USE OF COMPLEMENTARY AND ALTERNATIVE MEDICINE IN SPORTS AND EFFECTS OF *EURYCOMA LONGIFOLIA* JACK ON AEROBIC METABOLISM IN COLLEGIATE ATHLETES

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ABSTRACT

The use of herbs and plants as ergogenic aids in exercise is a common practice among athletes as this complementary or alternative medicines is believed to have properties that may improve the athlete's wellbeing and exercise performance. Locally, a traditional medicinal plant that is used to improve health and stamina is *Eurycoma longifolia* Jack (*E. longifolia*) or *Tongkat Ali*, however, lack of scientific evidence warrants an investigation of its acclaimed benefits in endurance exercise. The main aims of this study are to investigate the: (i) prevalence of complementary and alternative medicine among athletes in Malaysia, (ii) effect of acute and chronic consumption of *E. longifolia* on aerobic metabolisms during prolonged exercise, and (iii) lipid lowering effect of *E. longifolia* in an isolated cell culture.

Complementary and alternative medicine (CAM) is a subset of medicine which is generally regarded as a practice that includes a variety of treatment methods that is not mainstream conventional medicine. Since the prevalence of its usage has not been investigated among Malaysian national athletes, a survey was carried out on 231 sportsmen to examine the practice, reasons for using, and types of CAM therapies used. The survey showed that CAM is highly used among athletes in the past 12 months, especially in female athletes. This could be attributed to weight management aspiration and to a lesser extent menstrual related problems. It was also shown that athletes who consulted conventional physicians were often prescribed acupuncture as well. In addition, both male and female athletes preferred CAM to promote recovery, particularly the use of Vitamin C supplementation, and praying for health was preferred as self-help practices. The usage of supplements was preferred because they were readily available and affordable. Interestingly, it is shown that Malaysian athletes were similar to those from other countries, who rely on CAM usage to manage their well-being.

Prior studies have shown that endurance exercise capacity may be improved when fat oxidation is increased through interventions. *E. longifolia* has been hypothesised to improve fat metabolism and increase muscle strength during exercise, however, evidence to support these is still lacking. Using a randomised, double-blinded study, placebo-controlled design, the study investigated the acute effect of *E. longifolia* on aerobic metabolism in male athletes. Briefly, the athletes recruited were given either *E. longifolia* or placebo supplements, and ran on the treadmill for an hour at the speed based on 65% of their respectiveVO_2max. The expired gas was collected and analysed for carbohydrate and fat metabolisms while blood samples were withdrawn to determine free fatty acid (FFA), glycerol, TG, glucose, insulin, cholesterol (i.e. total cholesterol, HDL, LDL), testosterone, and cortisol concentrations. An acute 3-day *E. longifolia* supplementation (1.7 mg/kg body weight) resulted in increased fat metabolism during the moderate intensity exercise. From the blood analyses, increased glycerol and cortisol concentrations, while decreased FFA and LDL were observed. These suggest that *E. longifolia* group athletes were conditioned to favour fat metabolism as an energy source compared to glucose, as seen in the PG group. It is believed that athletes who are conditioned to use fat as an energy source would have better aerobic performance. Similarly, this phenomenon was also observed in prolonged usage of *E. longifolia* of up to 5 weeks. Therefore, *E. longifolia* is shown to benefit athletes who mainly depend on aerobic performance by disproportioning the metabolism towards fat burning for better energy yield.

To further examine lipid-lowering effect of *E. longifolia*, the study employed an *in vitro* model to investigate whether *E. longifolia* possesses lipid lowering potential or the changes was a physiological side-effect of the supplementation. The human hepatic cell line WRL-68 was treated with various concentrations (1, 2, 4 and 8 µg/ml) of the aqueous

E. longifolia root extract. Results demonstrated that intracellular fatty acids build up was reduced up to ~72%, in the treated cells compared to untreated control. This study therefore, unravelled the novel potential of intracellular lipid lowering effect of *E. longifolia*.

In conclusion, the epidemiology data of CAM practice among national athletes shows high usage mainly among female athletes. In view of improving sports performance, athletes prefer supplementation, while for self-help practice, prayers play a convincing role. These findings could also serve as one of the foundation in policy making in managing athletes. As a growing country in sports, *E. longifolia* can be widely used as an ergogenic aids to improve fat availability as energy source during aerobic exercise.

ABSTRAK

Penggunaan herba dan tumbuhan sebagai bahan bantuan ergogenik semasa sesi latihan adalah lazim di kalangan atlet kerana perubatan pelengkap atau alternatif ini dipercayai mempunyai ciri yang boleh meningkatkan kesejahteraan dan prestasi latihan.. Di negara ini, tumbuhan yang semakin popular penggunaannya ialah *Eurycoma longifolia* Jack (*E. longifolia*) atau Tongkat Ali, namun kekurangan bukti saintifik menyarankan siasatan ke atas manfaat yang disebutkan sebelumnya. Fokus utama kajian ini adalah untuk menyiasat: (i) penggunaan perubatan sampingan dan alternatif di kalangan atlet di Malaysia, (ii) Kesan penggunaan akut dan kronik *E. longifolia* metabolisma aerobik semasa senaman yang berpanjangan, dan (iii) kesan penurunan lipid oleh *E. longifolia* dalam isolasi sel kultur.

Sebagai permulaan, perubatan pelengkap dan alternatif (CAM) adalah subset perubatan yang secara umumnya dianggap sebagai pelbagai kaedah rawatan yang tidak termasuk dalan rangkuman perubatan konvensional. Pengamalan CAM telah menjadi semakin popular di negara Asia, namun penggunaannya antara atlet kebangsaan Malaysia belum lagi didokumentasikan. Oleh itu, tujuan kajian ini adalah untuk menyelidik penggunaan CAM antara atlet kebangsaan Malaysia. Penyelidikan dijalankan ke atas 231 atlet untuk mengkaji penggunaan, sebab dan jenis terapi CAM digunakan oleh atlet. Kaji selidik menunjukkan bahawa penggunaan CAM di kalangan atlet adalah sangat tinggi dalam tempoh 12 bulan lalu, terutamanya atlet wanita. Ini mungkin disebabkan oleh pengurusan berat badan dan kemungkinan juga masalah berkait menstruasi. Didapati juga atlet yang berjumpa dengan pakar perubatan sering menerima preskripsi akupunktur. Di samping itu, atlet lelaki dan perempuan lebih gemar menggunakan CAM untuk mempercepatkan proses pemulihan, terutamanya dengan menggunakan vitamin C sebagai suplemen, dan kaedah berdoa untuk kesihatan sebagai amalan bantu-kendiri. Ini kerana penggunaan suplemen untuk pemulihan dan peningkatan prestasi adalah mudah

didapati dan harganya berpatutan. Oleh itu, ia menunjukkan bahawa atlet Malaysia sama seperti atlet dari negara lain yang bergantung kepada penggunaan CAM untuk menguruskan kesejahteraan mereka.

Adalah penting untuk mengkaji aspek suplemen herba yang boleh memberi kesan positif ke atas prestasi latihan. Kajian sebelum ini telah mencadangkan bahawa kapasiti latihan daya tahan boleh diperbaiki apabila pengoksidaan lemak bertambah.. Herba EL dihipotesiskan berupaya meningkatkan metabolisme lemak dan meningkatkan kekuatan otot semasa latihan, tetapi setakat ini buktinya masih kurang. Melalui reka bentuk kajian persampelan secara rawak, *double-blind* dan plasebo terkawal, kajian ini menyiasat kesan akut *E. longifolia* metabolisme aerobik atlet lelaki. Secara umum, ahli sukan yang terlibat diberikan samada *E. longifolia* atau plasebo, dan berlari di atas treadmill selama sejam pada kelajuan berdasarkan 65% VO_2max masingnya. Gas pernafasan dikumpulkan untuk analisis metabolisme karbohidrat dan lemak, manakala darah digunakan untuk analisis asid lemak bebas (FFA), gliserol, trigliserida, glukosa, insulin, kolesterol (iaitu jumlah kolesterol, HDL, LDL), testosteron dan kortisol. Kajian akut 3 hari suplemen *E. longifolia* (1.7 mg / kg berat badan) mengakibatkan peningkatan dalam metabolisme lemak semasa intensiti senaman sederhana, penurunan ketara dalam FFA dan LDL dan peningkatan yang ketara gliserol dan kortisol daripada analisis darah. Ini menunjukkan bahawa atlet dalam kumpulan *E. longifolia* cenderung kepada metabolisme lemak sebagai sumber tenaga dan bukannya glukosa, seperti yang dilihat dalam kumpulan plasebo. Dalam kajian lain, telah disabitkan atlet yang mengutamakan penggunaan lemak sebagai sumber tenaga akan mempunyai prestasi aerobik yang lebih baik. Bersamaan dengan keputusan akut, fenomena ini juga dapat dilihat dalam penggunaan *E. longifolia* yang berpanjangan sehingga 5 minggu. Oleh itu, penggunaan *E. longifolia* ditunjukkan dapat memberi manfaat kepada atlet yang bergantung kepada

prestasi aerobik dengan pengalihan metabolisme terhadap pembakaran lemak untuk hasil tenaga yang lebih baik.

Untuk terus mengkaji kesan penurunan lipid oleh *E. longifolia*, kajian ini menggunakan model *in vitro* untuk menyiasat sama ada ekstrak akar *E. longifolia* mempunyai potensi penurunan lipid atau perubahan adalah kesan sampingan fisiologi daripada suplemen ini. Sel hepatik manusia WRL-68 telah digunakan dalam kajian ini dan dirawat dengan pelbagai kepekatan (1, 2, 4 dan 8 μg / ml) ekstrak akar *E. longifolia* akuas dijalankan ke atas sel-sel hati berlemak. Keputusan menunjukkan pengurangan asid lemak intrasel sehingga ~ 72% dalam sel dirawat berbanding dengan sel kawalan yang tidak dirawat. Kajian ini oleh itu, menunjukkan potensi novel EL dalam pengurangkan lipid intrasel.

Kesimpulan daripada kajian menunjukkan epidemiologi penggunaan CAM kalangan atlet negara adalah tinggi terutama di kalangan atlet wanita. Penggunaan suplemen lebih diutamakan, dan berdoa untuk kesihatan adalah menjadi amalan bantu-kendiri yang diutamakan. Penemuan ini akan menjadi salah satu asas dalam dasar membuat untuk pengurusan atlet. Sebagai sebuah negara yang berkembang dalam sukan, suplemen *E. longifolia* boleh digunakan secara meluas sebagai alat bantuan ergogenik untuk meningkatkan pembakaran lemak sebagai sumber tenaga semasa latihan aerobik.

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ADP	Adenosine Diphosphate
AMP	Adenosine Monophosphate
ANOVA	Analysis of Variance
ASEAN	Association of Southeast Asian Nations
ATCC	American Type Culture Collection
ATP	Adenosine Triphosphate
CAM	Complementary and Alternative Medicine
CHO	Carbohydrate
CoA	Coenzyme A
CPET	Cardio Pulmonary Exercise Test
CPTI	Carnitine Palymitoyl Transporter I
DHEA	Dehyroepiandrosterone
DHT	5α-dihyrdrotestosterone
DMSO	Dimethyl sulfoxide
E. longifolia	*Eurycoma longifolia* Jack
ETC	Electron Transport Chain
FA	Fatty Acid
FFA	Free Fatty Acid
GSH	Gluthathione
HDL	High-density Lipoprotein
HED	Human Equivalence Dose
HSL	Hormone Sensitive Lipase
I-CAM-Q	International Complementary and Alternative Medicine Questionnaire
LC-MS	Liquid Chromatography – Mass Spectrometry
LD_{50}	Lethal Dose at 50%

LDL	Low-density Lipoprotein
LPL	Lipolipase Protein
K_m	Michaelis constant
MD	Medical Doctor
MGL	Monoglycerol Lipase
MY	Malaysia
NADH	Nicotinamide Adenine Dinucleotide
NCCAM	National Centre for Complementary and Alternative Medicine
NEFA	Non-Esterified Fatty Acids
NHIS	National Health Interview Survey
NHMS	National Health and Morbidity Survey
NY	New York
ParQ	Physical Activity Readiness Questionnaire
PG	Placebo
RQ	Respiratory Exchange Ratio
SD	Standard Deviation
SE	Standard Error
SEA	South East Asia
SHBG	Sex-hormone-binding-globulin
TC	Total Cholesterol
TCM	Traditional Chinese Medicine
TG	Triglycerides
USA	United States of America
UV	Ultraviolet
VCO_2	Volume of Carbon Dioxide Production
VO_2	Volume of Oxygen Consumption

VO_2max	Volume of Maximal Oxygen Uptake
WHO	World Health Organisation
1-RM	One Repetition Maximum

CHAPTER 1: GENERAL INTRODUCTION

Complementary and alternative medicine (CAM) is a group of diverse medical and health systems, products, and practices that are generally not considered as part of conventional medicine (Vidal, Carvalho, and Bispo 2013). The usage of CAM has gain popularity among general public and developed countries in the last few decades especially athletes as it helps in energy production and boosting performance (Alshagga *et al*. 2011, Vidal, Carvalho, and Bispo 2013). This could be due to many reasons such as, the only available health source, cultural and historical influences and those who are disadvantaged in both economically and socially (Bodeker and Burford 2006, Payyappallimana 2010).

A survey conducted on Malaysian population on CAM usage showed that there is a high prevalence in herb-based therapies for health related issues and health maintenance as herbs were shown to provide good source of protein, fat, minerals, crude fibres and energy (Siti *et al*. 2009, Kochhar, Nagi, and Sachdeva 2006). The use of herbs and plants as ergogenic aids in exercise and performance such as mahuang, ginseng, coffee beans and *Eurycoma longifolia* Jack (*E. longifolia*) or more commonly known as Tongkat Ali are quite common among athletes as these herbs and plants are believed to have ergogenic effect thus, helping in physical performance improvement (Chen, Muhamad, and Ooi 2012, Bucci 2000). Therefore, the purpose of this study is to examine the usage and the types of CAM therapies that are used by the national athletes. With such data, this will increase the knowledge about the epidemiology of CAM use and provide the foundation for policy making pertaining to sports management.

As mentioned above, *E. longifolia* has been claimed to enhance sports performance by promoting energy production and eliminating fatigue symptom physically and mentally (Ayu-Suzailiana *et al*. 2010, Chen, Muhamad, and Ooi 2012, Hamzah and Yusof 2003). Although it has been suggested that small peptides known as 'eurypeptides' in *E.*

longifolia helps in improving energy level by increasing the release rate of free testosterone from its binding hormone, sex-hormone-binding-globulin (SHBG) through regulation of testosterone concentration in the body, the actual mechanism have yet to be identified (Zanoli *et al*. 2009). Numerous studies has been conducted to determine the effect of *E. longifolia* on human performances however, there are still many grey areas that need to be clarified.

Over the past few years, investigations have been carried out to provide evidence that consumption of *E. longifolia* root extract can result in weight loss, increase fat oxidation and muscle strength while exercising (Hamzah and Yusof 2003, Henkel *et al*. 2014, Yusof *et al*. 2016, George *et al*. 2013). Thus, these findings suggest that consumption of *E. longifolia* might improve endurance performance in humans, possibly by increasing fat lipolysis. However, studies done on endurance exercises with consumption of *E. longifolia* supplementation did not show significant effect on endurance performance as this could be due to restricted parameters tested i.e. free fatty acids (FFA), glucose, to conclude the effect of *E. longifolia* on endurance performance (Muhamad, Ooi, and Chen 2015, Ooi *et al*. 2001).

Therefore, the main purpose of this study is to investigate the effects of *E. longifolia* root extract supplementation at an appropriate dosage (1.7 mg/kg of body weight) recommended by Li and co-researchers and to be consumed for 3 days before the experimental trial on recreational athletes that will trigger aerobic metabolism (Li *et al*. 2013). As previous studies done by the researchers showed significant results after 5 weeks intervention, we hypothesised that the consumption of *E. longifolia* root extract for 5 weeks at the dosage of 1.7 mg/kg of body weight would show improvement in aerobic metabolisms in recreational athletes.

