	.91667	.88042	.563	-1.3702	3.2035
		High dose	Control	.66667	.88042	.734	-1.6202	2.9535
			Low dose	-.91667	.88042	.563	-3.2035	1.3702
Anion gap	Tukey HSD	Control	Low dose	-.16667	1.05585	.986	-2.9092	2.5759
			High dose	.66667	1.05585	.805	-2.0759	3.4092
		Low dose	Control	.16667	1.05585	.986	-2.5759	2.9092
			High dose	.83333	1.05585	.715	-1.9092	3.5759
		High dose	Control	-.66667	1.05585	.805	-3.4092	2.0759
			Low dose	-.83333	1.05585	.715	-3.5759	1.9092
Urea	Tukey HSD	Control	Low dose	-.55000	.47113	.490	-1.7737	.6737
			High dose	-.36667	.47113	.722	-1.5904	.8571
		Low dose	Control	.55000	.47113	.490	-.6737	1.7737
			High dose	.18333	.47113	.920	-1.0404	1.4071
		High dose	Control	.36667	.47113	.722	-.8571	1.5904
			Low dose	-.18333	.47113	.920	-1.4071	1.0404
Creatinine	Tukey HSD	Control	Low dose	3.00000	7.68838	.920	-16.9703	22.9703
			High dose	5.00000	7.68838	.795	-14.9703	24.9703
		Low dose	Control	-3.00000	7.68838	.920	-22.9703	16.9703
			High dose	2.00000	7.68838	.963	-17.9703	21.9703
		High dose	Control	-5.00000	7.68838	.795	-24.9703	14.9703
			Low dose	-2.00000	7.68838	.963	-21.9703	17.9703

*. The mean difference is significant at the 0.05 level.

Table C.45: Multiple range tests: Acute toxicity assay- Renal function test for male rats (between different groups)

Sodium			Subset for alpha = 0.05
	Group	N	1
Tukey HSD[a]	Control	6	139.5000
	High dose	6	139.6667
	Low dose	6	140.3333
	Sig.		.158
Potassium			Subset for alpha = 0.05
	Group	N	1
Tukey HSD[a]	Control	6	4.8833
	High dose	6	4.9833
	Low dose	6	5.0167
	Sig.		.818
Chloride			Subset for alpha = 0.05
	Group	N	1
Tukey HSD[a]	Low dose	6	100.6667
	Control	6	101.3333
	High dose	6	102.1667
	Sig.		.054
Carbon dioxide			Subset for alpha = 0.05
	Group	N	1
Tukey HSD[a]	Control	6	20.8333
	High dose	6	21.5000
	Low dose	6	22.4167
	Sig.		.204
Anion gap			Subset for alpha = 0.05
	Group	N	1
Tukey HSD[a]	High dose	6	21.0000
	Control	6	21.6667
	Low dose	6	21.8333
	Sig.		.715
Urea			Subset for alpha = 0.05
	Group	N	1
Tukey HSD[a]	Control	6	5.9000
	High dose	6	6.2667
	Low dose	6	6.4500
	Sig.		.490
Creatinine			Subset for alpha = 0.05
	Group	N	1
Tukey HSD[a]	High dose	6	24.6667
	Low dose	6	26.6667
	Control	6	29.6667
	Sig.		.795

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 6.000.

Table C.46: One way analysis of variance (ANOVA): Effects of *P. giganteus* to prevent TAA-induced liver injury- Body and liver weights of experimental Rats (between different groups)

		Sum of Squares	df	Mean Square	F	Sig.
Body Weight	Between Groups	70702.222	5	14140.444	3.275	.018
	Within Groups	129512.333	30	4317.078		
	Total	200214.556	35			
Liver Weight	Between Groups	60.828	5	12.166	2.933	.028
	Within Groups	124.434	30	4.148		
	Total	185.261	35			
Liver weight to body weight ratio	Between Groups	13.500	5	2.700	33.350	.000
	Within Groups	2.429	30	.081		
	Total	15.928	35			

Table C.47: Multiple comparisons: Effects of *P. giganteus* to prevent TAA-induced liver injury- Body and liver weights of experimental rats (between different groups)

Dependent Variable		(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
							Lower Bound	Upper Bound
Body Weight	Tukey HSD	Control 1	Control 2	14.16667	37.93450	.999	-101.2147	129.5480
			TAA Control	88.33333	37.93450	.214	-27.0480	203.7147
			Silymarin Control	119.50000*	37.93450	.039	4.1187	234.8813
			Low Dose	101.16667	37.93450	.112	-14.2147	216.5480
			High Dose	78.16667	37.93450	.334	-37.2147	193.5480
		Control 2	Control 1	-14.16667	37.93450	.999	-129.5480	101.2147
			TAA Control	74.16667	37.93450	.390	-41.2147	189.5480
			Silymarin Control	105.33333	37.93450	.089	-10.0480	220.7147
			Low Dose	87.00000	37.93450	.228	-28.3813	202.3813
			High Dose	64.00000	37.93450	.550	-51.3813	179.3813
		TAA Control	Control 1	-88.33333	37.93450	.214	-203.7147	27.0480
			Control 2	-74.16667	37.93450	.390	-189.5480	41.2147
			Silymarin Control	31.16667	37.93450	.961	-84.2147	146.5480
			Low Dose	12.83333	37.93450	.999	-102.5480	128.2147
			High Dose	-10.16667	37.93450	1.000	-125.5480	105.2147
		Silymarin Control	Control 1	-119.50000*	37.93450	.039	-234.8813	-4.1187
			Control 2	-105.33333	37.93450	.089	-220.7147	10.0480
			TAA Control	-31.16667	37.93450	.961	-146.5480	84.2147
			Low Dose	-18.33333	37.93450	.996	-133.7147	97.0480
			High dose	-41.33333	37.93450	.882	-156.7147	74.0480
		Low Dose	Control 1	-101.16667	37.93450	.112	-216.5480	14.2147
			Control 2	-87.00000	37.93450	.228	-202.3813	28.3813
			TAA Control	-12.83333	37.93450	.999	-128.2147	102.5480
			Silymarin Control	18.33333	37.93450	.996	-97.0480	133.7147
			High Dose	-23.00000	37.93450	.990	-138.3813	92.3813
		High Dose	Control 1	-78.16667	37.93450	.334	-193.5480	37.2147
			Control 2	-64.00000	37.93450	.550	-179.3813	51.3813
			TAA Control	10.16667	37.93450	1.000	-105.2147	125.5480
			Silymarin Control	41.33333	37.93450	.882	-74.0480	156.7147
			Low Dose	23.00000	37.93450	.990	-92.3813	138.3813
Liver Weight	Tukey HSD	Control 1	Control 2	-.23667	1.17584	1.000	-3.8131	3.3398
			TAA Control	-3.16500	1.17584	.107	-6.7414	.4114

Table C.47, continued: Multiple comparisons: Effects of *P. giganteus* to prevent TAA-induced liver injury- Body and liver weights of experimental rats (between different groups)

			Silymarin Control	1.13167	1.17584	.926	-2.4448	4.7081
			Low Dose	-.59833	1.17584	.995	-4.1748	2.9781
			High Dose	-.86667	1.17584	.976	-4.4431	2.7098
		Control 2	Control 1	.23667	1.17584	1.000	-3.3398	3.8131
			TAA Control	-2.92833	1.17584	.159	-6.5048	.6481
			Silymarin Control	1.36833	1.17584	.850	-2.2081	4.9448
			Low Dose	-.36167	1.17584	1.000	-3.9381	3.2148
			High Dose	-.63000	1.17584	.994	-4.2064	2.9464
		TAA Control	Control 1	3.16500	1.17584	.107	-.4114	6.7414
			Control 2	2.92833	1.17584	.159	-.6481	6.5048
			Silymarin Control	4.29667*	1.17584	.011	.7202	7.8731
			Low Dose	2.56667	1.17584	.275	-1.0098	6.1431
			High Dose	2.29833	1.17584	.391	-1.2781	5.8748
		Silymarin Control	Control 1	-1.13167	1.17584	.926	-4.7081	2.4448
			Control 2	-1.36833	1.17584	.850	-4.9448	2.2081
			TAA Control	-4.29667*	1.17584	.011	-7.8731	-.7202
			Low Dose	-1.73000	1.17584	.684	-5.3064	1.8464
			High Dose	-1.99833	1.17584	.543	-5.5748	1.5781
		Low Dose	Control 1	.59833	1.17584	.995	-2.9781	4.1748
			Control 2	.36167	1.17584	1.000	-3.2148	3.9381
			TAA Control	-2.56667	1.17584	.275	-6.1431	1.0098
			Silymarin Control	1.73000	1.17584	.684	-1.8464	5.3064
			High Dose	-.26833	1.17584	1.000	-3.8448	3.3081
		High Dose	Control 1	.86667	1.17584	.976	-2.7098	4.4431
			Control 2	.63000	1.17584	.994	-2.9464	4.2064
			TAA Control	-2.29833	1.17584	.391	-5.8748	1.2781
			Silymarin Control	1.99833	1.17584	.543	-1.5781	5.5748
			Low Dose	.26833	1.17584	1.000	-3.3081	3.8448
Liver weight to body weight ratio	Tukey HSD	Control 1	Control 2	-.19412	.16427	.842	-.6938	.3055
			TAA Control	-1.84896*	.16427	.000	-2.3486	-1.3493
			Silymarin Control	-.70239*	.16427	.002	-1.2020	-.2027
			Low Dose	-1.13525*	.16427	.000	-1.6349	-.6356
			High Dose	-.94393*	.16427	.000	-1.4436	-.4443
		Control 2	Control 1	.19412	.16427	.842	-.3055	.6938
			TAA Control	-1.65483*	.16427	.000	-2.1545	-1.1552
			Silymarin Control	-.50826*	.16427	.044	-1.0079	-.0086
			Low Dose	-.94113*	.16427	.000	-1.4408	-.4415
			High Dose	-.74980*	.16427	.001	-1.2495	-.2502
		TAA Control	Control 1	1.84896*	.16427	.000	1.3493	2.3486
			Control 2	1.65483*	.16427	.000	1.1552	2.1545
			Silymarin Control	1.14657*	.16427	.000	.6469	1.6462
			Low Dose	.71371*	.16427	.002	.2141	1.2134
			High Dose	.90503*	.16427	.000	.4054	1.4047
		Silymarin Control	Control 1	.70239*	.16427	.002	.2027	1.2020
			Control 2	.50826*	.16427	.044	.0086	1.0079
			TAA Control	-1.14657*	.16427	.000	-1.6462	-.6469
			Low Dose	-.43286	.16427	.120	-.9325	.0668