Along with the compiling evidence that corroborates the therapeutic effects of this plant extract as efficacious concoction, it is noteworthy that novel potential for the use of

the plant extract in regulating fat metabolism has also been recently proposed. Results from *in vivo* and human trials are unable to determine if the decrease in lipid deposits was caused by the *E. longifolia* root extract or merely a side effect of the reported physiological changes. Therefore, this study adopts an *in vitro* model to investigate whether *E. longifolia* root extract possesses lipid lowering and/or absorption inhibitory potentials.

1.1 Objectives of the study

The use of CAM as ergogenic aids in exercise is common among athletes as these complementary or alternative medicines are believed to have properties that may improve the athlete's wellbeing and exercise performance. To date, the research concerning the use of CAM is limited in athletes. Although surveys have been done by researchers on the CAM utilization, many questions to be addressed as both failed to recognise the reason or situation that might lead athletes to seek CAM (Pike 2005, Nichols and Harrigan 2006).

Locally, *E. longifolia* is gaining popularity in its usage as ergogenic aids. However, lack of scientific evidence warrants an investigation of its acclaimed benefits in lipid-lowering effect. Previous studies done by researchers found that *E. longifolia* elicit ergogenic effect in strength exercises (Hamzah and Yusof 2003, Henkel *et al*. 2014, Yusof *et al*. 2016). To the best of our knowledge, there has not been any conclusive study conducted to relate the ergogenic effect of *E. longifolia* in endurance exercise.

Therefore, the main objectives of this report are:

1. To investigate the usage of complementary and alternative medicine among collegiate athletes in Malaysia,
2. To investigate the effect of acute consumption of *Eurycoma longifolia* Jack on aerobic metabolisms during prolonged exercise.
3. To investigate the effect of chronic consumption of *Eurycoma longifolia* Jack on aerobic metabolisms during prolonged exercise.
4. To investigate the lipid lowering effect of *Eurycoma longifolia* Jack in an isolated cell culture.

1.2 Hypotheses of the study

Null hypothesis (H_0)1:

There is no prevalence of CAM usage among Malaysian national collegiate athletes. The athletes did not use any form of CAM modalities in the past 12 months.

Alternative hypothesis (H_A) 1:

There is a high prevalence of CAM usage among Malaysian national collegiate athletes. The athletes used at least one kind of CAM modalities in the past 12 months.

Null hypothesis (H_0) 2:

The acute consumption of *E. longifolia* on aerobic metabolism during endurance running exercise did not differ from the placebo (PG). Consumption of *E. longifolia* did not promote lipolysis on the endurance athletes.

Alternative hypothesis (H_A) 2:

The acute consumption of *E. longifolia* on aerobic metabolism during endurance running exercise differ from the PG. Consumption of *E. longifolia* elicit promotes lipolysis on the endurance athletes.

Null hypothesis (H_0) 3:

The chronic consumption of *E. longifolia* on aerobic metabolism during endurance running exercise did not differ from the PG. Consumption of *E. longifolia* did not promote lipolysis on the endurance athletes.

Alternative hypothesis (H_A) 3:

The chronic consumption of *E. longifolia* on aerobic metabolism during endurance running exercise differ from the PG. Consumption of *E. longifolia* elicit promotes lipolysis on the endurance athletes.

Null hypothesis (H_0) 4:

The *E. longifolia* root extract did not have any lipid-lowering effect on hepatocyte cells.

Alternative hypothesis (H_A) 4:

The *E. longifolia* root extract elicit lipid-lowering effect on hepatocyte cells.

CHAPTER 2: LITERATURE REVIEW

2.1 Complementary and Alternative Medicine (CAM)

Complementary and alternative medicine (CAM) is defined by National Centre for Complementary and Alternative Medicine (NCCAM) as:

> "Complementary and alternative medicine (CAM) is a broad domain of healing resources that encompasses all health systems, modalities, and practices, and their accompanying theories and beliefs, other than those intrinsic to the politically dominant health system of a particular society or culture in a given historical period. CAM includes all such practices and ideas self-defined by their users as preventing or treating illness or promoting health and well-being. Boundaries within CAM and between the CAM domain and the domain of the dominant system are not always sharp or fixed" (Adler 1999).

Generally, people who opt for CAM therapies are seeking to improve their health and well-being, to relieve symptom which are chronic or to relieve the side effects that the conventional treatments has on them (Astin *et al*. 2000, Wolsko *et al*. 2002, Shen *et al*. 2002, Humpel and Jones 2006). There are also many other reasons for choosing CAM therapies such as cultural and historical influences, wanting a greater control over one's own health and the availability of the primary healthcare source in their country (Astin 1998, WHO 2015a).

According to the National Health Interview Survey (NHIS) in 2002, CAM treatment was often used to relieve back pain, head or chest cold, neck stiffness, joint pain, anxiety, depression or less commonly, to provide relief to cancer, cardiovascular diseases or lung diseases symptoms (Barnes *et al*. 2004, Saydah and Eberhardt 2006, Mao *et al*. 2007).

As for cultural and historical influences, China has been using acupuncture technique within the Traditional Chinese Medicine (TCM) over 2000 years ago to direct the flow of *Qi* or energy flow by inserting thin, long needles into the acupoints according to the theory of the meridians (Kong *et al*. 2007, Wu 1996).

In countries such as Africa, Latin America, and Asia, almost 80% of the population seek CAM therapies to meet their primary health care need and in India, CAM is the only available health source to 65% of the population (Payyappallimana 2010). Aside from that, CAM are now proven for quality, safety and efficacy thus, ensuring everyone have the access to CAM healthcare (WHO 2015a). In addition to that, it is culturally accepted and trusted by large number of people as it is close to home, accessible and most importantly, affordable (WHO 2015a). Thus, the population are not only restricted to conventional medicine but are able to enjoy the benefits of CAM as well.

In Asia, the usage of CAM may be the largest in the world. A survey conducted on the population of South East Asian countries showed that Malaysia (55.6%) demonstrated the highest percentage use of CAM therapies followed by Singapore (42.7%), Philippines (6.3%), Cambodia (5.4%), Vietnam (3.5%), Thailand (2.6%) and Indonesia (2.0%) (Peltzer and Pengpid 2015). Aside from that, researchers found that Malaysian population showed high prevalence in usage of CAM, particularly in herb-based therapies as herbs were shown to provide good source of protein, fat, minerals, crude fibres, and energy (Kochhar, Nagi, and Sachdeva 2006, Siti *et al*. 2009).

2.2 Prevalence of CAM use in Malaysia

In Malaysia, the increasing popularity and use of CAM have raised significantly over the years. A study conducted in 1996 by the Malaysia's National Health and Morbidity (NHMS) showed that 2.6% of the population visited CAM practitioners and 3.8% of the population visited both conventional and CAM practitioners (Siti *et al*. 2009). Another study conducted in 2004 using 6947 respondent showed that the prevalence of ever-used of CAM in their lifetime was 69.4% and at least 55.6% of the respondent had used CAM at least once in the last twelve months (Siti *et al*. 2009). More recently, NHMS 2015 showed in 7982 respondent, an estimate of 29.25% of the population visited CAM practitioners and 21.51% of the population used CAM therapies within the last twelve months with consultation. Further, the survey also showed that female users had a higher percentage (23.89%) compared to male user (19.33%) and the urban population had higher percentage of use (22.64%) compared to the rural population (18.23%). By ethnicity, the Chinese showed the highest prevalence (32.98%), followed by the Malay (31.36%) and 'Others' (24.44%) (NHMS 2015).

The main reason of the high prevalence of CAM use in Malaysia is likely due to the fact that Malaysian population practices CAM to conventional treatment. Malaysia is diverse in ethnicity and still follows generations of traditional healing practices supported by natural resources (Siti *et al*. 2009). The usage of CAM in treatments and maintaining wellness are deeply rooted and influenced by its multicultural nature (Ching *et al*. 2013). For example, based on the NHMS 2015, 68.04% of the population used Malay herbs to maintain wellness, 17.53% for treatment and 14.43% for combination whereas for Chinese herbs, 60.80% of the population used it to maintain wellness, 21.59% for treatment and 17.61% for both (NHMS 2015).

Another reason that contributes to the high prevalence of CAM use in Malaysia could be due to religion. Islam is found to be one of the strongest predictors of CAM therapies (Ching *et al*. 2013, Hamidah *et al*. 2009). The usage of CAM is embedded into the Muslim belief system and cultural heritage thus, it has been deeply integrated in their lives (Ching *et al*. 2013). As Muslim also believe in the sense of sympathy, empathy and respect of the rights to those who is in need, many CAM practitioners, especially from the rural area often prescribe CAM therapies to patients without any payment or at a minimum charge (Alatas 1967, Jeannot and Khairul Anuar 2012). Since Malaysia is an Islamic country, this may explain the high prevalence of CAM use in Malaysia.

2.3 Prevalence of CAM use in athletes

The high prevalence of CAM usage among athletes is not surprising as competitive athletes are highly motivated to speed up their recovery period from injuries and illness that may affect their ability to train and compete (Nichols and Harrigan 2006). The use of CAM therapies among athletes are often linked to boosting performance or to exceed the limit of the athletes' ability. According to White and co-researchers, athletes are a group of people that are very susceptible to conventional medicine (White *et al*. 1998). The researchers also claimed that athletes that are leading may explore alternative treatments which would give them advantage.

Athletes often view their body as an instrument as what they can do with their body (i.e. the fastest speed that they can run or the degree of flexibility of their body depending on the size of their body and muscle type) and judging the appearance of their body to suit the types of sports that they are involved (Franzoi 1995). Aside from that, the researcher also claimed that athletes value the opportunities which allows them to use

their body to perform and are more concerned about their bodies compared to non-athletes in terms of physical performances and ability.

According to Pike, female athletes are more likely to use CAM compared to male athletes. Pike reported that in her study, 59% of female athletes had used CAM therapies compared to 10% in male athletes. The researcher hypothesised that this could be due to team practitioners are rarely assigned to women's sports and team which resulted in female athletes seeking practitioners on their own, be it the conventional treatment or alternative treatment or the female athletes may find CAM therapies more gender-appropriate as it is perceived by many to empower and engage individuals more so than most biomedical treatments and, as such, may attract those who have suffered the most as the result of patriarchal structures in sport and biomedicine (Pike 2005).

Another study found that 56% of the subjects used at least one kind of CAM therapy in the past 12 months and the most commonly used modalities are massage, chiropractic, lomilomi (Hawaiian traditional massage) and acupuncture (Nichols and Harrigan 2006).. The researchers also reported that women visit medical doctors who prescribed CAM more frequently compared to men This could be due to gynaecological problems, more likely to care about their health or to improve their body image, in which all these factors could affect their performances as an athlete (Shih *et al*. 2012).

The commonly used CAM therapies by athletes includes natural products such as herbs and plants, supplements such as vitamins and minerals, self-help practices such as yoga, meditation and massage and seeking CAM practitioners such as acupuncturist or chiropractors. In 2007, Atkinson found that sports supplements such as creatine, whey protein, thermogenic and testosterone enhancer are commonly used by athletes to increase muscle mass whereas in 2012, Kong and co-researchers reported that Chinese massage and herbal ointment resulted in significant improvement in athletes who suffered from low back pain and muscle stiffness (Kong *et al*. 2012, Atkinson 2007).

This shows that CAM is frequently used by athletes as self-medication or performance booster (Theberge 2008b). Regardless the consequences, athletes will try their best to get back to the field no matter what it takes even if it conflicts with their health.

2.4 Energy substrate and metabolic pathway

Carbohydrate (CHO) and fat are the two main energy substrates responsible for the continuous resynthesis of adenosine triphosphate (ATP) during endurance exercise. During exercise, CHO contributes to 4 kcal/g of energy whereas for fat, 9 kcal/g of energy (Hunt and Stubbs 1975). The use of fat and CHO requires mobilisation of glycogen and triglyceride (TG) from the adipose tissue, liver, and skeletal muscle. These endogenous reserves are then delivered to muscle mitochondria for oxidation to produce ATP (Coyle 1995).

2.4.1 Carbohydrate metabolism

Glycogen present in the liver and skeletal muscle are the main storage form of CHO (Martin and Klein 1998). During exercise, most of the glycogen metabolised are derived from the intramuscular stores. The muscle glycogen concentration ranges between 10 – 30 g/kg of muscle mass (~10.4 MJ potential energy) and 80 g in the liver (~1.25 MJ potential energy). With the limited storage of glycogen, the energy supply from CHO is exhaustible during endurance exercises (Martin and Klein 1998, Newsholme and Leech 1984). With the rising exercise intensity, CHO in the form of glucose and glycogen plays an important role in supplying energy (Holloszy and Coyle 1984). CHO oxidation accounts for 10 - 15% of energy production in low exercise intensity (~30% VO_2max), 70 - 80% of energy production in high exercise intensity (~85% VO_2max) and 100% of

energy production at exercise intensities that are 100% VO_2max and above (Jensen and Richter 2012, Romijn *et al*. 1993, Holloszy and Kohrt 1996).

During endurance exercise, plasma glucose and those derived from glycogenolysis are phosphorylated to glucose-6-phosphate and further degraded through glycolysis to yield 2 moles of pyruvate and a net yield of 2 X ATP. Pyruvate is then transported into mitochondria to be converted to acetyl coenzyme A (CoA) and enter the Krebs cycle to further produce 2 X ATP and hydrogen atoms to be transported by the reduced coenzyme nicotinamide adenine dinucleotide (NADH) to the electron transport chain (ETC). With the presence of oxygen, the ETC accepts NADH to generate the proton motive force which will then be used to synthesise ATP and adenosine diphosphate (ADP). In ETC, 34 X ATP are produced (Jensen and Richter 2012, Robergs, Ghiasvand, and Parker 2004, Rose and Richter 2005).

2.4.2 Fat metabolism

Most energy in the body are stored as adipose tissue or TG. By far, TG is the largest energy reserve storing 40 X more than the amount of energy stored as glycogen in the skeletal muscle and liver. As a result, TG serves as an important source of energy during prolonged endurance exercise. In low exercise intensity (~25% VO_2max), lipolysis can increase two- to fivefold above resting level and remains relatively stable until 65% VO_2max. The increased demand of energy during endurance exercises are met by an increased rate of lipolysis; a process whereby TG are hydrolysed into FFA and glycerol.

The release of FFA from TG into the circulation to be used as a fuel during endurance exercises is regulated by the enzyme hormone sensitive lipase (HSL) and monoglycerol lipase (MGL) through a cascade of cellular signals (Horowitz 2003, Stephens, Constantin-Teodosiu, and Greenhaff 2007). In the adipocytes, phosphorylated

HSL moves to the surface of the lipid droplet. Along with phosphorylated perilipins, a family of proteins located on the surface of the droplets, HSL catalyse the hydrolysis of TG to yield two mole of FFA and one mole of monoglyceride. The monoglyceride is then hydrolysed by MGL into one glycerol and one FFA (Horowitz 2003).

Once the fatty acid (FA) are available in the cytoplasm, they must pass through the inner and outer membranes of the mitochondria before being oxidised. In comparison with the short and medium chain of FAs which are able to diffuse easily into the mitochondria, long chain of FAs requires transporters to diffuse into the mitochondria. Catalysed by acyl CoA synthetase, the long chain FAs are activated by addition of CoA to form long chain FA acyl-CoA. Then, carnitine palymitoyl transporter I (CPTI) found on the outer membrane of the mitochondria convert FA-acyl CoA to acyl-carnitine, which are then able to pass through the mitochondria via acylcarnitine translocase. While in the inner membrane of mitochondria, acyl-carnitine undergoes transesterification to become FA-acyl CoA which are then free to undergo β-oxidation to form acetyl CoA for ATP production (Saggerson and Carpenter 1981).

2.5 Substrate utilisation during endurance exercise

The rate of energy production and ATP turnover plays an important role during endurance exercise through aerobic and anaerobic metabolism (Joyner and Coyle 2008). During endurance exercise, fat and CHO are responsible for the ATP production in human skeletal muscle (Coyle *et al*. 1985). The utilisation of fat and CHO during endurance exercise is determined by many factors such as gender, dietary intake and mainly, exercise intensity (Ruby and Robergs 1994, Van Baak 1999, Tarnopolsky 2000, Carter, Rennie, and Tarnopolsky 2001, Coyle *et al*. 1985).

As the exercise intensity increases, there will be a shift in energy substrate utilisation and mobilisation whereby higher relative contribution of CHO oxidation to energy expenditure and lower relative contribution of fat oxidation to energy expenditure (Martin and Klein 1998). In human studies, high exercise intensity showed absolute rates of hepatic glucose production, glycogenolysis of skeletal muscle and increased whole body CHO oxidation (Coggan 1991). In contrast, moderate intensity exercise showed increase in whole body fat oxidation.

Previous studies have shown that the contribution of fat was higher at rest whereas the contribution of CHO was higher during high-intensity training (> 65% VO_2max) (Romijn *et al*. 1993, Krogh and Lindhard 1920). Using isotope tracers and indirect calorimetry methods, Romijn and co-workers showed that high exercise intensity (85% VO_2max) favours glucose metabolism whereas low and moderate exercise intensity (25 and 65% VO_2max) favours fat metabolism in trained cyclists (Romijn *et al*. 1993). Further study using the same methods has been conducted by van Loon and fellow researchers demonstrated that whole body fat oxidation increased at 55% maximal workload (W_{max}) whereas glucose oxidation rised at 75% W_{max} (van Loon *et al*. 2001). Similarly, a study conducted by Achten and team found that the maximal fat oxidation rate was at 64 ± 4% VO_2max in moderately active men during graded cycling exercise. Aside from that, it was also shown that there is a steep declination in fat oxidation and gradual inclination in CHO oxidation as the intensity of the exercise increased (Achten, Gleeson, and Jeukendrup 2002).

It is suggested that during high exercise intensity, the declination of muscle free carnitine concentration reduces long chain FA oxidation thus, muscle glycogen has become the main fuel to meet the immediate demand of energy (van Loon *et al*. 2001, Romijn *et al*. 1993, Myopathy 1973). The decrease of fat oxidation during high exercise intensity can also be explained by decline availability of non-esterified fatty acids

(NEFA) concentrations. However, raising the NEFA concentration using lipid emulsion does not completely restore the rate of fat oxidation even at moderate intensity (Romijn *et al*. 1995). The suppression of fat oxidation during high intensity is likely to be related to the fatty acid metabolism within skeletal muscle itself (Martin and Klein 1998). Based on the previous studies, the optimal intensity to induce fat oxidation would be at 65% VO_2max.

2.6 Effect of herbs on fat metabolism in endurance exercise

Herbs have been used throughout history as ergogenic aid to increase fat metabolism as well as improving endurance exercise training among athletes. Herbs is defined as a plant which can be used for medicine, cooking, cosmetics and as a scent or dye (Chen, Muhamad, and Ooi 2012). There are many types of herbs available on the sports nutrition market nowadays that offer nutritional support to endurance exercise training such as *E. longifolia*, *Panax* ginseng, mahuang (Chinese ephedra) and coffee beans (Bucci 2000, Chen, Muhamad, and Ooi 2012). Of the herbs mentioned above, *E. longifolia* has recently gained popularity among athletes in reducing fat free mass and increasing muscle mass.

2.7 *Eurycoma longifolia* Jack

The plant, *E. longifolia* is a tropical herbal plant primitive to South-East Asian countries such as Malaysia, Indonesia, and Vietnam. The plant extract, especially root, has claimed to possess various medicinal properties such as anticoagulant, antimalarial, antipyretic, aphrodisiac, antibacterial, anticancer, antihyperglycemic and antianxiety (Osman *et al*. 2003, Ang and Sim 1997, 1998a, b, Ang, Cheang, and Yusof 2000, Ang and Lee 2002, Ang *et al*. 2002, Chan *et al*. 1989, Chan *et al*. 1986, Farouk and Benafri

2007, Husen, Pihie, and Nallappan 2004). Regular consumption of *E. longifolia* root extract is also believed to increase testosterone concentration thus, attracting the interest of body builders to increase muscle mass and strength.

Recently, the demand for *E. longifolia* products has increased tremendously mainly, highlighting the aphrodisiac effects of the root extract. The dried *E. longifolia* roots are known to cost somewhere between 20 to 25 US dollar/kg whereas the water extracts of the roots are known to cost about 26 US dollars per bottle of 60 capsules. Due to the bitterness of the *E. longifolia* root extract, most of the products are available either in the form of raw crude powder as capsules or as an additive in coffee mixture (Kaur, Kumaresan, and Sarmidi 2003).

2.8 Taxonomy of *E. longifolia*

Although there are four different species of Tongkat Ali which includes *E. longifolia*, *Entomophthora apiculata, Polyathia bullata* and *Goniothalamus* sp., *E. longifolia* is more commonly used for traditional medicinal purposes (Fazlin, Ahmad, and Lim 2002). In Malaysia, *E. longifolia* can be found in Langkawi, Melaka, Johor, Pahang and Terengganu (Bhat and Karim 2010).

The plant, *E. longifolia* is a slow growing herbal plant which could reach up to 18 meters in height and only bearing fruits after 2 - 3 years of cultivation (Figure 2.1). Each leaf compound of the plant consists of 30 – 40 leaflets which is about 5 – 20 cm long and 1.5 – 6 cm wide. Although the complete maturation of the plant take up to 25 years, the roots of the plant are harvested after 4 years for commercial use. As the root of *E. longifolia* penetrates deep into the soil, harvesting of the roots requires a lot of time (between 8 to 10 hours) and to be carried out manually. The *E. longifolia* plant grows

well with presence of partial shade, adequate amount of water and survives on a variety of soil but preferably, acidic well-drained soil (Bhat and Karim 2010, Ismail *et al*. 1999).

In Malaysia, *E. longifolia* has been declared as a protected plant due to its acclaimed benefits as herbal remedies for various illness. Most parts of the plants are used traditionally as remedies however, the roots of the plant are the most valuable component as it has been proven to restore energy and vitality, enhancing blood flow and functioning as a cure for various types of diseases (Perry 1980, Darise *et al*. 1982). In the olden days, the roots of the plants are cut into small portions, boiled and consumed as a tea. However, due to the bitterness, intense boiling and addition of honey, sugar syrups or dates are used to reduce the bitterness. It is suggested by traditional healers that the more bitter, the better its efficacy (Bhat and Karim 2010).

Figure 2. 1: *Eurycoma longifolia* Jack plant in a natural habitat (Bhat and Karim 2010)

2.9 Chemical components of *E. longifolia*

Most of the pharmacological effect are related to the biological active compound of the plant, especially the roots. According to Kuo and his co-researchers, sixty five phenolic compounds have been isolated and characterised from *E. longifolia*. For instance, quassinoids, β-carboline alkaloids, canthin-6-one alkaloids, titerpene-type tirucallane, squalene derivatives, eurycolactone, eurycomalactone, laurycolactone, biphenyl neo-ligan and bioactive steroids (Kuo *et al*. 2004).

Among the compounds mentioned, the bitter tasting quassinoids are majorly found in the roots of *E. longifolia* (Figure 2.2). The quassinoids, namely 13β-dihydroxyeurycomanol, 21-dihydroxyeurycomanol, 5α-trihydroxyklaineanone, 14β-trihydroxyklaineanone, 15β-trihydroxyklaineanone, eurycomalide A, eurycomalide B, eurycomanol-2-*O*-β-d-glycopyranoside, eurycomanol, eurycomanone A and eurycomanone B are found to possess antimalarial and antiulcer activity (Ang, Chan, and Mak 1995b, Chan *et al*. 1989, Chan *et al*. 1986, Tada *et al*. 1991, Kuo *et al*. 2004).

Aside from that, quassinoids that are isolated from the leaves such as lonilactone, 6-dehydro lonilactone, 14,15β-dihydroxyklaineanone, 11-dehydroklaineanone, 15-β-*O*-acetyl-14-hydroxyklaineanone, 12-epi-dehydroklaineanone and 15β-hydroxyklaineanone, are claimed to possess anti-tumour and anti-parasitic properties (Jiwajinda *et al*. 2001).

In addition, 2 isomeric and 2 biphenyls of biphenylneolignans (2,2′-dimethoxy-4-(3-hydroxy-1-propenyl)-4′-(1,2,3-trihydroxypropyl) diphenyl ethers, 2-hydroxy-3,2′-dimethoxy-4′-(2,3-epoxy-1-hydroxypropyl)-5-(3-hydroxy-1-propenyl)-biphenyl, 2-hydroxy-3,2′,6′-trimethoxy-4′- (2,3-epoxy-1-hydroxypropyl)-5-(3-hydroxy-1-propenyl)-biphenyl) and 5 canthin-6-one alkaloids (9,10-dimethoxycanthin-6-one, 10-hydroxy-9-methoxycanthin-6-one, 11-hydroxy-10-methoxycanthin-6-one, 5,9-dimethoxycanthin-6-

one and 9-methoxy-3-methylcanthin-5,6-dione) are isolated from the bark and wood of *E. longifolia* (Morita *et al*. 1992, Mitsunaga *et al*. 1994).

2.10 Analytical Methods

Due to the small amount of metabolites present in *E. longifolia*, liquid chromatography with mass spectrometry (LC-MS) are recognised as the most suitable tool for analysing the quassinoids bio-constituents of the plant. Numerous studies have reported using photodiode array or fluorescence and ultraviolet (UV) detection but none of the methods are sensitive enough to detect the nonchromophoric bioactive constituents such as eurycomanol (Choo and Chan 2002, Chan *et al*. 1998, Tan, Yuen, and Chan 2002, Rehman, Choe, and Yoo 2016).

Using three LC-MS hybrid systems (QTof, QTrap, and TripleTof), Chua and co-researchers are able to scan and detect the targeted metabolites, such as alkaloid, quassinoids, triterpene and biphenylneolignans from *E. longifolia* extracts (Chua *et al*. 2011). In addition, Teh and co-researchers developed and optimised a liquid chromatography with mass spectrometry method which allows simultaneous determination of bioactive compound from *E. longifolia* extracts (Teh, Murugaiyah, and Chan 2011). The LC-MS method simultaneously determine six major quassinoids from *E. longifolia* namely, eurycomanone, eurycomalactone, 13α(21)-epoxyeurycomanone, 13,21-dihydroeurycomanone, 14,15β-dihydroxyklaineanone and longilactone (Teh, Murugaiyah, and Chan 2011).

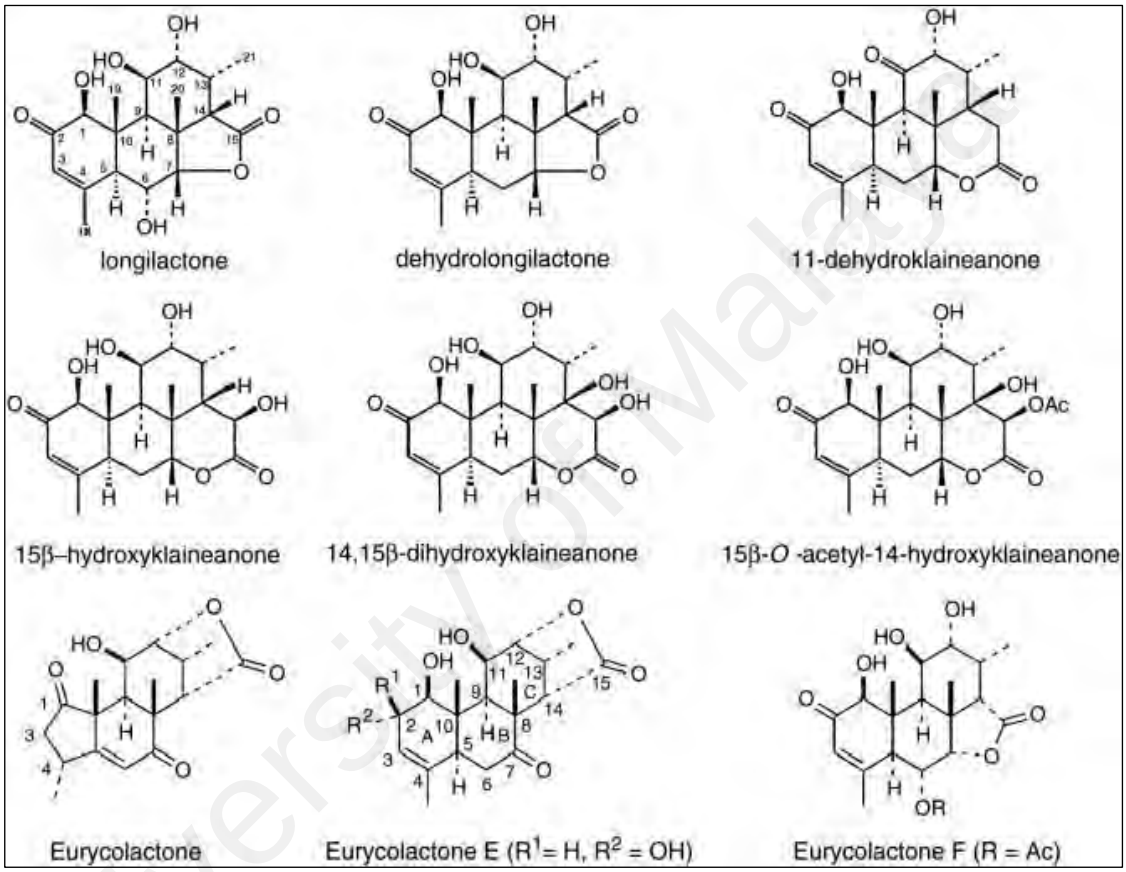


Figure 2. 2: Chemical structure and compound of the quassinoids isolated from *Eurycoma longifolia* Jack (Bhat and Karim 2010).

2.11 Pharmacological properties

2.11.1 Aphrodisiac properties

The root extract of *E. longifolia* has been popular for its aphrodisiac properties as it is claimed to improve strength, stamina and power during sexual activities (Ang and Lee 2002, Ang, Cheang, and Yusof 2000). Using *E. longifolia* roots extract of different fractions (chloroform, methanol, water and butanol) to investigate the libido of two hundred and forty sexually experienced male rats aged 3 – 4 months with various doses (200, 400 and 800 mg/kg of body weight, 2 times daily for 10 days), Ang and Sim reported that the mounting frequency of the rats was dose-dependent and those with chloroform fractions were observed to have the highest mounting frequency. However, no erections, intromission and ejaculation were observed in the 20 min observation period. With the results obtained, the researchers claimed that *E. longifolia* could be a potent sex arousal stimulator with the absence of genital sensation feedback (Ang and Sim 1997).

Later on, Ang and Lee performed another investigation to determine the changes in sexual behaviour of middle-aged rats with different fractions of *E. longifolia* root extracts. The investigation reported that 800 mg/kg of *E. longifolia* increases orientation activities of the male rats towards the female rats (anogenital sniffing, licking and mounting). It is also observed that the male rats increased their genital grooming and restrict themselves to a particular area of the cage (Ang and Lee 2002).

Sexual motivation are also increased after the consumption of *E. longifolia* root extract. Administering of 500 mg/kg of *E. longifolia* root extracts with different fractions (chloroform, methanol, butanol and water) daily for 10 days on middle-aged male mice are shown to increase momentary response in 50% of the mice (Ang, Lee, and Kiyoshi 2003).

In another study, Ang and co-researchers investigated the effects of *E. longifolia* root extracts on the sex qualities of the middle-aged rats. An administration of 0.5 g/kg of body weight with various fractions of extract daily for 12 weeks using electrical copulation cage demonstrated that the sex qualities of the rats were enhanced by decreasing the hesitation time as compared with controls. Along with that, the researchers also found that more than 50% of the male rats responded to the right choice of partner after 2 weeks post-treatment (Ang, Ngai, and Tan 2003).

The sexual arousal of sluggish old male rats (24 months old, retired breeder) were also investigated by Ang and co-researchers. The rats were administered with 200, 400 or 800 mg/kg of body weight of different fractions of *E. longifolia* root extracts 2 times daily for 10 days. As the rats performed acts such as yawning or stretching, it will be considered as identity for sexual arousal. At 800 mg/kg of body weight of the *E. longifolia* extract, the researchers observed that 50% of the rats performed yawning and 16.7% of the rats performed stretching, which indicates that *E. longifolia* extracts possessed aphrodisiac properties (Ang, Lee, and Kiyoshi 2004).