Table C.47, continued: Multiple comparisons: Effects of *P. giganteus* to prevent TAA-induced liver injury- Body and liver weights of experimental rats (between different groups)

		High Dose	-.24154	.16427	.685	-.7412	.2581
	Low Dose	Control 1	1.13525*	.16427	.000	.6356	1.6349
		Control 2	.94113*	.16427	.000	.4415	1.4408
		TAA Control	-.71371*	.16427	.002	-1.2134	-.2141
		Silymarin Control	.43286	.16427	.120	-.0668	.9325
		High Dose	.19132	.16427	.850	-.3083	.6910
	High Dose	Control 1	.94393*	.16427	.000	.4443	1.4436
		Control 2	.74980*	.16427	.001	.2502	1.2495
		TAA Control	-.90503*	.16427	.000	-1.4047	-.4054
		Silymarin Control	.24154	.16427	.685	-.2581	.7412
		Low Dose	-.19132	.16427	.850	-.6910	.3083

*. The mean difference is significant at the 0.05 level.

Table C.48: Multiple range tests: Effects of *P. giganteus* to prevent TAA-induced liver injury- Body and liver weights of experimental rats (between different groups)

Body weight			Subset for alpha = 0.05	
	Group	N	1	2
Tukey HSD[a]	Silymarin Control	6	265.0000	
	Low Dose	6	283.3333	283.3333
	TAA Control	6	296.1667	296.1667
	High Dose	6	306.3333	306.3333
	Control 2	6	370.3333	370.3333
	Control 1	6		384.5000
	Sig.		.089	.112

Liver weight			Subset for alpha = 0.05	
	Group	N	1	2
Tukey HSD[a]	Silymarin Control	6	8.5533	
	Control 1	6	9.6850	9.6850
	Control 2	6	9.9217	9.9217
	Low Dose	6	10.2833	10.2833
	High Dose	6	10.5517	10.5517
	TAA Control	6		12.8500
	Sig.		.543	.107

Liver weight to body weight ratio			Subset for alpha = 0.05		
	Group	N	1	2	3
Tukey HSD[a]	Control 1	6	2.5087		
	Control 2	6	2.7028		
	Silymarin Control	6		3.2111	
	High Dose	6		3.4526	
	Low Dose	6		3.6439	
	TAA Control	6			4.3576
	Sig.		.842	.120	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 6.000.

Table C.49: One way analysis of variance (ANOVA): Effects of *P. giganteus* to prevent TAA-induced liver injury- liver function test (between different groups)

		Sum of Squares	df	Mean Square	F	Sig.
ALP	Between Groups	118811.139	5	23762.228	64.646	.000
	Within Groups	11027.167	30	367.572		
	Total	129838.306	35			
ALT	Between Groups	1533.889	5	306.778	13.255	.000
	Within Groups	694.333	30	23.144		
	Total	2228.222	35			
AST	Between Groups	8301.889	5	1660.378	4.642	.003
	Within Groups	10731.333	30	357.711		
	Total	19033.222	35			
GGT	Between Groups	266.756	5	53.351	10.273	.000
	Within Groups	155.800	30	5.193		
	Total	422.556	35			
Total bilirudin	Between Groups	95.739	5	19.148	19.923	.000
	Within Groups	28.833	30	.961		
	Total	124.572	35			
Total protein	Between Groups	191.889	5	38.378	15.700	.000
	Within Groups	73.333	30	2.444		
	Total	265.222	35			
Albumin	Between Groups	34.806	5	6.961	5.995	.001
	Within Groups	34.833	30	1.161		
	Total	69.639	35			

Table C.50: Multiple comparisons: Effects of *P. giganteus* to prevent TAA-induced liver injury- liver function test (between different groups)

							95% Confidence Interval	
Dependent Variable		(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	Lower Bound	Upper Bound
ALP	Tukey HSD	Control 1	Control 2	-9.00000	11.06906	.963	-42.6676	24.6676
			TAA Control	-148.00000*	11.06906	.000	-181.6676	-114.3324
			Silymarin Control	-136.83333*	11.06906	.000	-170.5009	-103.1657
			Low Dose	-95.50000*	11.06906	.000	-129.1676	-61.8324
			High Dose	-96.50000*	11.06906	.000	-130.1676	-62.8324
		Control 2	Control 1	9.00000	11.06906	.963	-24.6676	42.6676
			TAA Control	-139.00000*	11.06906	.000	-172.6676	-105.3324
			Silymarin Control	-127.83333*	11.06906	.000	-161.5009	-94.1657
			Low Dose	-86.50000*	11.06906	.000	-120.1676	-52.8324
			High Dose	-87.50000*	11.06906	.000	-121.1676	-53.8324
		TAA Control	Control 1	148.00000*	11.06906	.000	114.3324	181.6676
			Control 2	139.00000*	11.06906	.000	105.3324	172.6676
			Silymarin Control	11.16667	11.06906	.911	-22.5009	44.8343
			Low Dose	52.50000*	11.06906	.001	18.8324	86.1676
			High Dose	51.50000*	11.06906	.001	17.8324	85.1676
		Silymarin Control	Control 1	136.83333*	11.06906	.000	103.1657	170.5009
			Control 2	127.83333*	11.06906	.000	94.1657	161.5009
			TAA Control	-11.16667	11.06906	.911	-44.8343	22.5009
			Low Dose	41.33333*	11.06906	.009	7.6657	75.0009
			High Dose	40.33333*	11.06906	.012	6.6657	74.0009
		Low Dose	Control 1	95.50000*	11.06906	.000	61.8324	129.1676
			Control 2	86.50000*	11.06906	.000	52.8324	120.1676

Table C.50, continued: Multiple comparisons: Effects of *P. giganteus* to prevent TAA-induced liver injury- liver function test (between different groups)

			TAA Control	-52.50000*	11.06906	.001	-86.1676	-18.8324
			Silymarin Control	-41.33333*	11.06906	.009	-75.0009	-7.6657
			High Dose	-1.00000	11.06906	1.000	-34.6676	32.6676
		High Dose	Control 1	96.50000*	11.06906	.000	62.8324	130.1676
			Control 2	87.50000*	11.06906	.000	53.8324	121.1676
			TAA Control	-51.50000*	11.06906	.001	-85.1676	-17.8324
			Silymarin Control	-40.33333*	11.06906	.012	-74.0009	-6.6657
			Low Dose	1.00000	11.06906	1.000	-32.6676	34.6676
ALT	Tukey HSD	Control 1	Control 2	.50000	2.77756	1.000	-7.9482	8.9482
			TAA Control	-18.83333*	2.77756	.000	-27.2815	-10.3851
			Silymarin Control	-9.83333*	2.77756	.015	-18.2815	-1.3851
			Low Dose	-8.66667*	2.77756	.042	-17.1149	-.2185
			High Dose	-6.83333	2.77756	.168	-15.2815	1.6149
		Control 2	Control 1	-.50000	2.77756	1.000	-8.9482	7.9482
			TAA Control	-19.33333*	2.77756	.000	-27.7815	-10.8851
			Silymarin Control	-10.33333*	2.77756	.010	-18.7815	-1.8851
			Low Dose	-9.16667*	2.77756	.027	-17.6149	-.7185
			High Dose	-7.33333	2.77756	.118	-15.7815	1.1149
		TAA Control	Control 1	18.83333*	2.77756	.000	10.3851	27.2815
			Control 2	19.33333*	2.77756	.000	10.8851	27.7815
			Silymarin Control	9.00000*	2.77756	.032	.5518	17.4482
			Low Dose	10.16667*	2.77756	.011	1.7185	18.6149
			High Dose	12.00000*	2.77756	.002	3.5518	20.4482
		Silymarin Control	Control 1	9.83333*	2.77756	.015	1.3851	18.2815
			Control 2	10.33333*	2.77756	.010	1.8851	18.7815
			TAA Control	-9.00000*	2.77756	.032	-17.4482	-.5518
			Low Dose	1.16667	2.77756	.998	-7.2815	9.6149
			High Dose	3.00000	2.77756	.885	-5.4482	11.4482
		Low Dose	Control 1	8.66667*	2.77756	.042	.2185	17.1149
			Control 2	9.16667*	2.77756	.027	.7185	17.6149
			TAA Control	-10.16667*	2.77756	.011	-18.6149	-1.7185
			Silymarin Control	-1.16667	2.77756	.998	-9.6149	7.2815
			High Dose	1.83333	2.77756	.985	-6.6149	10.2815
		High Dose	Control 1	6.83333	2.77756	.168	-1.6149	15.2815
			Control 2	7.33333	2.77756	.118	-1.1149	15.7815
			TAA Control	-12.00000*	2.77756	.002	-20.4482	-3.5518
			Silymarin Control	-3.00000	2.77756	.885	-11.4482	5.4482
			Low Dose	-1.83333	2.77756	.985	-10.2815	6.6149
AST	Tukey HSD	Control 1	Control 2	13.00000	10.91957	.838	-20.2129	46.2129
			TAA Control	-34.66667*	10.91957	.037	-67.8796	-1.4538
			Silymarin Control	-18.00000	10.91957	.575	-51.2129	15.2129
			Low Dose	-10.00000	10.91957	.939	-43.2129	23.2129
			High Dose	.00000	10.91957	1.000	-33.2129	33.2129
		Control 2	Control 1	-13.00000	10.91957	.838	-46.2129	20.2129
			TAA Control	-47.66667*	10.91957	.002	-80.8796	-14.4538
			Silymarin Control	-31.00000	10.91957	.078	-64.2129	2.2129