In human trials, a randomised, double-blind, placebo-controlled, parallel group study was carried out on 109 men aged between 30 – 55 years old to investigate the aphrodisiac properties of *E. longifolia* root extracts. The groups were administrated with either 300 mg of *E. longifolia* water extract or PG for 12 weeks. The *E. longifolia* group demonstrated significant higher score in erectile-function-domain, sexual libido and seminal fluid analysis (sperm mortality and volume) compared to the PG group (Ismail *et al*. 2012).

In another study, the sexual performance and well-being was investigated on men aged 40 – 65 years old. Using a randomised, double-blind, placebo-controlled study design, the volunteers were administrated with 200 mg of *E. longifolia* extracts (Physta®) for 12 weeks. The results demonstrated significant improvement in sexual intercourse

attempt diary score, erection hardness scale score, sexual health inventory score and ageing male symptom scale score. The study concluded that *E. longifolia* extract was well-tolerated and effective in enhancing sexual performances in healthy volunteers.

The water extract of *E. longifolia* is also shown to relieve aging male symptom among elderly men. In a study, Tambi and co-researchers administered 200 mg of *E. longifolia* water extract on a group of men who suffered from late-onset hypogonadism for a month. The results of the study demonstrated significant improvement in the ageing male symptom scores as well as serum testosterone concentration (Tambi, Imran, and Henkel 2012).

2.11.2 Antimalarial properties

The effect of *E. longifolia* root extracts on antimalarial properties have also been studied extensively in the past. In 2013, World Health Organisation (WHO) reported that there were 207 million cases of malaria and 627 000 deaths caused by *Plasmodium* sp., especially *Plasmodium falciparum* malaria (WHO 2015b). Interestingly, previous study have reported that *E. longifolia* extracts has good antimalarial effect against *Plasmodium* sp. or the intermittent fever.

Chan and co-researchers investigated the effects of *E. longifolia* extract for antiplasmodial activity against drug resistance Thailand strain (K-1) of *Plasmodium falciparum* under *in vitro* condition. The study found that isolated metabolites, 10-hydroxycanthin-6-one, eurycomalactone, eurycomanone and eurycomanol from the *E. longifolia* extract demonstrated antimalarial activities (Chan *et al*. 1986). Aside from that, Kardono and co-researchers found that two compounds from the *E. longifolia* extract, eurycomanone and 7-methoxy-β-carboline-1-propionic acid exhibit significant antimalarial activity against *Plasmodium falciparum* strains (Kardono *et al*. 1991).

In 2009, Wernsdofer and co-researchers analysed the effects of *E. longifolia* extracts against *Plasmodium falciparum* isolates. A standardise extract of *E. longifolia* containing there major quassinoids (eurycomanone, 13,21-dihydroeurycomanone and 13α(21)-epoxyeurycomanone) were evaluated for the antiplasmodial activity against the isolates and the activity was compared with that of artemisinin, using thirty eight fresh parasite isolates and inhibition assessment of schizont maturation.

The results showed that those with *E. longifolia* extract demonstrated higher inhibitory activity than the three quassinoids isolated from the plant, suggesting that the antimalarial effect could be due to synergism between quassinoids or presence of unidentified compound in the *E. longifolia* extract (Wernsdorfer *et al*. 2009).

In a study conducted by Ang and his co-researchers, the effects of daily replacement of culture medium containing *E. longifolia* extract against six Malaysian *Plasmodium falciparum* isolates was investigated. Ang reported that the antimalarial activity of *E. longifolia* extract was dose-dependent and a complete inhibition were observed after 3 days of post-treatment (Ang, Chan, and Mak 1995a, b). At certain dosage and incubation period, the methanol extract of *E. longifolia* showed decreased gluthathione content (GSH) in both infected and healthy erythrocytes. The decreased GSH of host and parasite showed *in vitro* growth inhibition of *Plasmodium falciparum* and thus, screening activity of GSH can be one of the procedures in evaluating the antimalarial properties of herbal products (Mar *et al*. 2005).

2.11.3 Antimicrobial properties

Farouk and co-researchers demonstrated that the alcoholic and acetone extracts of *E. longifolia* leaves and stem are effective against both gram-positive and gram-negative bacteria *Escherichia coli* and *Salmonella typhi*. In addition, the aqueous leaves extract of

E. longifolia also showed antibacterial activity against *Staphylococcus aureus* and *Serratia marscesens.* Although the root of *E. longifolia* possess various medical properties, it does not exhibit antibacterial activity against both gram-positive and gram-negative bacteria (Farouk and Benafri 2007).

In another study, the extract of *E. longifolia* and *L. pumila* leaves were analysed for their antibacterial activity against the human pathogenic gram positive and gram negative bacteria, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Both extracts were prepared in different solvents (acetone, methanol, ethanol, and phosphate buffer and concentrations ranging from 5 to 100 mg/mL. The results demonstrated that most of the extracts exhibit relatively high antibacterial activity with the inhibition zone ranging from 7 to 25 mm against the tested bacteria. Aside from that, 25 mg/mL of extracts in phosphate buffer and 75 mg/mL of extracts in ethanol are showed to inhibit growth of *Pseudomonas aeruginosa* and *Staphylococcus aureus* respectively (Farouk, Nawi, and Hassan 2008).

Aside from that, Kong and co-researchers found that *E. longifolia* extract improved the survival of *Staphylococcus aureus*–infected worms by 2.8 fold, suggesting that the extract could either possibly activate the host immunity to eliminate the bacteria or to interfere with the factors that prevent pathogen accumulation (Kong *et al*. 2014).

2.11.4 Anti-anxiety properties

The anti-anxiety properties of *E. longifolia* extract was investigated in mice using various types of behaviour tests which includes, the open field test (emotional state), elevated plus-maze test (anxiety and anxiogenic drug test) and anti-fighting test (Ang and Cheang 1999).

As for humans, the effects of *E. longifolia* extract was tested on 63 subjects (32 men and 31 women) for stress hormone and mood state. The results showed that daily

supplementation of 200 mg/day of *E. longifolia* extract for 4 weeks demonstrated significant improvement in stress hormone profile and certain mood state parameters compared with PG (Talbott *et al*. 2013).

2.11.5 Antidiabetic properties

In a study, *E. longifolia* extract was administrated to streptozotocin-induced adult rats and normoglycaemic rats to determine the glucose lowering effect. The results showed that 150 mg/kg of body weight of two different form of *E. longifolia* extracts; freeze-dried (TA-a) and spray-dried (TA-b), demonstrated blood glucose decreased in streptozotocin-induced adult rats. In normoglycaemic rats, no significant reduction was observed when administrated with the same amount of *E. longifolia* extracts (Husen, Pihie, and Nallappan 2004).

Consumption of *E. longifolia* is shown to increase insulin sensitivity which could benefit those who are already suffering from diabetes. Lahrita and co-researchers investigated on the effect of *E. longifolia* extract on glucose uptake enhancement and lipid suppression in 3T3-L1 adipocytes. The investigation showed that 50 µg/mL of *E. longifolia* extract increased insulin sensitivity by enhancing glucose uptake more than 200% and supressed lipid accumulation in a concentration-dependent manner. The results of the investigation suggest that consumption of *E. longifolia* extract could benefit the treatment of diabetes by suppressing lipid production as well as increasing insulin sensitivity (Lahrita, Kato, and Kawabata 2015).

2.12 Pharmacokinetic properties of *E. longifolia*

2.12.1 Absorption

In animal research, the bioavailability of the constituent eurycomanone was investigated (Low *et al*. 2005). The eurycomanone was detected in the plasma and declining to zero within eight hours following intravenous injection. As for oral administration, the C_{max} and T_{max} values were 0.33 ± 0.03 µg/mL and 4.40 ± 0.98 h, respectively. Plasma concentration was lower following oral administration compared to intravenous injection despite at much higher oral dose (five times the dose). The investigation concludes that the oral bioavailability of eurycomanone is relatively poor compared to intravenous injection.

In another animal study, the constituent 13α(21)-epoxy-eurycomanone had a higher C_{max} compared to eurycomanone (1.61 ± 0.41 µg/mL vs 0.53 ± 0.10 mcg/mL) following oral administration of a standardise *E. longifolia* extract. The higher C_{max} in 13α(21)-epoxy-eurycomanone could be due to increased membrane permeability compared to eurycomanone (higher log Kw value of – 0.43 vs – 1.46 at pH 1). However, following the oral administration of the standardise *E. longifolia* extract, both constituent 13α(21)-epoxy-eurycomanone and eurycomanone were not detected in the plasma (Low *et al*. 2011).

2.12.2 Distribution and excretion of *E. longifolia*

In the animal study conducted by Low and co-researchers, it is found that the volume of distribution of eurycomanone was relatively high (0.68 ± 0.30 L/kg). The results of the study suggest that eurycomanone is well distributed in the extravascular fluid (Low *et al*. 2005).

Following the intravenous injection of the *E. longifolia* extract, Low and co-researchers found that the mean elimination rate constant and clearance of the eurycomanone were 0.88 ± 0.19 h^{-1} and 0.39 ± 0.08 L/h/kg, respectively (Low *et al*. 2005).

2.12.3 Half-life of *E. longifolia*

Following an intravenous injection of a standardised *E. longifolia* extract in an animal study, Low and co-researchers found that the constituent, 13α(21)-epoxy-eurycomanone had longer biological half-life compared to eurycomanone (0.75 ± 0.25 h vs 0.35 ± 0.04 h) due to lower elimination rate constant (Low *et al*. 2011). In contrast, another study conducted reported that the biological half-life of eurycomanone was 1.00 ± 0.26 h (Low *et al*. 2005).

2.13 Safety and toxicity of *E. longifolia*

The plant, EL have been used as a CAM for generations in Malaysia. It is only in the late 1990s that its safe dosage and toxicity profile are investigated. In animal studies, the acute toxicity study conducted by Satayavivad and co-researchers found that oral Lethal Dose (LD_{50}) of alcoholic *E. longifolia* extract in mice is between 1500 – 2000 mg/kg of body weight whereas for water *E. longifolia* extract, the LD_{50} is more than 3000 mg/kg of body weight (Satayavivad *et al*. 1998). The researchers further showed that daily consumption of ethanolic *E. longifolia* extract of 200 mg/kg of body weight and aqueous *E. longifolia* extract of 300 mg/kg of body weight were not toxic. However, dosages of *E. longifolia* extract above 1200 mg/kg of body weight exhibit hepatotoxic

effects in rate due to the n-butanol fraction of *E. longifolia*, mainly eurycomanone (Chan, Low, and San Ho 2007, Shuid *et al*. 2011).

As the composition of ethanolic, n-butanolic- and aqueous-based fractions of *E. longifolia* extracts differs from one another, the LD_{50} as well as daily effective dosage would also vary among fractions. Therefore, the water-based fraction is considered to be the safest as the LD_{50} of water-based fraction is relatively high (> 3000 mg/kg of body weight) compared to the other fractions (Rehman, Choe, and Yoo 2016).

Choudhary and co-researchers investigated the acute, subacute and subchronic toxicity of aqueous *E. longifolia* extract (Physta®) on male and female Wistar rats. Both rats were treated with *E. longifolia* extract concentrations ranging from 250 mg/kg of body weight to 2000 mg/kg of body weight for 90 days. The results demonstrated that there were no significant changes in blood chemistry and haematological parameters. There were also no histopathological changes observed and in the acute toxicity test, no changes were observed in mortality ad behaviour of the rats (Choudhary *et al*. 2012).

The Endocrine Society states that prostate cancer are regarded as a contradiction of any testosterone treatment (Bhasin *et al*. 1997). Considering that consumption of *E. longifolia* extract can increase serum testosterone concentration, there could be a potential risk that elderly men might cause prostatic problems (Rehman, Choe, and Yoo 2016).

Using a randomised, double-blind, placebo-controlled study design, Ismail and co-researchers reported that there were no differences in the serum prostate-specific antigen level in treated group compared to PG (Ismail *et al*. 2012). Aside from that, Li and co-researchers revealed that neither mutagenicity nor clastogenicity was identified and the acute LD_{50} was more than 6 g/kg of body weight of *E. longifolia* extract. In an investigation carried out by Li and co-researchers, after 4 weeks subacute and 13 weeks subchronic exposure of 0, 0.6, 1.2 and 2 g/kg of body weight *E. longifolia* extract did not

exhibit any adverse effects in respect to body weight, haematology, urinalysis, macropathology, histopathology or serum biochemistry parameters. Although the calculated accepted daily for *E. longifolia* extract is up to 1.2 g/adult/ day, Li and co-researchers recommended that 1.7 mg/kg of body weight would be sufficient to elicit the benefits of *E. longifolia* extract (Li *et al*. 2013).

With regards to pancreas, Hamoud and Qamar suggest that low dosage of *E. longifolia* extract does not have any side effect or any deleterious effect on the pancreatic tissue and can be considered as a safe herbal supplementation so far the pancreas in human being is concerned (Hamoud and Qamar 2013).

With regards to liver and renal function, Chen and co-researchers reported that 400 mg/day of standardise *E. longifolia* water extract (Physta®) for 6 weeks did not show significant changes to liver and renal functions. The results suggest that *E. longifolia* water extract supplementation at 400 mg/day for 6 weeks is safe and does not exhibit toxicity effect on the liver and renal functions (Chen *et al*. 2014).

As the toxicity studies are done on animals, the Food and Drug Administration (FDA) suggested that the extrapolation of animal doses to human doses can be correctly performed through normalisation to BSA, which is normally presented in mg/m^2. The human dose can be approximately calculated by using the formula and conversion list (Table 2.1) suggested by the FDA (FDA 2005).

Human Equivalent Dose (HED) (mg/kg) = Animal dose (mg/kg) [Animal K*m* / Human K*m*].

Table 2. 1: Conversion of Animal doses to human equivalent doses (HED) based on body surface area.

Species	To Convert Animal Dose in mg/kg to Dose in mg/m^2, Multiply by k_m	To Convert Animal Dose in mg/kg to HED[a] in mg/kg, Either:	
		Divide Animal Dose By	Multiply Animal Dose By
Human	37	---	---
Child (20kg)[b]	25	---	---
Mouse	3	12.3	0.08
Hamster	5	7.4	0.13
Rat	6	6.2	0.16
Ferret	7	5.3	0.19
Guinea pig	8	4.6	0.22
Rabbit	12	3.1	0.32
Dog	20	1.8	0.54
Primates:			
Monkey[c]	12	3.1	0.32
Marmoset	6	6.2	0.16
Squirrel monkey	7	5.3	0.19
Baboon	20	1.8	0.54
Micro-pig	27	1.4	0.73
Mini-pig	35	1.1	0.95

The HED can be calculated from the following formula: HED = animal dose in mg/kg x (animal weight in kg/human weight in kg). The km value in the table is provided for reference only since healthy children will rarely be volunteers for phase 1 study (FDA 2005).

2.14 The use of *E. longifolia* as ergogenic aid

The ergogenic effects of *E. longifolia* on exercise is scarce in the literature. As increased testosterone is shown in animal models, researchers have been suggesting that consumption of *E. longifolia* could benefit athletes in promoting stamina, increasing muscle mass and muscle strength (Chan *et al*. 2009).

2.14.1 Mechanism to describe the ergogenic effect of *E. longifolia*

Although previous works have been carried out to investigate the acclaimed benefits of EL extract, the exact mechanism of *E. longifolia* as ergogenic aid have not been identified. Zanoli and co-researchers suggested that *E. longifolia* contains small peptides known as 'eurypeptides' which help in improving energy level of rodents. The mechanism of action is related to the release of free testosterone from its binding hormone, SHBG thus, stimulating lipolysis (Zanoli *et al*. 2009).

The mechanism action of increment in serum testosterone level with *E. longifolia* supplementation is still unclear but there appears to be more than one mechanisms that have been suggested by researchers. Ali and Saad suggested that eurypeptides found in *E. longifolia* activate the CYP17 enzyme which is responsible for the metabolism of pregnenolone and 17-OH-pregnenolone to produce more dehyroepiandrosterone (DHEA). From there, progesterone and 17-OH-progesterone is further metabolised into 4-androstenedione and testosterone (Ali and Saad 1993).

In another study, Low and co-researchers claimed that the enhanced testosterone production happen when there is an inhibition of phosphodiesterase and aromatase by eurycomanone in the Leydig cells. In the study, male rats were treated with 25 mg kg^{-1} of eurycomanone which significantly increased testosterone level in the plasma while a dosage of 50 mg kg^{-1} showed adverse effect. It was seen that 25 mg kg^{-1} of eurycomanone

stimulated higher LH secretion, leading to an increase in testosterone level (Low *et al*. 2013).

As testosterone are known as fat reducing hormone, it could inhibit the uptake of lipid and lipoprotein lipase (LPL), as well as increasing the number of lipolytic β-adrenergic receptors to stimulate lipolysis and therefore, contribute to energy production through fat metabolism (De Pergola 2000).

2.14.2 Effects of *E. longifolia* on fat metabolism in cell

Lahrita and co-researchers found that methanol *E. longifolia* extract suppressed lipid production in 3T3-L1 adipocytes of rats. The root of *E. longifolia* were extracted with 50% aqueous methanol and dissolved in 50% DMSO when tested in the adipose cells. Using the Oil-Red-O staining technique, *E. longifolia* extract showed lipid inhibition activity and the inhibition was not attributed to their cytotoxicity in the adipose cells (Lahrita, Kato, and Kawabata 2015).

2.14.3 Effect of *E. longifolia* on fat metabolism in animals

Solomon and co-researchers investigated the *in vivo* effects of *E. longifolia* in body and organ weight as well as sperm qualities of male rats. In the study, 42 rats were divided into 3 groups; control, low dose (200 mg/kg of body weight of aqueous *E. longifolia* extract) and high dose (800 mg/kg of body weight of aqueous *E. longifolia* extract). The rats were fed for 14 days and sacrificed. The results showed that there was a significant decrease in body weight (5.7%) and omentum fat (31.9%) as well as increased in sperm concentrations (25.1%) (Solomon, Erasmus, and Henkel 2014).

2.14.4 Effect of *E. longifolia* on exercise and fat metabolism in humans

Although there are improvement in strength training after the consumption of *E. longifolia*, studies have also shown that *E. longifolia* might not be an effective ergogenic aid for improving endurance performances. In regards to endurance exercises, Ooi and co-researchers reported that acute effect of *E. longifolia* in herbal drinks could not enhance endurance performance in cycling. In the study, each trained young cyclist ingested either 0.67 mg of either *E. longifolia* or PG and cycle as long as possible at 70% VO_2max for the first hour and 80% VO_2max until exhaustion during the experimental trial. The study found that there is no significant differences in terms of cycling performance, physiological response (i.e. heart rate, oxygen consumption), plasma glucose and lactate concentrations between the supplements. The results suggest that acute supplementation of *E. longifolia* in herbal drinks did not exhibit any potential benefits in improving cycling performance and physiological responses (Ooi *et al*. 2001).

As an extension the above study, Ayu-Suzailiana and co-researchers investigated the effect of *E. longifolia* on recreational athletes for seven days. In this study, each subject were required to ingest either 150 mg/day of *E. longifolia* or PG daily as well as one hour before the experimental trial. The result did not show any significant changes in performances and physiological responses. It was speculated that the lack of significant results could be due to low dosage or the short consumption period of the supplementation (Ayu-Suzailiana *et al*. 2010).

In regards to strength training, Hamzah and Yusof investigated the effect of *E. longifolia* on muscle strength and size in 14 young healthy men. In this study, the participants were given 100 mg/day of either *E. longifolia* or PG and asked to perform an intense strength training programme on alternate days for five weeks. The results showed

that fat free mass, muscle strength and arm circumferences increased significantly in those who ingested *E. longifolia* (Hamzah and Yusof 2003).

Aside from that, Henkel and co-researchers investigated the effect of *E. longifolia* on testosterone level and aging males' symptoms on elderly people. In the study, 25 (13 male and 12 females) physically active seniors were supplemented with 400 mg/day of either *E. longifolia* or PG for 5 weeks. The results demonstrated significant increase in total and free testosterone concentrations as well as muscular force in both men and women. The study confirmed the benefits of *E. longifolia* in enhancing muscle strength and mass (Henkel *et al*. 2014).

Yusof and co-researchers investigated the effect of *E. longifolia* on muscle strength and performance function in middle-aged women. In this study, 31 women were randomly assigned to ingest either 100 mg of *E. longifolia* or PG for 12 weeks. Following the strength training, *E. longifolia* only showed significant results in stair climb. The researchers conclude that *E. longifolia* supplementation did not enhance muscle strength gain in middle-aged women following a strength training programme (Yusof *et al*. 2016).

CHAPTER 3: HIGH PREVALENCE OF CAM USAGE AMONG MALAYSIAN NATIONAL COLLEGIATE ATHLETES

3.1 Introduction

Complementary and alternative medicine (CAM) is a group of diverse medical and health systems, products, and practices that are generally not considered as part of conventional medicine (Vidal, Carvalho, and Bispo 2013). CAM can be defined as "diagnosis, treatment and/or prevention which complements mainstream medicine by contributing to a common whole, by satisfying a demand not met by orthodoxy or by diversifying the conceptual frameworks of medicine" (Ernst 2000). A vast spectrum of population resort to CAM mainly to regain health status or to boost performance.

In the last few decades, the usage of CAM has gain popularity among general public and developed countries (Alshagga *et al*. 2011, Vidal, Carvalho, and Bispo 2013). The reasons that explain the increased popularity of CAM usage could be due to the primary health source of the country, cultural and historical influences or complementary therapy (WHO 2015a). Around 80% of the population in Africa, Asia and Latin America have incorporated CAM to help meet their primary health care need and in India, CAM is the only available health source to 65% of the population (Payyappallimana 2010). Besides that, ethnic minorities who are disadvantaged in both economically and socially depend on CAM as their primary health care choice (Bodeker and Burford 2006).

CAM is now proven for quality, safety and efficacy thus, ensuring everyone have the access to CAM healthcare (WHO 2015a). In addition, it is also culturally accepted and trusted by large number of people as it is close to home, accessible and most importantly, affordable (WHO 2015a). In other words, geographically, the use of CAM is localised based on the factors mentioned, however with recent globalisation trend, the use is not just limited to the resources available locally. This in turn creates a possible

avenues to those where different population may enjoy the benefits of CAM which are not locally found.

The use of CAM by the people in Asia is possibly the largest in the world. Among South East Asian countries, Malaysia demonstrated the highest percentage (55.6%) in the prevalence of Traditional Medicine and CAM according to published report by the Association of Southeast Asian Nations (ASEAN) (Peltzer and Pengpid 2015). This is followed by Singapore (42.7%), Philippines (6.3%), Cambodia (5.4%), Vietnam (3.5%), Thailand (2.6%) and Indonesia (2.0%) (Peltzer and Pengpid 2015). Aside from that, a survey conducted on Malaysian population showed high prevalence in usage of CAM, particularly in herb-based therapies for both health related issues (88.9%) and health maintenance (87.3%) (Siti *et al*. 2009). Traditional plants and herbs were shown to provide good source of protein, fat, minerals, crude fibres and energy (Kochhar, Nagi, and Sachdeva 2006). This in turn maybe relevant among athletes as it can help with energy production and boost performances.

Theberge shows that athletes often consult CAM practitioners for advices on therapeutic treatments available (Theberge 2008b). CAM users among athletes are shown to be very much dependent on the social context of athletes; such as influence from friends or recommendation from coaches (Kimmerle *et al*. 2012). There are many reasons that lead to the popularity of CAM usage among athletes. Studies suggest that this could be due to dissatisfaction towards conventional medicine, their desire to get back to the field no matter what it takes, and to leave no option untried (Ernst, Willoughby, and Weihmayr 1995, Nichols and Harrigan 2006). Besides that, it is also found that athletes often focus on achieving peak performance even if it conflicts with their health (Schnell *et al*. 2014, Theberge 2008b).

In general CAM is frequently used by athletes as self-medication or performance booster (Theberge 2008a). In a study conducted by Nichols and Harrigan, it is found that

56% of the subjects used at least one kind of CAM therapy in the past 12 months and the most commonly used modalities are massage, chiropractic, lomilomi (Hawaiian traditional massage) and acupuncture (Nichols and Harrigan 2006). Kong and co-workers reported that Chinese massage and herbal ointment showed significant improvement in athletes who suffers from low back pain and muscle stiffness (Kong *et al*. 2012). Another study by Atkinson found that sports supplements such as creatine, whey protein, thermogenics and testosterone enhancer are commonly used by athletes to increase muscle mass (Atkinson 2007).

To our knowledge, in Malaysia, studies have shown that there are high prevalence of CAM usage by the general population, however, no study has explored CAM usage among national athletes. Therefore, the purpose of this study is to examine the usage and the types of CAM therapies that are used by the national athletes. With such data, this will increase the knowledge about the epidemiology of CAM use and provide the foundation for policy making pertaining to sports management.

3.2 Methods

3.2.1 Participants

A total number of 231 national athletes who represented Malaysia in various sports were approached and asked to complete the questionnaires with the assumption that they had experience using CAM thus; they could provide a more reliable response. The recommended sample size, 210, was calculated using PS: Power and Sample Size Calculations software (W.D. Dupont & W.D Plummer. Nashville, TN) based on the total Malaysian athletes who had participated in the SEA Games 2013 with a pre-determined margin of error of 5% and confidence level of 95%. Additional inclusion criteria were those aged between 18-30 years old and understood (written and spoken) English language.

The survey was approved by the Human Research Ethics Committees of the University of Malaya. Participants were approached via sports club, sports institute and coaches with permission to conduct the survey. The researchers introduced the survey described as it is a survey to study CAM usage among athletes. As part of the consent process, involvement was entirely voluntarily and they could withdraw at any time or skip any of the survey question. Participants were asked to respond directly to the questionnaire to increase accuracy. Questionnaires were collected once it was completed and coded with identification numbers to ensure confidentiality and anonymity.

3.2.2 Questionnaire

The questionnaire (I-CAM-Q) assesses the use of CAM and the reason for CAM use was adapted from Quand and co-researchers (Quandt *et al*. 2009) and can be found in Appendix E. I-CAM-Q isclassified into four major categories: (1) visiting health care providers (i.e. physician, chiropractor, homeopath, acupuncturist, herbalist, spiritual

healer); (2) CAM treatments received from physicians/medical doctors (MDs); (3) use of herbal medicine and dietary supplements, including tablets, capsules and liquids; and (4) self-help practices (i.e. meditation, yoga, qigong, tai chi, relaxation technique, visualization, attended traditional health ceremony, praying for own health). Detailed description of CAM providers, treatments and self-help practises used to guide the participants during the survey are listed in Tables 3.1 – 3.3.

3.2.3 Measures

Participants were classified as CAM users if they had used at least one therapy mode in any of the three major categories. Demographic variables included were age, gender, ethnicity, types of sports involved, experience of involvement (years) and medical information (i.e. last medical check-up, cholesterol and blood pressure check-up, exercise for 30 minutes three or more times per week, cigarette smoker). The degree of satisfaction in the survey depends on whether the service provided by the health care providers, treatments received, type of supplements used and self-help practises practised by the participants meets the participant's expectation.

3.2.4 Statistical analyses

All statistical analyses were performed using SPSS20.0 (IBM Corp. Armonk, NY). The difference between gender with respect to visiting healthcare provider, receiving complementary therapies from physicians, use of herbal medicine and dietary supplement and self-help practices were tested using Pearson Chi-Square test. Results were considered significant at $p < 0.05$.

Table 3. 1: Definition of Complementary and Alternative Medicine (CAM) health providers.

CAM Providers	Definition
Physician	Medical doctors who practice medicine.
Chiropractor	Healthcare who focused on diagnosis and treatment of neuromuscular disorder, especially spine.
Homeopath	Healthcare who use medicine that cause the symptom of disease in healthy people to cure similar symptom in sick people.
Acupuncturist	Healthcare who used small needle for therapeutic/ preventive purposes.
Herbalist	Healthcare who use medicinal herbs for treatment purposes.
Spiritual Healer	Healthcare who allow the energy of spirit or its own body to rejuvenate the others.
Physiotherapist	Healthcare who help affected injuries/ illness through movement and exercise.

Table 3. 2: Definitions of Complementary and Alternative Medicine (CAM) treatments by physicians/ MDs.

CAM Treatments	**Definition**
Manipulation	Moving and jolting joints/ massage to relieve pressure on joints, reduce inflammation and improve nerve.
Homeopathy	Treatment of using natural substances in a healthy person that would produce similar symptom of a disease to treat the sick people.
Acupuncture	Treatment of inserting small needles in the body to relieve pain.
Herbs	Treatment of using medicinal herbs to cure the illness.
Spiritual Healing	Treatment that use energy medicine by channelling healing energy to patients.

Table 3. 3: Definitions of self-help practices.

Self-help Practices	Definition
Meditation	A practise where an individual train their mind to promote relaxation.
Yoga	A practise that involves physical, mental and spiritual as complementary intervention for relaxation and health.
Qi Gong	A practise that focused on physical posture, breathing technique and focused intention using inner energy.
Tai Chi	A practise that involved graceful movement focussing on slow focused manner and deep breathing.
Visualisation	The formation of image of something that is going to happen.
Relaxation technique	A practise that decrease the effect of stress on mind and body.
Attending traditional healing ceremony	A practise that involve rituals, including prayers and sage of traditional medicine and practises.
Praying for own health	A practise that asking god for good health/ improve health.

3.3 Results

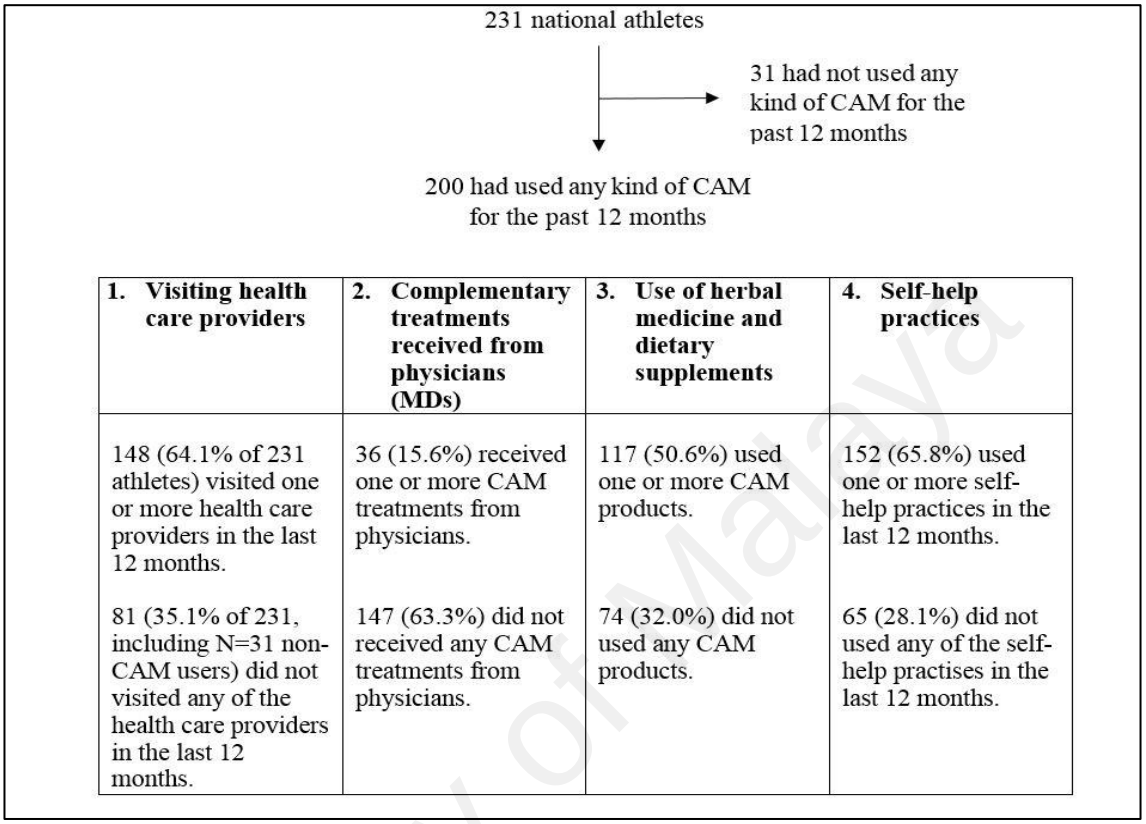
3.3.1 Demographic characteristics

Overall, there were 231 national athletes (n=128 male, and n=103 female, which was 55.4 and 44.6% respectively) voluntarily participated in this questionnaire survey. The mean age of participants was 22.6 years (SD±3.0). 49.8% were Malay, 42.9% Chinese, 5.6% Indian, 0.4% Sikh, 0.4% Iban and 0.4% Sino-Kadazan ethnicities.