Table C.50, continued: Multiple comparisons: Effects of *P. giganteus* to prevent TAA-induced liver injury- liver function test (between different groups)

			Low Dose	-23.00000	10.91957	.311	-56.2129	10.2129
			High Dose	-13.00000	10.91957	.838	-46.2129	20.2129
		TAA Control	Control 1	34.66667*	10.91957	.037	1.4538	67.8796
			Control 2	47.66667*	10.91957	.002	14.4538	80.8796
			Silymarin Control	16.66667	10.91957	.651	-16.5462	49.8796
			Low Dose	24.66667	10.91957	.242	-8.5462	57.8796
			High Dose	34.66667*	10.91957	.037	1.4538	67.8796
		Silymarin Control	Control 1	18.00000	10.91957	.575	-15.2129	51.2129
			Control 2	31.00000	10.91957	.078	-2.2129	64.2129
			TAA Control	-16.66667	10.91957	.651	-49.8796	16.5462
			Low Dose	8.00000	10.91957	.976	-25.2129	41.2129
			High Dose	18.00000	10.91957	.575	-15.2129	51.2129
		Low Dose	Control 1	10.00000	10.91957	.939	-23.2129	43.2129
			Control 2	23.00000	10.91957	.311	-10.2129	56.2129
			TAA Control	-24.66667	10.91957	.242	-57.8796	8.5462
			Silymarin Control	-8.00000	10.91957	.976	-41.2129	25.2129
			High Dose	10.00000	10.91957	.939	-23.2129	43.2129
		High Dose	Control 1	.00000	10.91957	1.000	-33.2129	33.2129
			Control 2	13.00000	10.91957	.838	-20.2129	46.2129
			TAA Control	-34.66667*	10.91957	.037	-67.8796	-1.4538
			Silymarin Control	-18.00000	10.91957	.575	-51.2129	15.2129
			Low Dose	-10.00000	10.91957	.939	-43.2129	23.2129
GGT	Tukey HSD	Control 1	Control 2	-.20000	1.31572	1.000	-4.2019	3.8019
			TAA Control	-7.66667*	1.31572	.000	-11.6685	-3.6648
			Silymarin Control	-5.00000*	1.31572	.008	-9.0019	-.9981
			Low Dose	-2.16667	1.31572	.576	-6.1685	1.8352
			High Dose	-1.83333	1.31572	.731	-5.8352	2.1685
		Control 2	Control 1	.20000	1.31572	1.000	-3.8019	4.2019
			TAA Control	-7.46667*	1.31572	.000	-11.4685	-3.4648
			Silymarin Control	-4.80000*	1.31572	.012	-8.8019	-.7981
			Low Dose	-1.96667	1.31572	.670	-5.9685	2.0352
			High Dose	-1.63333	1.31572	.813	-5.6352	2.3685
		TAA Control	Control 1	7.66667*	1.31572	.000	3.6648	11.6685
			Control 2	7.46667*	1.31572	.000	3.4648	11.4685
			Silymarin Control	2.66667	1.31572	.352	-1.3352	6.6685
			Low Dose	5.50000*	1.31572	.003	1.4981	9.5019
			High Dose	5.83333*	1.31572	.001	1.8315	9.8352
		Silymarin Control	Control 1	5.00000*	1.31572	.008	.9981	9.0019
			Control 2	4.80000*	1.31572	.012	.7981	8.8019
			TAA Control	-2.66667	1.31572	.352	-6.6685	1.3352
			Low Dose	2.83333	1.31572	.289	-1.1685	6.8352
			High Dose	3.16667	1.31572	.186	-.8352	7.1685
		Low Dose	Control 1	2.16667	1.31572	.576	-1.8352	6.1685
			Control 2	1.96667	1.31572	.670	-2.0352	5.9685
			TAA Control	-5.50000*	1.31572	.003	-9.5019	-1.4981
			Silymarin Control	-2.83333	1.31572	.289	-6.8352	1.1685
			High Dose	.33333	1.31572	1.000	-3.6685	4.3352

		High Dose	Control 1	1.83333	1.31572	.731	-2.1685	5.8352

Table C.50, continued: Multiple comparisons: Effects of *P. giganteus* to prevent TAA-induced liver injury- liver function test (between different groups)

			Control 2	1.63333	1.31572	.813	-2.3685	5.6352
			TAA Control	-5.83333*	1.31572	.001	-9.8352	-1.8315
			Silymarin Control	-3.16667	1.31572	.186	-7.1685	.8352
			Low Dose	-.33333	1.31572	1.000	-4.3352	3.6685
Total bilirudin	Tukey HSD	Control 1	Control 2	-.20000	.56601	.999	-1.9216	1.5216
			TAA Control	-4.76667*	.56601	.000	-6.4882	-3.0451
			Silymarin Control	-2.80000*	.56601	.000	-4.5216	-1.0784
			Low Dose	-2.60000*	.56601	.001	-4.3216	-.8784
			High Dose	-2.40000*	.56601	.002	-4.1216	-.6784
		Control 2	Control 1	.20000	.56601	.999	-1.5216	1.9216
			TAA Control	-4.56667*	.56601	.000	-6.2882	-2.8451
			Silymarin Control	-2.60000*	.56601	.001	-4.3216	-.8784
			Low Dose	-2.40000*	.56601	.002	-4.1216	-.6784
			High Dose	-2.20000*	.56601	.006	-3.9216	-.4784
		TAA Control	Control 1	4.76667*	.56601	.000	3.0451	6.4882
			Control 2	4.56667*	.56601	.000	2.8451	6.2882
			Silymarin Control	1.96667*	.56601	.018	.2451	3.6882
			Low Dose	2.16667*	.56601	.007	.4451	3.8882
			High Dose	2.36667*	.56601	.003	.6451	4.0882
		Silymarin control	Control 1	2.80000*	.56601	.000	1.0784	4.5216
			Control 2	2.60000*	.56601	.001	.8784	4.3216
			TAA control	-1.96667*	.56601	.018	-3.6882	-.2451
			Low dose	.20000	.56601	.999	-1.5216	1.9216
			High dose	.40000	.56601	.980	-1.3216	2.1216
		Low dose	Control 1	2.60000*	.56601	.001	.8784	4.3216
			Control 2	2.40000*	.56601	.002	.6784	4.1216
			TAA control	-2.16667*	.56601	.007	-3.8882	-.4451
			Silymarin control	-.20000	.56601	.999	-1.9216	1.5216
			High dose	.20000	.56601	.999	-1.5216	1.9216
		High dose	Control 1	2.40000*	.56601	.002	.6784	4.1216
			Control 2	2.20000*	.56601	.006	.4784	3.9216
			TAA control	-2.36667*	.56601	.003	-4.0882	-.6451
			Silymarin control	-.40000	.56601	.980	-2.1216	1.3216
			Low dose	-.20000	.56601	.999	-1.9216	1.5216
Total protein	Tukey HSD	Control 1	Control 2	-1.50000	.90267	.566	-4.2456	1.2456
			TAA control	5.50000*	.90267	.000	2.7544	8.2456
			Silymarin control	3.83333*	.90267	.002	1.0878	6.5789
			Low dose	2.33333	.90267	.132	-.4122	5.0789
			High dose	2.16667	.90267	.188	-.5789	4.9122
		Control 2	Control 1	1.50000	.90267	.566	-1.2456	4.2456
			TAA control	7.00000*	.90267	.000	4.2544	9.7456
			Silymarin control	5.33333*	.90267	.000	2.5878	8.0789
			Low dose	3.83333*	.90267	.002	1.0878	6.5789
			High dose	3.66667*	.90267	.004	.9211	6.4122
		TAA control	Control 1	-5.50000*	.90267	.000	-8.2456	-2.7544
			Control 2	-7.00000*	.90267	.000	-9.7456	-4.2544
			Silymarin control	-1.66667	.90267	.453	-4.4122	1.0789
			Low dose	-3.16667*	.90267	.016	-5.9122	-.4211

			High dose	-3.33333*	.90267	.010	-6.0789	-.5878
		Silymarin control	Control 1	-3.83333*	.90267	.002	-6.5789	-1.0878

Table C.50, continued: Multiple comparisons: Effects of *P. giganteus* to prevent TAA-induced liver injury- liver function test (between different groups)