The 25 sports represented were archery, athletic, badminton, baseball, basketball, chess, diving, dodgeball, fencing, floorball, football, futsal, handball, hockey, karate, netball, softball, squash, table tennis, taekwondo, tennis, tenpin bowling, triathlon, volleyball and weight lifting. Most sports involved both male and female athletes.

3.3.2 Use of CAM

Of the 231 participants, 200 (86.6%) used at least one type of CAM in the past 12 months (Figure 3.1). Of the 200 participants who reported using at least, one kind of CAM, 103 were men and 97 women.The most frequently used types of CAM was self-help practices (65.8%), followed by visiting healthcare providers (64.1%) and use of herbal medicine and dietary supplementary (50.6%). The difference between men and women was significant ($\chi^2 = 9.23$, df = 1, p=0.02; $p < 0.05$). By comparing the expected and observed values, we deduced that women were more likely to use CAM (expected count 89, actual count 97) than men (expected count 111, actual count 103).



1. Visiting health care providers	2. Complementary treatments received from physicians (MDs)	3. Use of herbal medicine and dietary supplements	4. Self-help practices
148 (64.1% of 231 athletes) visited one or more health care providers in the last 12 months.	36 (15.6%) received one or more CAM treatments from physicians.	117 (50.6%) used one or more CAM products.	152 (65.8%) used one or more self-help practices in the last 12 months.
81 (35.1% of 231, including N=31 non-CAM users) did not visited any of the health care providers in the last 12 months.	147 (63.3%) did not received any CAM treatments from physicians.	74 (32.0%) did not used any CAM products.	65 (28.1%) did not used any of the self-help practises in the last 12 months.

Figure 3. 1: Break down of Complementary and Alternative Medicine among the National Athletes participating in I-CAM-Q.

3.3.3 Visiting healthcare providers

Number of participants visited one or more healthcare providers in the last 12 months was 148; 81 were men and 67 women. The most frequently visited healthcare providers were the physicians (54.1%) (Table 3.4). Physicians were visited 60 times ranging 0 to 12 times in the past 3 months. The main reason for visiting was to treat an acute illness or condition that lasted less than a month (43.3%). Results showed that 48.2% were very satisfied with the treatment given by the physicians in the past 12 months. There were no significant difference in gender and visiting health care providers ($p < 0.05$).

3.3.4 Complementary Therapies from Physicians/ MDs

Of the 125 participants who visited the physicians, 36 received one or more CAM treatments. Among these, 16 were men and 20 women. The most frequent complementary therapies received from physicians were acupuncture (15.3%) (Table 3.5). Participants were treated with acupuncture ranging from 2 to 5 times in the past 3 months. The main reason for seeing the last provider were to treat an acute illness or condition that lasted less than a month (68.8%). Results showed that 83.3% were very satisfied with the complementary therapies that they received from the physicians. There was no significant difference between gender and receiving complementary therapies from physicians ($p < 0.05$).

Table 3. 4: Visitation to health care providers

CAM Provider	Visited CAM provider in the last 12 months (N, %)	No. of visit in the past 3 months (N, range)	Main reason of last saw the provider			Satisfaction		
			Acute illness (N, %)	Chronic illness (N, %)	Well-being (N, %)	Very (N, %)	Somewhat (N, %)	Not at all (N, %)
Physician	125 (54.1%)	60 (0-12)	45 (43.3%)	25 (24.0%)	27 (26.0%)	55 (48.2%)	52 (45.6%)	1 (0.9%)
Chiropractor	7 (3.0%)	6 (1-5)	0 (0%)	5 (71.4%)	2 (28.6%)	0 (0%)	7 (100%)	0 (0%)
Homeopath	1 (0.4%)	1 (1)	1 (100%)	0 (0%)	0 (0%)	0 (0%)	1 (100%)	0 (0%)
Acupuncturist	31 (13.4%)	12 (1-10)	15 (53.6%)	8 (28.6%)	5 (17.9%)	14 (48.3%)	15 (51.7%)	0 (0%)
Herbalist	11 (4.8%)	4 (0-3)	8 (80.0%)	0 (0%)	2 (20.0%)	8 (72.7%)	3 (27.3%)	0 (0%)
Spiritual Healer	4 (1.7%)	0 (0)	0 (0%)	1 (50.0%)	1 (50.0%)	2 (50.0%)	2 (50.0%)	0 (0%)
Physiotherapist	7 (3.0%)	5 (2-14)	1 (14.3%)	3 (42.9%)	2 (28.6%)	5 (71.4%)	2 (28.6%)	0 (0%)

Table 3. 5: Complementary therapies from physician (MDs)

CAM Treatments	Used CAM treatments in the last 12 months (N, %)	No. of treatments in the past 3 months (N, range)	Main reason of last received this treatment			Satisfaction		
			Acute illness (N, %)	Chronic illness (N, %)	Well-being (N, %)	Very (N, %)	Somewhat (N, %)	Not at all (N, %)
Manipulation	10 (8.1%)	4 (1-5)	3 (33.3%)	3 (33.3%)	3 (33.3%)	8 (88.9%)	0 (0%)	1 (11.1%)
Homeopathy	1 (0.8%)	1 (1)	1 (100%)	0 (0%)	0 (0%)	0 (0%)	1 (0.4%)	0 (0%)
Acupuncture	19 (15.3%)	5 (2-5)	11 (68.8%)	5 (31.3%)	0 (0%)	10 (83.3%)	2 (16.7%)	0 (0%)
Herbs	13 (10.5%)	2 (1)	7 (63.3%)	2 (18.2%)	2 (18.2)	6 (54.5%)	4 (36.4%)	0 (0%)
Spiritual Healing	2 (1.6%)	0 (0)	0 (0%)	0 (0%)	2(100%)	2 (100%)	0 (0%)	0 (0%)

3.3.5 Use of herbal medicine and dietary supplementation

The reported number using at least, one kind of herbal medicine and dietary supplementation was 117; 62 men and 55 women (Table 3.6). The most frequently used was Vitamin C (13.9%). The main reason for using was mostly to improve well-being (96.8%). Results showed that 48.6% were very satisfied with the outcome from consuming Vitamin C. There was no significant difference between gender and usage of herbal medicine and dietary supplementation ($p < 0.05$).

3.3.6 Self-help Practices

Of the 152 who reported using one or more kind of self-help practices, 72 were men and 80 female. The most frequent used self-help practices were praying for own health (51.5%) (Table 3.7). Participants prayed for own health 30 times ranging 1 to 90 times in the past 3 months. The main reason for visiting was to increase well-being (95.9%). Results showed that 71.7% were very satisfied with the outcome of the self-help practices they practice in the past 12 months. There were significant difference between gender and self-help practices ($\chi^2 = 11.639$, df = 2, p=0.03; $p < 0.05$). By comparing the expected and observed values, we deduced that women were more likely to use self-help practices (expected count 68, actual count 80) than men (expected count 84, actual count 72).

Table 3. 6: Use of herbal medicine and dietary supplements

Supplementation	Currently using (N, %)	Main reason of last use of supplements			Satisfaction		
		Acute illness (N, %)	Chronic illness (N, %)	Well-being illness (N, %)	Very (N, %)	Somewhat (N, %)	Not at all (N, %)
Vitamin C	32 (13.9%)	1 (3.2%)	0 (0%)	30 (96.8%)	17 (48.6%)	13 (37.1%)	5 (14.3%)
Multivitamin & Minerals	25 (10.8%)	4 (16.0%)	0 (0%)	21 (84.0%)	15 (51.7%)	11 (37.9%)	3 10.3%)
Protein Powder	15 (6.5%)	0 (0%)	1 (6.7%)	14 (93.3%)	11 (68.8%)	4 (25.0%)	1 (6.3%)
Omega 3 Fish Oil	9 (3.9%)	0 (0%)	1 (10.0%)	9 (90.0%)	4 (44.4%)	2 (22.2%)	3 (33.3%)
Meal Replacement Drink	8 (3.5%)	0 (0%)	0 (0%)	8 (100%)	6 (75.0%)	1 (12.5%)	1 (12.5%)
Recovery Drink	8 (3.5%)	0 (0%)	0 (0%)	8 (100%)	6 (75.0%)	2 (25.0%)	0 (0%)
Power Gel	7 (3.0%)	1 (14.3%)	0 (0%)	6 (85.7%)	5 (55.5%)	3 (33.3%)	1 (11.1%)
Power Bar	6 (2.6%)	1 (16.7%)	0 (0%)	5 (83.3%)	4 (57.1%)	3 (42.6%)	0 (0%)
Glucosamine	5 (2.2%)	2 (40.0 %)	1 (20.0%)	2 (40.0%)	0 (0%)	5 (100%)	0 (0%)

Table 3. 6 (continue): Use of herbal medicine and dietary supplement

Calcium	4 (1.7%)	0 (0%)	1 (25.0%)	3 (75.0%)	3 (75.0%)	1 (25.0%)	0 (0%)
Evening Primrose Oil	3 (1.3%)	0 (0%)	0 (0%)	3 (100%)	2 (66.7%)	1 (33.3%)	0 (0%)
Vitamin B complex	3 (1.3%)	0 (0%)	0 (0%)	3 (100%)	2 (66.7%)	1 (33.3%)	0 (0%)
Amino Acid	2 (0.7%)	0 (0%)	0 (0%)	2 (100%)	1 (100%)	0 (0%)	0 (0%)
L-Carnitine	2 (0.7%)	0 (0%)	1 (100%)	0 (0%)	1 (50.0%)	1 (50.0%)	0 (0%)
Vitamin E	2 (0.7%)	0 (0%)	0 (0%)	2 (100%)	1 (50.0%)	1 (50.0%)	0 (0%)
Fat burner	1 (0.4%)	0 (0%)	0 (0%)	1 (100%)	1 (100%)	0 (0%)	0 (0%)
Gingko biloba	1 (0.4%)	0 (0%)	0 (0%)	1 (100%)	0 (0%)	1 (100%)	0 (0%)
Magnesium	1 (0.4%)	0 (0%)	0 (0%)	1 (100%)	0 (0%)	1 (100%)	0 (0%)
Chlorophyll	1 (0.4%)	0 (0%)	0 (0%)	1 (100%)	1 (100%)	0 (0%)	0 (0%)
Chlorella	1 (0.4%)	0 (0%)	0 (0%)	1 (100%)	1 (100%)	0 (0%)	0 (0%)
Probiotics	1 (0.4%)	0 (0%)	0 (0%)	1 (100%)	0 (0%)	1 (100%)	0 (0%)

Table 3. 7: Self-help practices

Self-help practices	Used self-help practices in the last 12 months (N, %)	No. of practices in the past 3 months (N, range)	Main reason of last use of self-help			Satisfaction		
			Acute illness (N, %)	Chronic illness (N, %)	Well-being (N, %)	Very (N, %)	Somewhat (N, %)	Not at all (N, %)
Meditation	19 (8.2%)	9 (2-90)	2 (10.5%)	0 (0%)	17 (89.5%)	15 (78.9%)	4 (21.1%)	0 (0%)
Yoga	23 (10.0%)	14 (2-30)	2 (8.7%)	0 (0%)	21 (91.3%)	14 (60.9%)	7 (30.4%)	0 (0%)
Qigong	0 (0%)	0 (0)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Tai Chi	2 (0.9%)	0 (0)	0 (0%)	2 (100%)	0 (0%)	0 (0%)	2 (100%)	0 (0%)
Visualization	73 (31.6%)	28 (1-40)	4 (6.1%)	2 (3.0%)	60 (90.9%)	36 (52.2%)	31 (44.9%)	0 (0%)
Relaxation technique	88 (38.1%)	33 (3-40)	1 (1.3%)	2 (2.6%)	73 (96.1%)	59 (75.6%)	19 (24.4%)	0 (0%)
Attended traditional healing ceremony	6 (2.6%)	4 (1-15)	2 (50%)	0 (0%)	2 (50%)	4 (66.7%)	2 (33.3%)	0 (0%)
Praying for own health	119 (51.5%)	30 (1-90)	2 (2.0%)	2 (2.0%)	94 (95.9%)	71 (71.7%)	26 (26.3%)	0 (0%)

3.4 Discussion

The main finding of this study shows that high percentage of Malaysian athletes, 86.6% (n=200) have reported using at least, one kind of CAM is the past 12 months. Among the users, more female than male athletes resort CAM use instead of conventional medicine. In Malaysia, physicians often prescribe acupuncture to injured athletes. However, on their own accord the athletes rather opt for supplementation for their well-being, Vitamin C being the most popular CAM treatment. Interestingly, praying for health is most commonly self-help practice mode of CAM among Malaysian athletes.

The high prevalence of CAM usage is not surprising as competitive athletes are highly motivated to do anything to speed up recovery from injuries and illness that may interfere their training session or competition (Nichols and Harrigan 2006). Aside from that, factors that may contribute to the high prevalence of CAM usage may be due to family or peer recommendation, looking for a solution to chronic health problem that cannot be improved by conventional medicine, concerned with possible side effect from conventional medicine or not satisfied with the conventional care (Nicholl, Coleman, and Williams 1995, Spigelblatt 1995).

Our study also finds that significantly, more women reported using CAM compared to men. In a study conducted by Nichols and Harrigan on collegiate athletes, the researchers reported that women visit medical doctors who prescribed CAM more frequently compared to men (Nichols and Harrigan 2006). Aside from that, an association between the usage of CAM and women has also been reported in other studies involving diseases and national surveys (Barnes *et al*. 2004, Hanssen *et al*. 2005, Härtel and Volger 2004, Mackenzie *et al*. 2003). This may be due to firstly, gynaecological problems including pre-menstrual syndrome or menstrual discomfort; secondly, it is also reported that women are more likely to care about their health and likely to use any form of health

care compared to men, and thirdly, women may use CAM to reduce body weight, waist circumference and body mass index to improve their body image, in which all these factors could affect their performances as an athlete (Shih *et al*. 2012).

The physician was the most commonly visited as reported by 54.1% of the athletes. This could be due to a large number of physicians are either referring or practising some well-known CAM therapies (Astin 1998). According to Winslow and Shapiro physicians who have education and experience on CAM are likely to respond positively to the patient inquiries and are more likely to recommend CAM therapies than physicians who do not practice CAM therapies (Winslow and Shapiro 2002).

In this study, the highest rate of complementary treatments from physicians is acupuncture (15.3%). This finding is in line with the results from other studies investigating CAM use in general population and also those involving diseases (AlBedah *et al*. 2013, Opheim *et al*. 2012). In 2007, Schneider and co-workers suggested in their systematic review that, acupuncture may have a positive effect on both quality life and clinical symptom (Schneider, Streitberger, and Joos 2007). According to Han, acupuncture applied to certain body sites may accelerate the release of specific neuropeptides in the central nervous system and therefore, activating the self-healing mechanism which could be indirect, creating a positive effect on both quality life and clinical symptom suggested by Schneider and co-researchers (Han 2003, Schneider, Streitberger, and Joos 2007). Although acupuncture is increasingly popular, it is not risk-free for the patient. White and co-workers mentioned that some minor events such as needle lost, fainting or exacerbation of symptom could happen due to the technique of the acupuncturist (White *et al*. 2001).

Vitamin C are commonly used by athletes to improve well-being in this study. In order to be competitive, athletes often underwent training and exercise training is well

known to increase free radicals which could lead to muscle injury. Vitamin C is a popular supplement among athletes as it has certain biological functions that can influence physical performance such as synthesis of carnitine which convey long adipose acid chain into the mitochondria, facilitates the convey and uptake of non-heme iron at the mucosa, potent antioxidant that regenerates oxidized by-products and enhance immune function. Other than that, Vitamin C may exert permissive effects on physiologic functions that facilitate instauration from excruciating training and thus, promoting performance (Lukaski 2004, Kotze *et al*. 1977). A study conducted on soccer players by Zoppi and co-researchers found that Vitamin C supplementation may reduce lipid peroxidation and muscle damage during high-intensity efforts but did not significantly improve their performance (Zoppi *et al*. 2006). This is further supported by Peters and co-researchers that Vitamin C may enhance upper respiratory tract infection resistant which is common after a race in ultramarathon runners and may lower the risk of infections in those who are living in a sedentary lifestyle (Peters *et al*. 1993).