			Control 2	-5.33333*	.90267	.000	-8.0789	-2.5878
			TAA control	1.66667	.90267	.453	-1.0789	4.4122
			Low dose	-1.50000	.90267	.566	-4.2456	1.2456
			High dose	-1.66667	.90267	.453	-4.4122	1.0789
		Low dose	Control 1	-2.33333	.90267	.132	-5.0789	.4122
			Control 2	-3.83333*	.90267	.002	-6.5789	-1.0878
			TAA control	3.16667*	.90267	.016	.4211	5.9122
			Silymarin control	1.50000	.90267	.566	-1.2456	4.2456
			High dose	-.16667	.90267	1.000	-2.9122	2.5789
		High dose	Control 1	-2.16667	.90267	.188	-4.9122	.5789
			Control 2	-3.66667*	.90267	.004	-6.4122	-.9211
			TAA control	3.33333*	.90267	.010	.5878	6.0789
			Silymarin control	1.66667	.90267	.453	-1.0789	4.4122
			Low dose	.16667	.90267	1.000	-2.5789	2.9122
Albumin	Tukey HSD	Control 1	Control 2	-.33333	.62212	.994	-2.2256	1.5589
			TAA control	2.33333*	.62212	.009	.4411	4.2256
			Silymarin control	.66667	.62212	.889	-1.2256	2.5589
			Low dose	-.50000	.62212	.965	-2.3922	1.3922
			High dose	-.33333	.62212	.994	-2.2256	1.5589
		Control 2	Control 1	.33333	.62212	.994	-1.5589	2.2256
			TAA control	2.66667*	.62212	.002	.7744	4.5589
			Silymarin control	1.00000	.62212	.600	-.8922	2.8922
			Low dose	-.16667	.62212	1.000	-2.0589	1.7256
			High dose	.00000	.62212	1.000	-1.8922	1.8922
		TAA control	Control 1	-2.33333*	.62212	.009	-4.2256	-.4411
			Control 2	-2.66667*	.62212	.002	-4.5589	-.7744
			Silymarin control	-1.66667	.62212	.110	-3.5589	.2256
			Low dose	-2.83333*	.62212	.001	-4.7256	-.9411
			High dose	-2.66667*	.62212	.002	-4.5589	-.7744
		Silymarin control	Control 1	-.66667	.62212	.889	-2.5589	1.2256
			Control 2	-1.00000	.62212	.600	-2.8922	.8922
			TAA control	1.66667	.62212	.110	-.2256	3.5589
			Low dose	-1.16667	.62212	.436	-3.0589	.7256
			High dose	-1.00000	.62212	.600	-2.8922	.8922
		Low dose	Control 1	.50000	.62212	.965	-1.3922	2.3922
			Control 2	.16667	.62212	1.000	-1.7256	2.0589
			TAA control	2.83333*	.62212	.001	.9411	4.7256
			Silymarin control	1.16667	.62212	.436	-.7256	3.0589
			High dose	.16667	.62212	1.000	-1.7256	2.0589
		High dose	Control 1	.33333	.62212	.994	-1.5589	2.2256
			Control 2	.00000	.62212	1.000	-1.8922	1.8922
			TAA control	2.66667*	.62212	.002	.7744	4.5589
			Silymarin control	1.00000	.62212	.600	-.8922	2.8922
			Low dose	-.16667	.62212	1.000	-2.0589	1.7256

*. The mean difference is significant at the 0.05 level.

Table C.51: Multiple range tests: Effects of *P. giganteus* to prevent TAA-induced liver injury- liver function test (between different groups)

ALP			Subset for alpha = 0.05			
	Group	N	1	2	3	
Tukey HSD[a]	Control 1	6	70.1667			
	Control 2	6	79.1667			
	Low dose	6		165.6667		
	High dose	6		166.6667		
	Silymarin control	6			207.0000	
	TAA control	6			218.1667	
	Sig.		.963	1.000	.911	
ALT			Subset for alpha = 0.05			
	Group	N	1	2	3	
Tukey HSD[a]	Control 2	6	46.0000			
	Control 1	6	46.5000			
	High dose	6	53.3333	53.3333		
	Low dose	6		55.1667		
	Silymarin control	6		56.3333		
	TAA control	6			65.3333	
	Sig.		.118	.885	1.000	
AST			Subset for alpha = 0.05			
	Group	N	1	2		
Tukey HSD[a]	Control 2	6	151.0000			
	Control 1	6	164.0000			
	High dose	6	164.0000			
	Low dose	6	174.0000	174.0000		
	Silymarin control	6	182.0000	182.0000		
	TAA control	6		198.6667		
	Sig.		.078	.242		
GGT			Subset for alpha = 0.05			
	Group	N	1	2	3	
Tukey HSD[a]	Control 1	6	5.0000			
	Control 2	6	5.2000			
	High dose	6	6.8333	6.8333		
	Low dose	6	7.1667	7.1667		
	Silymarin control	6		10.0000	10.0000	
	TAA control	6			12.6667	
	Sig.		.576	.186	.352	
Total bilirudin			Subset for alpha = 0.05			
	Group	N	1	2	3	
Tukey HSD[a]	Control 1	6	2.4000			
	Control 2	6	2.6000			
	High dose	6		4.8000		
	Low dose	6		5.0000		
	Silymarin control	6		5.2000		
	TAA control	6			7.1667	
	Sig.		.999	.980	1.000	
Total protein			Subset for alpha = 0.05			
Tukey HSD[a]	Group	N	1	2	3	4
	TAA control	6	63.8333			
	Silymarin control	6	65.5000	65.5000		
	Low dose	6		67.0000	67.0000	
	High dose	6		67.1667	67.1667	

Table C.51, continued: Multiple range tests: Effects of *P. giganteus* to prevent TAA-induced liver injury- liver function test (between different groups)

	Control 1	6			69.3333	69.3333
	Control 2	6				70.8333
	Sig.		.453	.453	.132	.566

Albumin			Subset for alpha = 0.05	
	Group	N	1	2
Tukey HSD[a]	TAA control	6	11.1667	
	Silymarin control	6	12.8333	12.8333
	Control 1	6		13.5000
	Control 2	6		13.8333
	High dose	6		13.8333
	Low dose	6		14.0000
	Sig.		.110	.436

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 6.000.

Table C.52: One way analysis of variance (ANOVA): Effects of *P. giganteus* to prevent TAA-induced liver injury- oxidative stress (between different groups)

		Sum of Squares	df	Mean Square	F	Sig.
MDA	Between Groups	35217.524	5	7043.505	11.789	.000
	Within Groups	17924.622	30	597.487		
	Total	53142.146	35			
DNA damage	Between Groups	530993.058	5	106198.612	4.639	.003
	Within Groups	686796.552	30	22893.218		
	Total	1217789.609	35			

Table C.53: Multiple comparisons: Effects of *P. giganteus* to prevent TAA-induced liver injury- oxidative stress (between different groups)

Dependent Variable		(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
							Lower Bound	Upper Bound
MDA	Tukey HSD	Control 1	Control 2	-.35995	14.11249	1.000	-43.2844	42.5645
			TAA control	-81.53024*	14.11249	.000	-124.4547	-38.6058
			Silymarin control	5.79470	14.11249	.998	-37.1298	48.7192
			Low dose	3.99947	14.11249	1.000	-38.9250	46.9239
			High dose'	1.54090	14.11249	1.000	-41.3836	44.4654
		Control 2	Control 1	.35995	14.11249	1.000	-42.5645	43.2844
			TAA control	-81.17029*	14.11249	.000	-124.0948	-38.2458
			Silymarin control	6.15465	14.11249	.998	-36.7698	49.0791
			Low dose	4.35942	14.11249	1.000	-38.5651	47.2839
			High dose'	1.90086	14.11249	1.000	-41.0236	44.8253
		TAA control	Control 1	81.53024*	14.11249	.000	38.6058	124.4547
			Control 2	81.17029*	14.11249	.000	38.2458	124.0948
			Silymarin control	87.32494*	14.11249	.000	44.4005	130.2494
			Low dose	85.52971*	14.11249	.000	42.6052	128.4542
			High dose'	83.07114*	14.11249	.000	40.1467	125.9956
		Silymarin control	Control 1	-5.79470	14.11249	.998	-48.7192	37.1298
			Control 2	-6.15465	14.11249	.998	-49.0791	36.7698
			TAA control	-87.32494*	14.11249	.000	-130.2494	-44.4005
			Low dose	-1.79523	14.11249	1.000	-44.7197	41.1292
			High dose'	-4.25379	14.11249	1.000	-47.1783	38.6707
		Low dose	Control 1	-3.99947	14.11249	1.000	-46.9239	38.9250
			Control 2	-4.35942	14.11249	1.000	-47.2839	38.5651

Table C.53, continued: Multiple comparisons: Effects of *P. giganteus* to prevent TAA-induced liver injury- oxidative stress (between different groups)

		TAA control	-85.52971*	14.11249	.000	-128.4542	-42.6052
		Silymarin control	1.79523	14.11249	1.000	-41.1292	44.7197
		High dose'	-2.45856	14.11249	1.000	-45.3830	40.4659
	High dose'	Control 1	-1.54090	14.11249	1.000	-44.4654	41.3836
		Control 2	-1.90086	14.11249	1.000	-44.8253	41.0236
		TAA control	-83.07114*	14.11249	.000	-125.9956	-40.1467
		Silymarin control	4.25379	14.11249	1.000	-38.6707	47.1783
		Low dose	2.45856	14.11249	1.000	-40.4659	45.3830
DNA damage	Tukey HSD	Control 1	Control 2	-21.02215	87.35601	1.000	-286.7237	244.6794
			TAA control	-339.59290*	87.35601	.006	-605.2944	-73.8914
			Silymarin control	-88.96994	87.35601	.908	-354.6715	176.7316
			Low dose	-205.72989	87.35601	.204	-471.4314	59.9717
			High dose'	-30.77945	87.35601	.999	-296.4810	234.9221
		Control 2	Control 1	21.02215	87.35601	1.000	-244.6794	286.7237
			TAA control	-318.57075*	87.35601	.012	-584.2723	-52.8692
			Silymarin control	-67.94779	87.35601	.969	-333.6493	197.7538
			Low dose	-184.70774	87.35601	.307	-450.4093	80.9938
			High dose'	-9.75730	87.35601	1.000	-275.4588	255.9442
		TAA control	Control 1	339.59290*	87.35601	.006	73.8914	605.2944
			Control 2	318.57075*	87.35601	.012	52.8692	584.2723
			Silymarin control	250.62296	87.35601	.073	-15.0786	516.3245
			Low dose	133.86301	87.35601	.647	-131.8385	399.5646
			High dose'	308.81345*	87.35601	.015	43.1119	574.5150
		Silymarin control	Control 1	88.96994	87.35601	.908	-176.7316	354.6715
			Control 2	67.94779	87.35601	.969	-197.7538	333.6493
			TAA control	-250.62296	87.35601	.073	-516.3245	15.0786
			Low dose	-116.75995	87.35601	.763	-382.4615	148.9416
			High dose'	58.19050	87.35601	.984	-207.5110	323.8920
		Low dose	Control 1	205.72989	87.35601	.204	-59.9717	471.4314
			Control 2	184.70774	87.35601	.307	-80.9938	450.4093
			TAA control	-133.86301	87.35601	.647	-399.5646	131.8385
			Silymarin control	116.75995	87.35601	.763	-148.9416	382.4615
			High dose'	174.95044	87.35601	.364	-90.7511	440.6520
		High dose'	Control 1	30.77945	87.35601	.999	-234.9221	296.4810
			Control 2	9.75730	87.35601	1.000	-255.9442	275.4588
			TAA control	-308.81345*	87.35601	.015	-574.5150	-43.1119
			Silymarin control	-58.19050	87.35601	.984	-323.8920	207.5110
			Low dose	-174.95044	87.35601	.364	-440.6520	90.7511

*. The mean difference is significant at the 0.05 level.

Table C.54: Multiple range tests: Effects of *P. giganteus* to prevent TAA-induced liver injury- oxidative stress (between different groups)

MDA			Subset for alpha = 0.05	
Tukey HSD[a]	Group	N	1	2
	Silymarin control	6	286.8124	
	Low dose	6	288.6076	
	High dose	6	291.0662	
	Control 1	6	292.6071	
	Control 2	6	292.9670	
	TAA control	6		374.1373
	Sig.		.998	1.000
DNA damage			Subset for alpha = 0.05	
Tukey HSD[a]	Group	N	1	2
	Control 1	6	2452.8695	
	Control 2	6	2473.8916	
	High dose	6	2483.6489	
	Silymarin control	6	2541.8394	2541.8394
	Low dose	6	2658.5994	2658.5994
	TAA control	6		2792.4624
	Sig.		.204	.073

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 6.000.

Table C.55: One way analysis of variance (ANOVA): Effects of *P. giganteus* to treat TAA-induced liver injury- Body and liver weights of experimental rats (between different groups)

		Sum of Squares	df	Mean Square	F	Sig.
Body weight	Between Groups	43360.222	5	8672.044	1.297	.292
	Within Groups	200652.000	30	6688.400		
	Total	244012.222	35			
Liver weight	Between Groups	59.851	5	11.970	1.471	.228
	Within Groups	244.043	30	8.135		
	Total	303.894	35			
Body to liver weight ratio	Between Groups	17.107	5	3.421	45.009	.000
	Within Groups	2.280	30	.076		
	Total	19.388	35			

Table C.56: Multiple comparisons: Effects of *P. giganteus* to treat TAA-induced liver injury- Body and liver weights of experimental rats (between different groups)

Dependent Variable		(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
							Lower Bound	Upper Bound
Body weight	Tukey HSD	Control 1	Control 2	27.16667	47.21723	.992	-116.4490	170.7823
			TAA control	103.00000	47.21723	.276	-40.6157	246.6157
			Silymarin Control	65.16667	47.21723	.738	-78.4490	208.7823
			Low dose	74.66667	47.21723	.617	-68.9490	218.2823
			High dose	81.66667	47.21723	.524	-61.9490	225.2823
		Control 2	Control 1	-27.16667	47.21723	.992	-170.7823	116.4490
			TAA control	75.83333	47.21723	.601	-67.7823	219.4490
			Silymarin Control	38.00000	47.21723	.964	-105.6157	181.6157
			Low dose	47.50000	47.21723	.912	-96.1157	191.1157
			High dose	54.50000	47.21723	.854	-89.1157	198.1157
		TAA control	Control 1	-103.00000	47.21723	.276	-246.6157	40.6157
			Control 2	-75.83333	47.21723	.601	-219.4490	67.7823

Table C.56, continued: Multiple comparisons: Effects of *P. giganteus* to treat TAA-induced liver injury- Body and liver weights of experimental rats (between different groups)

			Silymarin Control	-37.83333	47.21723	.965	-181.4490	105.7823
			Low dose	-28.33333	47.21723	.990	-171.9490	115.2823
			High dose	-21.33333	47.21723	.997	-164.9490	122.2823
		Silymarin Control	Control 1	-65.16667	47.21723	.738	-208.7823	78.4490
			Control 2	-38.00000	47.21723	.964	-181.6157	105.6157
			TAA control	37.83333	47.21723	.965	-105.7823	181.4490
			Low dose	9.50000	47.21723	1.000	-134.1157	153.1157
			High dose	16.50000	47.21723	.999	-127.1157	160.1157
		Low dose	Control 1	-74.66667	47.21723	.617	-218.2823	68.9490
			Control 2	-47.50000	47.21723	.912	-191.1157	96.1157
			TAA control	28.33333	47.21723	.990	-115.2823	171.9490
			Silymarin Control	-9.50000	47.21723	1.000	-153.1157	134.1157
			High dose	7.00000	47.21723	1.000	-136.6157	150.6157
		High dose	Control 1	-81.66667	47.21723	.524	-225.2823	61.9490
			Control 2	-54.50000	47.21723	.854	-198.1157	89.1157
			TAA control	21.33333	47.21723	.997	-122.2823	164.9490
			Silymarin Control	-16.50000	47.21723	.999	-160.1157	127.1157
			Low dose	-7.00000	47.21723	1.000	-150.6157	136.6157
Liver weight	Tukey HSD	Control 1	Control 2	.81167	1.64669	.996	-4.1969	5.8202
			TAA control	-3.27167	1.64669	.373	-8.2802	1.7369
			Silymarin Control	-1.58333	1.64669	.926	-6.5919	3.4252
			Low dose	-1.16500	1.64669	.980	-6.1736	3.8436
			High dose	-1.49833	1.64669	.941	-6.5069	3.5102
		Control 2	Control 1	-.81167	1.64669	.996	-5.8202	4.1969
			TAA control	-4.08333	1.64669	.162	-9.0919	.9252
			Silymarin Control	-2.39500	1.64669	.694	-7.4036	2.6136
			Low dose	-1.97667	1.64669	.833	-6.9852	3.0319
			High dose	-2.31000	1.64669	.725	-7.3186	2.6986
		TAA control	Control 1	3.27167	1.64669	.373	-1.7369	8.2802
			Control 2	4.08333	1.64669	.162	-.9252	9.0919
			Silymarin Control	1.68833	1.64669	.906	-3.3202	6.6969
			Low dose	2.10667	1.64669	.794	-2.9019	7.1152
			High dose	1.77333	1.64669	.887	-3.2352	6.7819
		Silymarin Control	Control 1	1.58333	1.64669	.926	-3.4252	6.5919
			Control 2	2.39500	1.64669	.694	-2.6136	7.4036
			TAA control	-1.68833	1.64669	.906	-6.6969	3.3202
			Low dose	.41833	1.64669	1.000	-4.5902	5.4269
			High dose	.08500	1.64669	1.000	-4.9236	5.0936
		Low dose	Control 1	1.16500	1.64669	.980	-3.8436	6.1736
			Control 2	1.97667	1.64669	.833	-3.0319	6.9852
			TAA control	-2.10667	1.64669	.794	-7.1152	2.9019
			Silymarin Control	-.41833	1.64669	1.000	-5.4269	4.5902
			High dose	-.33333	1.64669	1.000	-5.3419	4.6752
		High dose	Control 1	1.49833	1.64669	.941	-3.5102	6.5069
			Control 2	2.31000	1.64669	.725	-2.6986	7.3186

			TAA control	-1.77333	1.64669	.887	-6.7819	3.2352

Table C.56, continued: Multiple comparisons: Effects of *P. giganteus* to treat TAA-induced liver injury- Body and liver weights of experimental rats (between different groups)

			Silymarin Control	-.08500	1.64669	1.000	-5.0936	4.9236
			Low dose	.33333	1.64669	1.000	-4.6752	5.3419
Liver weight to body weight ratio	Tukey HSD	Control 1	Control 2	.03336	.15918	1.000	-.4508	.5175
			TAA control	-1.95302*	.15918	.000	-2.4372	-1.4689
			Silymarin Control	-.92762*	.15918	.000	-1.4118	-.4435
			Low dose	-.96352*	.15918	.000	-1.4477	-.4794
			High dose	-1.18833*	.15918	.000	-1.6725	-.7042
		Control 2	Control 1	-.03336	.15918	1.000	-.5175	.4508
			TAA control	-1.98638*	.15918	.000	-2.4706	-1.5022
			Silymarin Control	-.96098*	.15918	.000	-1.4451	-.4768
			Low dose	-.99688*	.15918	.000	-1.4810	-.5127
			High dose	-1.22169*	.15918	.000	-1.7059	-.7375
		TAA control	Control 1	1.95302*	.15918	.000	1.4689	2.4372
			Control 2	1.98638*	.15918	.000	1.5022	2.4706
			Silymarin Control	1.02541*	.15918	.000	.5412	1.5096
			Low dose	.98950*	.15918	.000	.5053	1.4737
			High dose	.76469*	.15918	.001	.2805	1.2489
		Silymarin Control	Control 1	.92762*	.15918	.000	.4435	1.4118
			Control 2	.96098*	.15918	.000	.4768	1.4451
			TAA control	-1.02541*	.15918	.000	-1.5096	-.5412
			Low dose	-.03590	.15918	1.000	-.5201	.4483
			High dose	-.26071	.15918	.581	-.7449	.2235
		Low dose	Control 1	.96352*	.15918	.000	.4794	1.4477
			Control 2	.99688*	.15918	.000	.5127	1.4810
			TAA control	-.98950*	.15918	.000	-1.4737	-.5053
			Silymarin Control	.03590	.15918	1.000	-.4483	.5201
			High dose	-.22481	.15918	.720	-.7090	.2594
		High dose	Control 1	1.18833*	.15918	.000	.7042	1.6725
			Control 2	1.22169*	.15918	.000	.7375	1.7059
			TAA control	-.76469*	.15918	.001	-1.2489	-.2805
			Silymarin Control	.26071	.15918	.581	-.2235	.7449
			Low dose	.22481	.15918	.720	-.2594	.7090

*. The mean difference is significant at the 0.05 level.