The most commonly reported self-help practices is praying for own health (51.5%). A high percentage in praying could be due to cultural differences or religion practise (Opheim *et al*. 2012). However, there is controversy whether praying for own health should be included in CAM therapies as this would dramatically increase the percentage of CAM user in the study (Tippens, Marsman, and Zwickey 2009). If prayers are not considered, relaxation technique (38.1%) will have the highest percentage, followed by visualization (31.6%). In a study conducted by the researchers on inflammatory bowel disease, the researchers suggested that there is an association between stress and usage of CAM therapies (Langhorst *et al*. 2007, Langmead, Chitnis, and Rampton 2002). Opheim and co-researchers state that stress management has been incorporated with relaxation which involves the interaction between the brain, mind, body and behaviour which in

return, helping them to cope with their stress and play a part in relapse prevention (Opheim *et al*. 2012).

There are few limitations to this study where participants were assumed to complete the survey accurately and attentively. Although the survey questions were explained one by one, some athletes may not have revealed everything they consumed or what they had been practising. As the survey was conducted on a wide range of sports, limiting factors such as, education status and gender of the participants may influence the outcomes of this survey.

3.5 Conclusion

As a conclusion, this survey demonstrates that there is a high prevalence of CAM usage among Malaysian national athletes. Although most athletes received their healthcare from physicians, there is also a number of them who received from other CAM practitioners. In this study, it can be seen that most athletes are drawn towards self-help practices compared to consuming supplements and visiting health care providers. Moreover, it is also found that higher percentage of women are using CAM therapies compared to men.

To understand the needs of future athletes, further research is recommended to identify the knowledge, behaviour and belief on the CAM therapies that the athletes are practising. Without understanding and the right knowledge, the athletes may not fully benefit from the CAM therapies that they are practising.

CHAPTER 4: *EURYCOMA LONGIFOLIA* JACK AND LIPOLYSIS IN COLLEGIATE ATHLETES – ACUTE STUDY

4.1 Introduction

In addition to its medicinal properties, *E. longifolia* has been used as an ergogenic supplement to promote energy metabolism, to improve sports performances and to manage body weight and composition (Chen, Muhamad, and Ooi 2012, Hamzah and Yusof 2003, Ayu-Suzailiana *et al*. 2010).

The two main sources of energy during muscular exercise are fat stored as TG comprised of three fatty acids attached to a molecule of glycerol, and CHO, stored as glycogen and glucose (Coyle 1995). Theoretically, those who are conditioned to yield energy from fat (9 kcal/g of fat) would outlast those conditioned to CHO (4 kcal/g of CHO). Due to the constrained amount of glycogen stored in muscle and liver, rapid usage and depletion would be expected in contrast to the amount of fat stored in the body (Hall *et al*. 2012, Lowery 2004). Therefore, increasing fat metabolism has attracted the attention of athletes who wish to manage their body composition and weight, extending endurance exercise and improving endurance related sports performances.

E. longifolia supplementation has been examined for its potential effects in fat metabolism (Hamzah and Yusof 2003, Pihie 2002, Solomon, Erasmus, and Henkel 2014). It has been suggested that the mechanism of action is related to the release of free testosterone from its binding hormone thus, stimulating lipolysis (Zanoli *et al*. 2009). Testosterone is known as fat reducing hormone as it could inhibit the uptake of lipid and LPL, as well as increasing the number of lipolytic β-adrenergic receptors to stimulates lipolysis therefore, contributing to energy production through fat metabolism (De Pergola 2000).

To date, studies conducted to determine the effect of *E. longifolia* on human performances is still lacking and scarce. Ooi and co-workers reported that the acute effect of *E. longifolia* did not enhance endurance performance in cycling. Nine healthy trained young male cyclists were given *E. longifolia* drink (containing 0.1 mg *E. longifolia*) and cycle until exhaustion on two occasions with 1-week intervals showed no improvement in aerobic performances when compared to the PG (Ooi *et al*. 2001). As for Muhamad and co-workers, 150 mg/day of *E. longifolia* was given for 7 days to twelve healthy male recreational athletes who run to exhaustion also did not demonstrate significant changes compared to PG group in terms of performance (Ayu-Suzailiana *et al*. 2010). Aside from that, both studies also showed no significant changes on plasma FFA and glucose compared to PG group. Studies done on *E. longifolia* thus far showed no significant effect on endurance performance as this could be due to restricted parameters tested i.e. FFA, glucose, and singular dose to conclude the effect of *E. longifolia* on endurance exercise. Based on this fact it is rational to investigate the effect of *E. longifolia* using relevant parameters (i.e. glycerol, TG, insulin, testosterone and cortisol) and to prescribe the supplement based on body weight (Li *et al*. 2013).

Therefore, this study was carried out to investigate the effects of *E. longifolia* root extract supplementation at the dosage of 1.7 mg/kg of body weight as recommended by Li and co-researchers on substrate utilisation and blood metabolites (Li *et al*. 2013). Collegiate athletes were supplemented with *E. longifolia* for 3 days before the experimental trial. It was hypothesised that acute *E. longifolia* supplementation would trigger metabolic responses in favour of lipid utilisation.

4.2 Methods

4.2.1 Participants

Twenty (n = 20) trained collegiate athletes (Mean ± SD; Age: 25.7 ± 3.8 years old, height: 169.8 ± 4.3 cm, weight: 63.8 ± 9.2 kg, and VO_2max: 54.9 ± 8.1 ml/min/kg) were recruited from universities and colleges in Malaysia. The recommended sample size, 14, was calculated using PS: Power and Sample Size Calculations software (W.D. Dupont & W.D Plummer. Nashville, TN). The inclusion criteria included participants who trained at least 3 times per week (30 mins/session), aged 18 - 30 years old, body mass index of between 18.5 – 25.0 kg/m^2 and a minimum VO_2max of 40 ml/kg/min. Participants were non-vegetarian, non-smoker, not on a weight reducing diets, not in contact with any cardiovascular or metabolic diseases and not consuming medication or drugs that are known to influence the metabolisms of CHO and lipid. Each participant was informed about the experimental design and the possible risks before given a consent form. The Physical Readiness Activity Questionnaire (ParQ) was also be given to confirm their overall health status.

4.2.2 Ethics statement

All participants were fully informed of the experimental trials and risk associated before given a written informed consent and participant information sheet for the study participation. All procedures and protocols were approved by University Malaya Research Ethics Committee (Reference Number: UM.TNC2/RC/H&E/UMREC-42), which can be found at Appendix A.

4.2.3 Experimental design

The research was designed using as a randomised, double-blinded, and placebo-controlled study model. The participants were randomly distributed into 2 groups. The participants were not apprised of their supplement whether they were receiving *E. longifolia* supplement or placebo (PG). The researcher was also blinded to which group that was receiving each treatment. Anthropometry and VO_2max were accessed during the preliminary study and each participant completed two experimental trials (baseline- days 0 and 3). Each trial consisted of consuming *E. longifolia* supplement at1.7 mg/kg of body weight by Li *et al.* (2013) or PG in the overnight fasted state (at least 8 hours) 1 hour before completing 60 mins steady state running exercise at 65% of their VO_2max.

4.2.4 Preliminary trial

Before the experimental trial, participants visited the Sports Nutrition and Physiology Laboratory at the Sports Centre, University of Malaya. The first pre-trial visit involved the measurements of body composition, a submaximal test and the VO_2max. These trials were conducted to determine the speed of the treadmill which corresponds to 65% of the participant's VO_2max. The speed established from the two tests were used as the speed of the experimental trial. The second pre-trial visit was familiarization of the exercise protocols and equipment used.

In the submaximal test, participants were asked to warm up on the treadmill (h/p/cosmos quasar, Germany) for 5 mins at the speed of 6 – 7 km/h. After that, participants were asked to put on the face mask and heart rate monitor. Gas collection started after the gas analyser (COSMED, Italy) reached a steady state. The participants ran 4 mins each for 20 mins (at 7, 8, 9, 10 km/h, respectively) and the speed was increased

by 1 km/h at the end of each 4 mins. Analysis of the expired gas was processed using the software installed in the gas analyser for oxygen consumption (**VO_2**) and carbon dioxide production (VCO_2). Heart rate was measured at the end of each 4 mins.

For measurement of VO_2max, participants were asked to run to volitional exhaustion with a continuous intensity increment on the treadmill. Appropriate speed was selected based on the submaximal test results and the test began with an increment of 0% for 3 mins. Participants ran for 3 mins each until exhaustion with the increment of 3% at the end of each 3 min. Heart rate was measured at the end of each 3 mins. VO_2max value was only to be accepted when: a) despite increasing workload, a plateau in oxygen uptake is obtained; b) age-predicted maximum HR with ± 10 beats/min and; and c) respiratory exchange ratio of > 1.1.

4.2.5 Supplementation

Participants were divided into two groups; *E. longifolia* (group 1) and PG (group 2). The *E. longifolia* standardised extract (Physta®, MY), batch number TA150101, was obtained from Biotropics Malaysia Berhad. The certificate of analysis is appended in appendix. The intervention supplements were provided in the form of capsules and each participant from *E. longifolia* was required to consume 1.7 mg/kg of body weight of *E. longifolia* or PG per day for 3 days. Both *E. longifolia* and PG were similar in terms of size and colour. The order of supplement was randomized by another researcher. The researcher was required to record the date, time and type of supplement taken by the subject before the trial. On the trial day, the remaining supplements were counted by the researcher to ensure the participants have adhered to the supplementation regimen.

4.2.6 Experimental trial

Participants arrived at the Sports Science Physiology laboratory two hours prior and measurement of body composition was taken using an electronic body composition analyser (Inbody 370, Korea). Aside from that, fasting blood, resting heart rate and expired air was measured and recorded. Participants were then given a standardize breakfast consisting of a piece of bread (Gardenia®) and a glass (250 ml) of water 60 mins prior to the trial.

During the experimental trial, participant ran at 65% of their respective VO_2max for 60 mins. Blood samples were collected after the experimental trial whereas, HR and expired air were collected and recorded every 15 mins throughout the running trials.

4.2.7 Blood analysis

Blood collected were stored in appropriate tubes and centrifuged at 3000 rpm for 15 mins at 4 °C (Heraus™ Multifuge™ X1 Centrifuge Series, USA). Aliquots of samples were transferred into Eppendorf tubes and stored at -80 °C for analysis of FFA, glycerol, TG, total cholesterol (TC), high-density lipoprotein (HDL), low-density lipoprotein (LDL), glucose, insulin, testosterone and cortisol. These parameters were analysed using ELISA kits based on the manual given and a spectrophotometer (Epoch, BioTek, USA).

4.2.8 Statistical analysis

All statistical analyses were performed using SPSS 20.0 (IBM Corp. Armonk, NY). Normality of the data was examined using Kolmogorov-Smirnov test and a two-way analysis of variance (ANOVA) were used to determine the effect of measured parameters and time and the changes of parameters over the time. Statistical significant was set and accepted at $p < 0.05$. All data are presented as mean values and standard deviation (mean ± SD). Aside from that, Cohen's d effect was also performed (Cohen, 1988).

4.3 Results

4.3.1 Body composition

The characteristic of the participants were listed in Table 4.1. By the end of the study, although there was a small decrement observed in *E. longifolia* group on VO_2max, body mass, skeletal muscle mass, body fat mass and percentage body fat, it was not statistically different compared to PG. On average, the *E. longifolia* group showed decrements in body mass ($d = 0.0$, small), skeletal muscle mass ($d = 0.0$, small), body fat mass ($d = 0.1$, small) and percentage body fat ($d = 0.1$, small).

4.3.2 Fat and Carbohydrate metabolisms

The rates of fat oxidation and CHO oxidation are based on the respiratory exchange ratio (RQ) provided by the gas analyser as RQ is the indicator of which fuel is metabolised to supply the body with energy. Fat oxidation during the experimental trials was significantly increased between treatments across time ($p < 0.05$). There was also a significant difference observed in fat oxidation between treatments on day 3 ($p < 0.05$). Fat oxidation in PG group was significantly lower in day 3 compared to day 0 ($p < 0.05$) (Table 4.2 and Figure 4.1). Similarly, CHO oxidation was significantly reduced between treatments across time ($p < 0.05$). CHO oxidation in PG group was significantly higher in day 3 compared to day 0 ($p < 0.05$) (Table 4.3 and Figure 4.2).

Table 4. 1: Body composition of *E. longifolia* – treated group and placebo group participants (mean ± SD)

Variables	*E. longifolia* (n = 9)			PG (n = 11)		
	Day 0	Day 3	Effect size (*d*)ŧ	Day 0	Day 3	Effect size (*d*)ŧ
Age (years)	25.0 ± 3.1	-	-	25.0 ± 3.8	-	-
VO_2max (ml/min/kg)	38.3 ± 8.3	39.1 ± 7.6	0.10	37.8 ± 3.3	38.4 ± 0.9	0.24
Body mass (kg)	64.3 ± 11.3	64.1 ± 10.7	0.02	63.6 ± 7.2	63.6 ± 7.2	0
Skeletal muscle mass (kg)	30.4 ± 4.7	30.5 ± 4.6	0.02	30.0 ± 3.9	29.9 ± 3.9	0.03
Body fat mass (kg)	10.6 ± 4.8	10.3 ± 4.6	0.06	10.2 ± 3.7	10.3 ± 3.6	0.03
Percentage body fat (kg)	15.9 ± 5.3	15.6 ± 5.3	0.06	15.9 ± 5.3	16.1 ± 5.2	0.04

E. longifolia = supplement group; PG = placebo group

ŧ Effect size (*d*) = effect size based on the formula by Cohen (1988)

Table 4. 2: Rates of fat oxidation (g/min) in *E. longifolia*-treated group (EL) and placebo group (PG) on days 0 and 3.

	E. longifolia				**PG**			
Day \ **Min**	**15**	**30**	**45**	**60**	**15**	**30**	**45**	**60**
Day 0 (g/min)	0.53	0.57	0.61	0.64	0.52	0.55	0.60	0.65
Day 3 (g/min)	0.59	0.65	0.72	0.73	0.46	0.49	0.54	0.54

Table 4. 3: Rates of carbohydrate (CHO) oxidation (g/min) in *E. longifolia*-treated group (EL) and placebo group (PG) on days 0 and 3.

	E. longifolia				**PG**			
Day \ **Min**	**15**	**30**	**45**	**60**	**15**	**30**	**45**	**60**
Day 0 (g/min)	1.49	1.40	1.27	1.15	1.42	1.35	1.15	1.06
Day 3 (g/min)	1.36	1.28	1.16	1.16	1.62	1.53	1.41	1.46

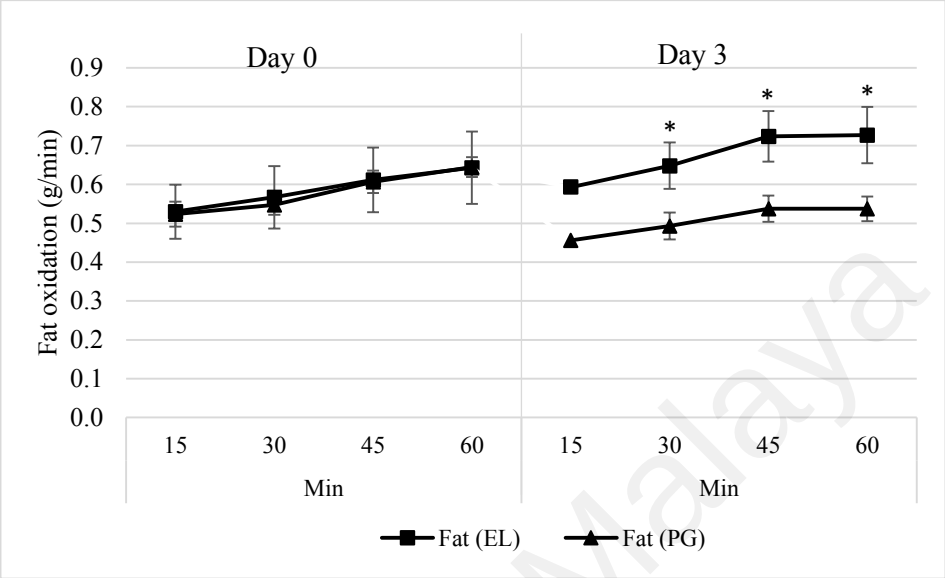


Figure 4. 1: Rates of fat oxidation (g/min) in *E. longifolia* - treated group (EL) and placebo group (PG). Data represented as (■) – EL and (▲) – PG at 65% VO_2max. * EL significantly different from PG ($p < 0.05$). Mean ± Standard error (SE), n = 20.