Table C.57: Multiple range tests: Effects of *P. giganteus* to treat TAA-induced liver injury- Body and liver weights of experimental rats (between different groups)

Body weight			Subset for alpha = 0.05
	Group	N	1
Tukey HSD[a]	TAA control	6	293.8333
	High dose	6	315.1667
	Low dose	6	322.1667
	Silymarin Control	6	331.6667
	Control 2	6	369.6667
	Control 1	6	396.8333
	Sig.		.276
Liver weight			Subset for alpha = 0.05
	Group	N	1

Tukey HSD[a]	Control 2	6	9.2000
	Control 1	6	10.0117

Table C.57, continued: Multiple range tests: Effects of *P. giganteus* to treat TAA-induced liver injury- Body and liver weights of experimental rats (between different groups)

	Low dose	6	11.1767		
	High dose	6	11.5100		
	Silymarin Control	6	11.5950		
	TAA control	6	13.2833		
	Sig.		.162		
Liver weight to body weight ratio Tukey HSD[a]			Subset for alpha = 0.05		
	Group	N	1	2	3
	Control 2	6	2.4826		
	Control 1	6	2.5160		
	Silymarin Control	6		3.4436	
	Low dose	6		3.4795	
	High dose	6		3.7043	
	TAA control	6			4.4690
	Sig.		1.000	.581	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 6.000.

Table C.58: One way analysis of variance (ANOVA): Effects of *P. giganteus* to treat TAA-induced liver injury- liver function test (between different groups)

		Sum of Squares	df	Mean Square	F	Sig.
ALP	Between Groups	8111.806	5	1622.361	6.231	.000
	Within Groups	7810.500	30	260.350		
	Total	15922.306	35			
ALT	Between Groups	604.806	5	120.961	11.700	.000
	Within Groups	310.167	30	10.339		
	Total	914.972	35			
AST	Between Groups	4644.472	5	928.894	5.189	.002
	Within Groups	5370.500	30	179.017		
	Total	10014.972	35			
GGT	Between Groups	111.222	5	22.244	20.222	.000
	Within Groups	33.000	30	1.100		
	Total	144.222	35			
Total bilirudin	Between Groups	6.917	5	1.383	4.220	.005
	Within Groups	9.833	30	.328		
	Total	16.750	35			
Total protein	Between Groups	665.139	5	133.028	6.218	.000
	Within Groups	641.833	30	21.394		
	Total	1306.972	35			
Albumin	Between Groups	79.583	5	15.917	4.914	.002
	Within Groups	97.167	30	3.239		
	Total	176.750	35			

Table C.59: Multiple comparisons: Effects of *P. giganteus* to treat TAA-induced liver injury- liver function test (between different groups)

Dependent Variable		(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
							Lower Bound	Upper Bound
ALP	Tukey HSD	Control 1	Control 2	1.00000	9.31576	1.000	-27.3348	29.3348
			TAA control	-40.50000*	9.31576	.002	-68.8348	-12.1652
			Silymarin control	-.16667	9.31576	1.000	-28.5014	28.1681
			Low dose	-18.66667	9.31576	.364	-47.0014	9.6681
			High dose	-4.50000	9.31576	.996	-32.8348	23.8348
		Control 2	Control 1	-1.00000	9.31576	1.000	-29.3348	27.3348
			TAA control	-41.50000*	9.31576	.001	-69.8348	-13.1652
			Silymarin control	-1.16667	9.31576	1.000	-29.5014	27.1681
			Low dose	-19.66667	9.31576	.309	-48.0014	8.6681
			High dose	-5.50000	9.31576	.991	-33.8348	22.8348
		TAA control	Control 1	40.50000*	9.31576	.002	12.1652	68.8348
			Control 2	41.50000*	9.31576	.001	13.1652	69.8348
			Silymarin control	40.33333*	9.31576	.002	11.9986	68.6681
			Low dose	21.83333	9.31576	.209	-6.5014	50.1681
			High dose	36.00000*	9.31576	.007	7.6652	64.3348
		Silymarin control	Control 1	.16667	9.31576	1.000	-28.1681	28.5014
			Control 2	1.16667	9.31576	1.000	-27.1681	29.5014
			TAA control	-40.33333*	9.31576	.002	-68.6681	-11.9986
			Low dose	-18.50000	9.31576	.374	-46.8348	9.8348
			High dose	-4.33333	9.31576	.997	-32.6681	24.0014
		Low dose	Control 1	18.66667	9.31576	.364	-9.6681	47.0014
			Control 2	19.66667	9.31576	.309	-8.6681	48.0014
			TAA control	-21.83333	9.31576	.209	-50.1681	6.5014
			Silymarin control	18.50000	9.31576	.374	-9.8348	46.8348
			High dose	14.16667	9.31576	.654	-14.1681	42.5014
		High dose	Control 1	4.50000	9.31576	.996	-23.8348	32.8348
			Control 2	5.50000	9.31576	.991	-22.8348	33.8348
			TAA control	-36.00000*	9.31576	.007	-64.3348	-7.6652
			Silymarin control	4.33333	9.31576	.997	-24.0014	32.6681
			Low dose	-14.16667	9.31576	.654	-42.5014	14.1681
ALT	Tukey HSD	Control 1	Control 2	3.66667	1.85642	.379	-1.9798	9.3131
			TAA control	-7.66667*	1.85642	.003	-13.3131	-2.0202
			Silymarin control	3.00000	1.85642	.595	-2.6465	8.6465
			Low dose	-5.16667	1.85642	.088	-10.8131	.4798
			High dose	-2.00000	1.85642	.886	-7.6465	3.6465
		Control 2	Control 1	-3.66667	1.85642	.379	-9.3131	1.9798
			TAA control	-11.33333*	1.85642	.000	-16.9798	-5.6869
			Silymarin control	-.66667	1.85642	.999	-6.3131	4.9798
			Low dose	-8.83333*	1.85642	.001	-14.4798	-3.1869
			High dose	-5.66667*	1.85642	.049	-11.3131	-.0202
		TAA control	Control 1	7.66667*	1.85642	.003	2.0202	13.3131
			Control 2	11.33333*	1.85642	.000	5.6869	16.9798
			Silymarin control	10.66667*	1.85642	.000	5.0202	16.3131
			Low dose	2.50000	1.85642	.757	-3.1465	8.1465
			High dose	5.66667*	1.85642	.049	.0202	11.3131
		Silymarin control	Control 1	-3.00000	1.85642	.595	-8.6465	2.6465
			Control 2	.66667	1.85642	.999	-4.9798	6.3131
			TAA control	-10.66667*	1.85642	.000	-16.3131	-5.0202

Table C.59, continued: Multiple comparisons: Effects of *P. giganteus* to treat TAA-induced liver injury- liver function test (between different groups)

			Low dose	-8.16667*	1.85642	.002	-13.8131	-2.5202
			High dose	-5.00000	1.85642	.106	-10.6465	.6465
		Low dose	Control 1	5.16667	1.85642	.088	-.4798	10.8131
			Control 2	8.83333*	1.85642	.001	3.1869	14.4798
			TAA control	-2.50000	1.85642	.757	-8.1465	3.1465
			Silymarin control	8.16667*	1.85642	.002	2.5202	13.8131
			High dose	3.16667	1.85642	.539	-2.4798	8.8131
		High dose	Control 1	2.00000	1.85642	.886	-3.6465	7.6465
			Control 2	5.66667*	1.85642	.049	.0202	11.3131
			TAA control	-5.66667*	1.85642	.049	-11.3131	-.0202
			Silymarin control	5.00000	1.85642	.106	-.6465	10.6465
			Low dose	-3.16667	1.85642	.539	-8.8131	2.4798
AST	Tukey HSD	Control 1	Control 2	5.00000	7.72478	.986	-18.4956	28.4956
			TAA control	-31.00000*	7.72478	.005	-54.4956	-7.5044
			Silymarin control	-5.16667	7.72478	.984	-28.6623	18.3290
			Low dose	-7.50000	7.72478	.924	-30.9956	15.9956
			High dose	-5.50000	7.72478	.979	-28.9956	17.9956
		Control 2	Control 1	-5.00000	7.72478	.986	-28.4956	18.4956
			TAA control	-36.00000*	7.72478	.001	-59.4956	-12.5044
			Silymarin control	-10.16667	7.72478	.774	-33.6623	13.3290
			Low dose	-12.50000	7.72478	.593	-35.9956	10.9956
			High dose	-10.50000	7.72478	.750	-33.9956	12.9956
		TAA control	Control 1	31.00000*	7.72478	.005	7.5044	54.4956
			Control 2	36.00000*	7.72478	.001	12.5044	59.4956
			Silymarin control	25.83333*	7.72478	.025	2.3377	49.3290
			Low dose	23.50000*	7.72478	.050	.0044	46.9956
			High dose	25.50000*	7.72478	.027	2.0044	48.9956
		Silymarin control	Control 1	5.16667	7.72478	.984	-18.3290	28.6623
			Control 2	10.16667	7.72478	.774	-13.3290	33.6623
			TAA control	-25.83333*	7.72478	.025	-49.3290	-2.3377
			Low dose	-2.33333	7.72478	1.000	-25.8290	21.1623
			High dose	-.33333	7.72478	1.000	-23.8290	23.1623
		Low dose	Control 1	7.50000	7.72478	.924	-15.9956	30.9956
			Control 2	12.50000	7.72478	.593	-10.9956	35.9956
			TAA control	-23.50000*	7.72478	.050	-46.9956	-.0044
			Silymarin control	2.33333	7.72478	1.000	-21.1623	25.8290
			High dose	2.00000	7.72478	1.000	-21.4956	25.4956
		High dose	Control 1	5.50000	7.72478	.979	-17.9956	28.9956
			Control 2	10.50000	7.72478	.750	-12.9956	33.9956
			TAA control	-25.50000*	7.72478	.027	-48.9956	-2.0044
			Silymarin control	.33333	7.72478	1.000	-23.1623	23.8290
			Low dose	-2.00000	7.72478	1.000	-25.4956	21.4956
GGT	Tukey HSD	Control 1	Control 2	-.16667	.60553	1.000	-2.0084	1.6751
			TAA control	-4.83333*	.60553	.000	-6.6751	-2.9916
			Silymarin control	.00000	.60553	1.000	-1.8418	1.8418
			Low dose	-2.00000*	.60553	.027	-3.8418	-.1582
			High dose	-.33333	.60553	.993	-2.1751	1.5084
		Control 2	Control 1	.16667	.60553	1.000	-1.6751	2.0084
			TAA control	-4.66667*	.60553	.000	-6.5084	-2.8249
			Silymarin control	.16667	.60553	1.000	-1.6751	2.0084
			Low dose	-1.83333	.60553	.052	-3.6751	.0084
			High dose	-.16667	.60553	1.000	-2.0084	1.6751
		TAA control	Control 1	4.83333*	.60553	.000	2.9916	6.6751

Table C.59, continued: Multiple comparisons: Effects of *P. giganteus* to treat TAA-induced liver injury- liver function test (between different groups)

			Control 2	4.66667*	.60553	.000	2.8249	6.5084
			Silymarin control	4.83333*	.60553	.000	2.9916	6.6751
			Low dose	2.83333*	.60553	.001	.9916	4.6751
			High dose	4.50000*	.60553	.000	2.6582	6.3418
		Silymarin control	Control 1	.00000	.60553	1.000	-1.8418	1.8418
			Control 2	-.16667	.60553	1.000	-2.0084	1.6751
			TAA control	-4.83333*	.60553	.000	-6.6751	-2.9916
			Low dose	-2.00000*	.60553	.027	-3.8418	-.1582
			High dose	-.33333	.60553	.993	-2.1751	1.5084
		Low dose	Control 1	2.00000*	.60553	.027	.1582	3.8418
			Control 2	1.83333	.60553	.052	-.0084	3.6751
			TAA control	-2.83333*	.60553	.001	-4.6751	-.9916
			Silymarin control	2.00000*	.60553	.027	.1582	3.8418
			High dose	1.66667	.60553	.094	-.1751	3.5084
		High dose	Control 1	.33333	.60553	.993	-1.5084	2.1751
			Control 2	.16667	.60553	1.000	-1.6751	2.0084
			TAA control	-4.50000*	.60553	.000	-6.3418	-2.6582
			Silymarin control	.33333	.60553	.993	-1.5084	2.1751
			Low dose	-1.66667	.60553	.094	-3.5084	.1751
Total bilirudin	Tukey HSD	Control 1	Control 2	-.16667	.33054	.996	-1.1720	.8387
			TAA control	-1.33333*	.33054	.004	-2.3387	-.3280
			Silymarin control	-.50000	.33054	.659	-1.5054	.5054
			Low dose	-.33333	.33054	.911	-1.3387	.6720
			High dose	-.16667	.33054	.996	-1.1720	.8387
		Control 2	Control 1	.16667	.33054	.996	-.8387	1.1720
			TAA control	-1.16667*	.33054	.016	-2.1720	-.1613
			Silymarin control	-.33333	.33054	.911	-1.3387	.6720
			Low dose	-.16667	.33054	.996	-1.1720	.8387
			High dose	.00000	.33054	1.000	-1.0054	1.0054
		TAA control	Control 1	1.33333*	.33054	.004	.3280	2.3387
			Control 2	1.16667*	.33054	.016	.1613	2.1720
			Silymarin control	.83333	.33054	.150	-.1720	1.8387
			Low dose	1.00000	.33054	.052	-.0054	2.0054
			High dose	1.16667*	.33054	.016	.1613	2.1720
		Silymarin control	Control 1	.50000	.33054	.659	-.5054	1.5054
			Control 2	.33333	.33054	.911	-.6720	1.3387
			TAA control	-.83333	.33054	.150	-1.8387	.1720
			Low dose	.16667	.33054	.996	-.8387	1.1720
			High dose	.33333	.33054	.911	-.6720	1.3387
		Low dose	Control 1	.33333	.33054	.911	-.6720	1.3387
			Control 2	.16667	.33054	.996	-.8387	1.1720
			TAA control	-1.00000	.33054	.052	-2.0054	.0054
			Silymarin control	-.16667	.33054	.996	-1.1720	.8387
			High dose	.16667	.33054	.996	-.8387	1.1720
		High dose	Control 1	.16667	.33054	.996	-.8387	1.1720
			Control 2	.00000	.33054	1.000	-1.0054	1.0054
			TAA control	-1.16667*	.33054	.016	-2.1720	-.1613
			Silymarin control	-.33333	.33054	.911	-1.3387	.6720
			Low dose	-.16667	.33054	.996	-1.1720	.8387
Total protein	Tukey HSD	Control 1	Control 2	.50000	2.67048	1.000	-7.6225	8.6225
			TAA control	12.66667*	2.67048	.001	4.5441	20.7892
			Silymarin control	6.50000	2.67048	.177	-1.6225	14.6225

			Low dose	7.00000	2.67048	.123	-1.1225	15.1225

Table C.59, continued: Multiple comparisons: Effects of *P. giganteus* to treat TAA-induced liver injury- liver function test (between different groups)

			High dose	4.16667	2.67048	.630	-3.9559	12.2892
		Control 2	Control 1	-.50000	2.67048	1.000	-8.6225	7.6225
			TAA control	12.16667*	2.67048	.001	4.0441	20.2892
			Silymarin control	6.00000	2.67048	.247	-2.1225	14.1225
			Low dose	6.50000	2.67048	.177	-1.6225	14.6225
			High dose	3.66667	2.67048	.742	-4.4559	11.7892
		TAA control	Control 1	-12.66667*	2.67048	.001	-20.7892	-4.5441
			Control 2	-12.16667*	2.67048	.001	-20.2892	-4.0441
			Silymarin control	-6.16667	2.67048	.222	-14.2892	1.9559
			Low dose	-5.66667	2.67048	.304	-13.7892	2.4559
			High dose	-8.50000*	2.67048	.036	-16.6225	-.3775
		Silymarin control	Control 1	-6.50000	2.67048	.177	-14.6225	1.6225
			Control 2	-6.00000	2.67048	.247	-14.1225	2.1225
			TAA control	6.16667	2.67048	.222	-1.9559	14.2892
			Low dose	.50000	2.67048	1.000	-7.6225	8.6225
			High dose	-2.33333	2.67048	.950	-10.4559	5.7892
		Low dose	Control 1	-7.00000	2.67048	.123	-15.1225	1.1225
			Control 2	-6.50000	2.67048	.177	-14.6225	1.6225
			TAA control	5.66667	2.67048	.304	-2.4559	13.7892
			Silymarin control	-.50000	2.67048	1.000	-8.6225	7.6225
			High dose'	-2.83333	2.67048	.893	-10.9559	5.2892
		High dose	Control 1	-4.16667	2.67048	.630	-12.2892	3.9559
			Control 2	-3.66667	2.67048	.742	-11.7892	4.4559
			TAA control	8.50000*	2.67048	.036	.3775	16.6225
			Silymarin control	2.33333	2.67048	.950	-5.7892	10.4559
			Low dose	2.83333	2.67048	.893	-5.2892	10.9559
Albumin	Tukey HSD	Control 1	Control 2	.33333	1.03905	.999	-2.8270	3.4937
			TAA control	4.00000*	1.03905	.007	.8396	7.1604
			Silymarin control	.50000	1.03905	.996	-2.6604	3.6604
			Low dose	2.50000	1.03905	.186	-.6604	5.6604
			High dose	.16667	1.03905	1.000	-2.9937	3.3270
		Control 2	Control 1	-.33333	1.03905	.999	-3.4937	2.8270
			TAA control	3.66667*	1.03905	.016	.5063	6.8270
			Silymarin control	.16667	1.03905	1.000	-2.9937	3.3270
			Low dose	2.16667	1.03905	.322	-.9937	5.3270
			High dose	-.16667	1.03905	1.000	-3.3270	2.9937
		TAA control	Control 1	-4.00000*	1.03905	.007	-7.1604	-.8396
			Control 2	-3.66667*	1.03905	.016	-6.8270	-.5063
			Silymarin control	-3.50000*	1.03905	.023	-6.6604	-.3396
			Low dose	-1.50000	1.03905	.701	-4.6604	1.6604
			High dose	-3.83333*	1.03905	.010	-6.9937	-.6730
		Silymarin control	Control 1	-.50000	1.03905	.996	-3.6604	2.6604
			Control 2	-.16667	1.03905	1.000	-3.3270	2.9937
			TAA control	3.50000*	1.03905	.023	.3396	6.6604
			Low dose	2.00000	1.03905	.407	-1.1604	5.1604
			High dose	-.33333	1.03905	.999	-3.4937	2.8270
		Low dose	Control 1	-2.50000	1.03905	.186	-5.6604	.6604
			Control 2	-2.16667	1.03905	.322	-5.3270	.9937
			TAA control	1.50000	1.03905	.701	-1.6604	4.6604
			Silymarin control	-2.00000	1.03905	.407	-5.1604	1.1604
			High dose'	-2.33333	1.03905	.248	-5.4937	.8270

	High dose	Control 1	-.16667	1.03905	1.000	-3.3270	2.9937
		Control 2	.16667	1.03905	1.000	-2.9937	3.3270

Table C.59, continued: Multiple comparisons: Effects of *P. giganteus* to treat TAA-induced liver injury- liver function test (between different groups)

	TAA control	3.83333*	1.03905	.010	.6730	6.9937
	Silymarin control	.33333	1.03905	.999	-2.8270	3.4937
	Low dose	2.33333	1.03905	.248	-.8270	5.4937

*. The mean difference is significant at the 0.05 level.

Table C.60: Multiple range tests: Effects of *P. giganteus* to treat TAA-induced liver injury- liver function test (between different groups)

ALP			Subset for alpha = 0.05			
	Group	N	1		2	
Tukey HSD[a]	Control 2	6	68.6667			
	Control 1	6	69.6667			
	Silymarin control	6	69.8333			
	High dose	6	74.1667			
	Low dose	6	88.3333		88.3333	
	TAA control	6			110.1667	
	Sig.		.309		.209	
ALT			Subset for alpha = 0.05			
	Group	N	1	2	3	4
Tukey HSD[a]	Control 2	6	45.0000			
	Silymarin control	6	45.6667	45.6667		
	Control 1	6	48.6667	48.6667	48.6667	
	High dose	6		50.6667	50.6667	
	Low dose	6			53.8333	53.8333
	TAA control	6				56.3333
	Sig.		.379	.106	.088	.757
AST			Subset for alpha = 0.05			
	Group	N	1		2	
Tukey HSD[a]	Control 2	6	148.1667			
	Control 1	6	153.1667			
	Silymarin control	6	158.3333			
	High dose	6	158.6667			
	Low dose	6	160.6667			
	TAA control	6			184.1667	
	Sig.		.593		1.000	
GGT			Subset for alpha = 0.05			
	Group	N	1	2	3	
Tukey HSD[a]	Control 1	6	3.0000			
	Silymarin control	6	3.0000			
	Control 2	6	3.1667	3.1667		
	High dose	6	3.3333	3.3333		
	Low dose	6		5.0000		
	TAA control	6			7.8333	
	Sig.		.993	.052	1.000	
Total bilirudin			Subset for alpha = 0.05			
	Group	N	1		2	
Tukey HSD[a]	Control 1	6	1.5000			
	Control 2	6	1.6667			
	High dose	6	1.6667			
	Low dose	6	1.8333		1.8333	
	Silymarin control	6	2.0000		2.0000	

	TAA control	6		2.8333
	Sig.		.659	.052

Table C.60, continued: Multiple range tests: Effects of *P. giganteus* to treat TAA-induced liver injury- liver function test (between different groups)

Total protein			Subset for alpha = 0.05	
	Group	N	1	2
Tukey HSD[a]	TAA control	6	54.5000	
	Low dose	6	60.1667	60.1667
	Silymarin control	6	60.6667	60.6667
	High dose	6		63.0000
	Control 2	6		66.6667
	Control 1	6		67.1667
	Sig.		.222	.123
Albumin			Subset for alpha = 0.05	
	Group	N	1	2
Tukey HSD[a]	TAA control	6	8.6667	
	Low dose	6	10.1667	10.1667
	Silymarin control	6		12.1667
	Control 2	6		12.3333
	High dose	6		12.5000
	Control 1	6		12.6667
	Sig.		.701	.186

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 6.000.

Table C.61: One way analysis of variance (ANOVA): Effects of *P. giganteus* to treat TAA-induced liver injury- oxidative stress (between different groups)

		Sum of Squares	df	Mean Square	F	Sig.
MDA	Between Groups	189827.914	5	37965.583	67.003	.000
	Within Groups	16998.653	30	566.622		
	Total	206826.567	35			
DNA damage	Between Groups	495468.769	5	99093.754	31.931	.000
	Within Groups	93100.419	30	3103.347		
	Total	588569.189	35			

Table C.62: Multiple comparisons: Effects of *P. giganteus* to treat TAA-induced liver injury- oxidative stress (between different groups)

Dependent Variable		(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
							Lower Bound	Upper Bound
MDA	Tukey HSD	Control 1	Control 2	27.28902	13.74314	.374	-14.5120	69.0901
			TAA control	-188.98664*	13.74314	.000	-230.7877	-147.1856
			Silymarin control	2.44379	13.74314	1.000	-39.3573	44.2449
			Low dose	-67.61160*	13.74314	.000	-109.4127	-25.8105
			High dose	-12.62626	13.74314	.939	-54.4273	29.1748
		Control 2	Control 1	-27.28902	13.74314	.374	-69.0901	14.5120
			TAA control	-216.27566*	13.74314	.000	-258.0767	-174.4746
			Silymarin control	-24.84523	13.74314	.476	-66.6463	16.9558
			Low dose	-94.90062*	13.74314	.000	-136.7017	-53.0996
			High dose	-39.91528	13.74314	.068	-81.7163	1.8858
		TAA control	Control 1	188.98664*	13.74314	.000	147.1856	230.7877
			Control 2	216.27566*	13.74314	.000	174.4746	258.0767
			Silymarin control	191.43043*	13.74314	.000	149.6294	233.2315

		Low dose	121.37504*	13.74314	.000	79.5740	163.1761
		High dose	176.36038*	13.74314	.000	134.5593	218.1614
	Silymarin control	Control 1	-2.44379	13.74314	1.000	-44.2449	39.3573
		Control 2	24.84523	13.74314	.476	-16.9558	66.6463

Table C.62, continued: Multiple comparisons: Effects of *P. giganteus* to treat TAA-induced liver injury- oxidative stress (between different groups)

		TAA control	-191.43043*	13.74314	.000	-233.2315	-149.6294
		Low dose	-70.05539*	13.74314	.000	-111.8565	-28.2543
		High dose	-15.07006	13.74314	.879	-56.8711	26.7310
	Low dose	Control 1	67.61160*	13.74314	.000	25.8105	109.4127
		Control 2	94.90062*	13.74314	.000	53.0996	136.7017
		TAA control	-121.37504*	13.74314	.000	-163.1761	-79.5740
		Silymarin control	70.05539*	13.74314	.000	28.2543	111.8565
		High dose	54.98534*	13.74314	.005	13.1843	96.7864
	High dose	Control 1	12.62626	13.74314	.939	-29.1748	54.4273
		Control 2	39.91528	13.74314	.068	-1.8858	81.7163
		TAA control	-176.36038*	13.74314	.000	-218.1614	-134.5593
		Silymarin control	15.07006	13.74314	.879	-26.7310	56.8711
		Low dose	-54.98534*	13.74314	.005	-96.7864	-13.1843
DNA damage	Tukey HSD	Control 1	Control 2	-57.97319	32.16285	.479	-155.7995	39.8532
			TAA control	-350.29732*	32.16285	.000	-448.1237	-252.4710
			Silymarin control	-202.87189*	32.16285	.000	-300.6982	-105.0455
			Low dose	-248.41985*	32.16285	.000	-346.2462	-150.5935
			High dose	-130.79233*	32.16285	.004	-228.6187	-32.9660
		Control 2	Control 1	57.97319	32.16285	.479	-39.8532	155.7995
			TAA control	-292.32412*	32.16285	.000	-390.1505	-194.4978
			Silymarin control	-144.89870*	32.16285	.001	-242.7250	-47.0723
			Low dose	-190.44666*	32.16285	.000	-288.2730	-92.6203
			High dose	-72.81913	32.16285	.240	-170.6455	25.0072
		TAA control	Control 1	350.29732*	32.16285	.000	252.4710	448.1237
			Control 2	292.32412*	32.16285	.000	194.4978	390.1505
			Silymarin control	147.42542*	32.16285	.001	49.5991	245.2518
			Low dose	101.87746*	32.16285	.037	4.0511	199.7038
			High dose	219.50499*	32.16285	.000	121.6786	317.3313
		Silymarin control	Control 1	202.87189*	32.16285	.000	105.0455	300.6982
			Control 2	144.89870*	32.16285	.001	47.0723	242.7250
			TAA control	-147.42542*	32.16285	.001	-245.2518	-49.5991
			Low dose	-45.54796	32.16285	.717	-143.3743	52.2784
			High dose	72.07957	32.16285	.250	-25.7468	169.9059
		Low dose	Control 1	248.41985*	32.16285	.000	150.5935	346.2462
			Control 2	190.44666*	32.16285	.000	92.6203	288.2730
			TAA control	-101.87746*	32.16285	.037	-199.7038	-4.0511
			Silymarin control	45.54796	32.16285	.717	-52.2784	143.3743
			High dose	117.62753*	32.16285	.011	19.8012	215.4539
		High dose	Control 1	130.79233*	32.16285	.004	32.9660	228.6187
			Control 2	72.81913	32.16285	.240	-25.0072	170.6455
			TAA control	-219.50499*	32.16285	.000	-317.3313	-121.6786
			Silymarin control	-72.07957	32.16285	.250	-169.9059	25.7468
			Low dose	-117.62753*	32.16285	.011	-215.4539	-19.8012

*. The mean difference is significant at the 0.05 level.

Table C.63: Multiple range tests: Effects of *P. giganteus* to treat TAA-induced liver injury- oxidative stress (between different groups)

MDA			Subset for alpha = 0.05		
Tukey HSD[a]	Group	N	1	2	3
	Control 2	6	278.4295		
	Silymarin control	6	303.2747		
	Control 1	6	305.7185		
	High dose	6	318.3447		
	Low dose	6		373.3301	
	TAA control	6			494.7051
	Sig.		.068	1.000	1.000

DNA damage			Subset for alpha = 0.05				
Tukey HSD[a]	Group	N	1	2	3	4	5
	Control 1	6	2402.9923				
	Control 2	6	2460.9655	2460.9655			
	High dose	6		2533.7846	2533.7846		
	Silymarin control	6			2605.8642	2605.8642	
	Low dose	6				2651.4121	
	TAA control	6					2753.2896
	Sig.		.479	.240	.250	.717	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 6.000.