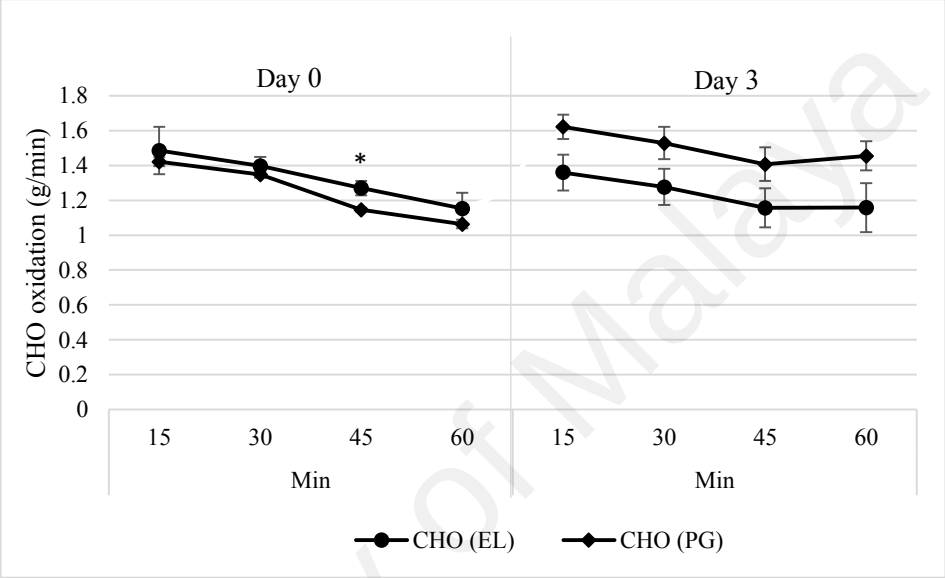


Figure 4. 2: Rates of carbohydrate (CHO) oxidation (g/min) in *E. longifolia* - treated group (EL) and placebo group (PG). Data represented as (●) – EL and (♦) – PG at 65% VO_2max. * EL significantly different from PG ($p < 0.05$). Mean ± SE, n = 20.

Table 4. 4: Energy expenditure (%) from fat and carbohydrate (CHO) in *E. longifolia* – treated group (EL) and placebo group (PG) on days 0 and 3.

	Fat		**Carbohydrate (CHO)**	
Energy Expenditure	***E. longifolia***	**PG**	***E. longifolia***	**PG**
Day 0 (%)	48.8	49.4	50.9	40.4
Day 3 (%)	59.3	47.7	50.3	52.3

Table 4. 5: Plasma and serum metabolites concentrations (mmol/l) in *E. longifolia* - treated group (EL) and placebo group (PG) on days 0 and 3.

Variables	***E. longifolia***		**PG**	
	Day 0	**Day 3**	**Day 0**	**Day 3**
Free fatty acid (FFA) (mmol/l)	0.21	0.04	0.14	0.17
Glycerol (mmol/l)	0.66	0.86	0.59	0.59
Triglycerides (TG) (mmol/l)	0.31	0.24	0.29	0.20
Glucose (mmol/l)	0.49	0.63	0.51	0.79
Testosterone (mmol/l)	0.63	0.86	0.82	0.59
Cortisol (mmol/l)	6.41	9.31	5.87	4.67

4.3.3 Energy Expenditure

Energy expenditure from fat in *E. longifolia* increases significantly whereas, energy expenditure from CHO decreases on day 3 compared to day 0 ($p < 0.01$). The results also demonstrated treatment effect on day 3 whereby energy from fat was significantly higher PG and energy from CHO was significantly lower in *E. longifolia* compared to PG ($p < 0.01$) (Table 4.4) (Figures 4.3 and 4.4).

4.3.4 Plasma and serum metabolites concentrations

Plasma FFA concentrations showed significant treatment x duration interactions ($p < 0.05$) (Table 4.5andFigure 4.5). Plasma FFA concentrations in *E. longifolia* group on day 3 was significantly lower compared to day 0 ($p < 0.05$). In addition, differences were observed between treatments on day 3 whereby, plasma FFA concentrations in *E. longifolia* group was significantly lower compared to PG group ($p < 0.05$).

Plasma glycerol concentrations showed no significant treatment x duration interactions and duration effects, but there was a significant difference between treatments (Table 4.5) (Figure 4.6) where a significant increase was observed in *E. longifolia* compared to PG group on day 3 ($p < 0.05$). Plasma TG concentrations showed no significant treatment x duration interactions, however there was a significant duration effect (Table 4.5 and Figure 4.7), where a significant reduction of plasma TG in *E. longifolia* group was observed on day 3 compared to day 0 ($p < 0.05$).

As for plasma glucose concentrations, the results showed no significant treatment x duration interactions, however, there was a significant duration effect (Table 4.5 and Figure 4.8), where a significant increment of plasma glucose in PG group was observed on day 3 compared to day 0 ($p < 0.05$).

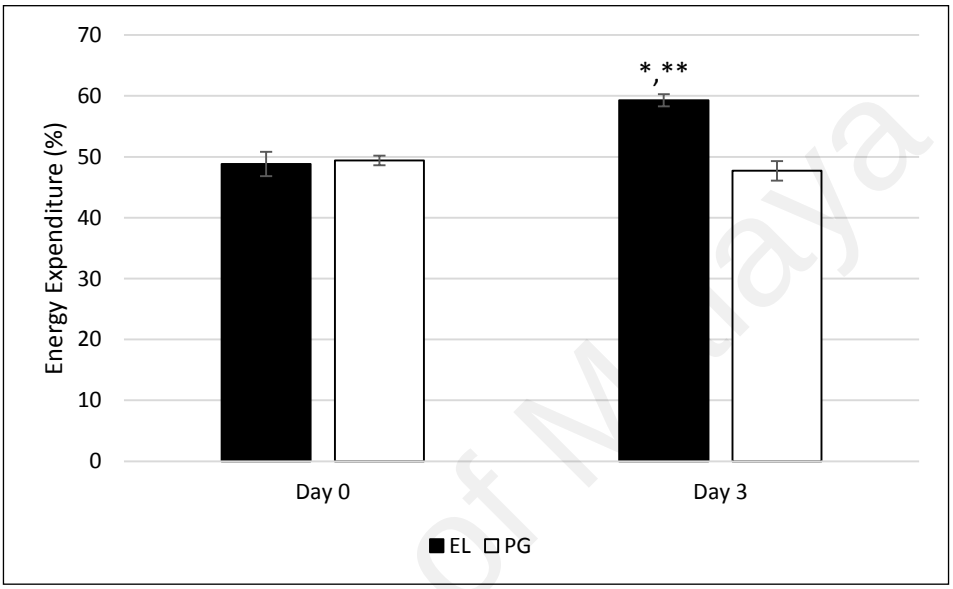


Figure 4. 3: Energy expenditure from fat (%) in *E. longifolia* - treated group (EL) and placebo group (PG). Data represented as black bar – EL and white bar – PG. *Significant different between days 0 and 3 ($p < 0.01$). ** Significant different between EL and PG on day 3 ($p < 0.01$). Mean ± SE, n = 20.

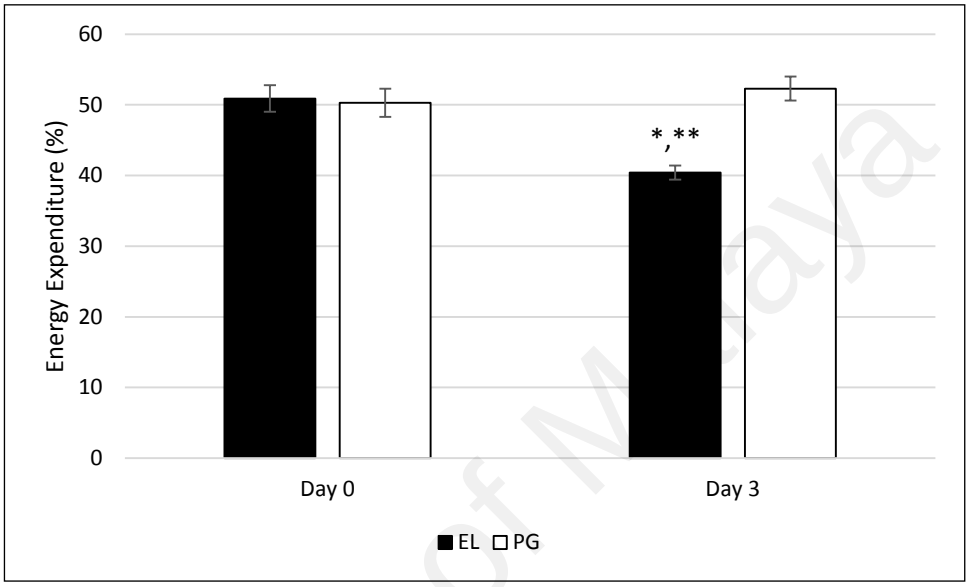


Figure 4. 4: Energy expenditure from carbohydrate (CHO) (%) in *E. longifolia* - treated group (EL) and placebo group (PG). Data represented as black bar – EL and white bar – PG. *Significant different between days 0 and 3 ($p < 0.01$). ** Significant different between EL and PG on day 3 ($p < 0.01$). Mean ± SE, n = 20.

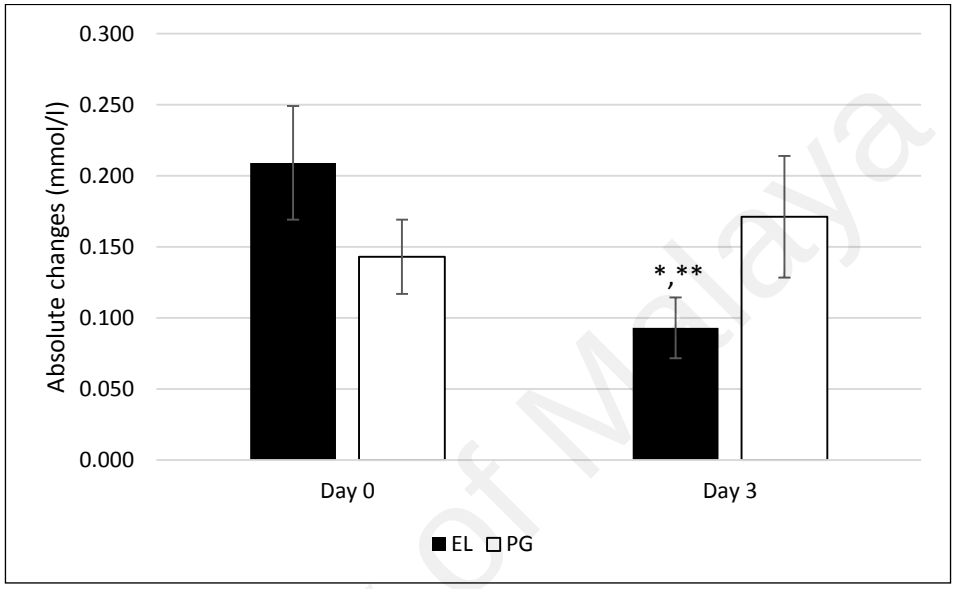


Figure 4. 5: Plasma concentration of free fatty acid (FFA) (mmol/l) in *E. longifolia* - treated group (EL) and placebo group (PG). Data represented as black bar – EL and white bar – PG. *Significant different between days 0 and 3 ($p < 0.05$). ** Significant different between EL and PG on day 3 ($p < 0.05$). Mean ± SE, n = 20.

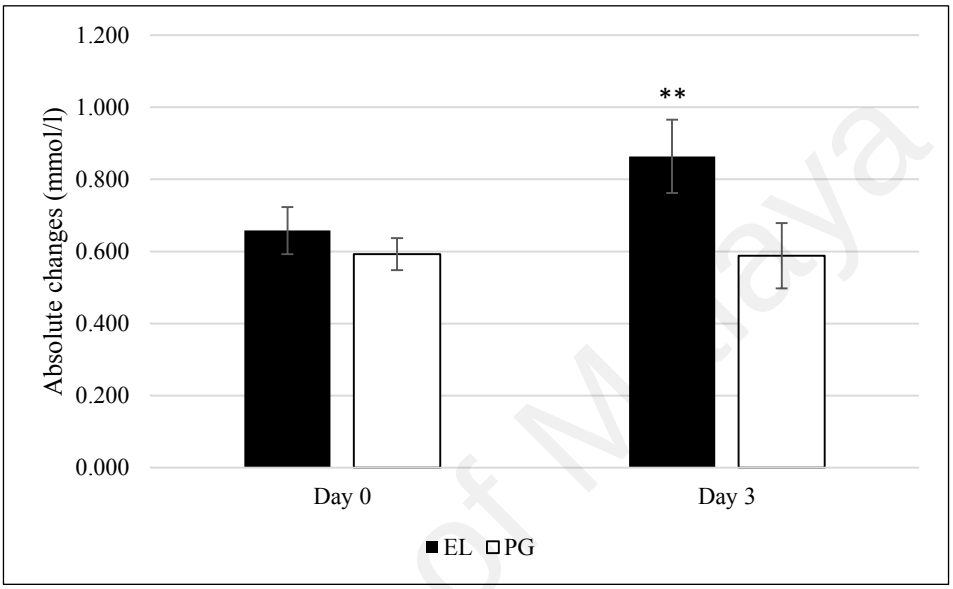


Figure 4. 6: Plasma concentration of glycerol (mmol/l) in *E. longifolia* - treated group (EL) and placebo group (PG). Data represented as black bar – EL and white bar – PG. ** Significant different between EL and PG on day 3 ($p < 0.05$). Mean ± SE, n = 20.

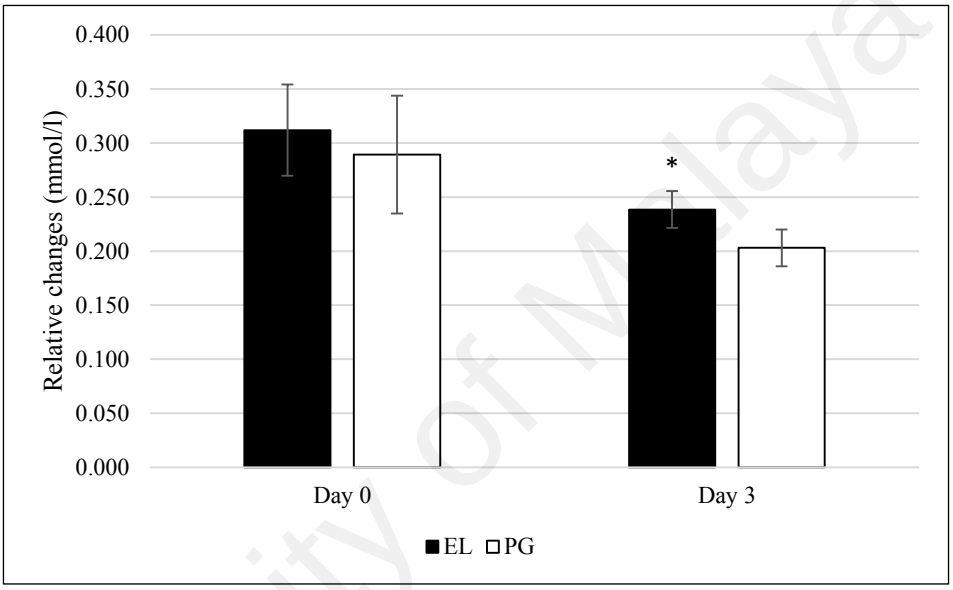


Figure 4. 7: Plasma concentration of triglycerides (TG) (mmol/l) in *E. longifolia* - treated group (EL) and placebo group (PG). Data represented as black bar – EL and white bar – PG. *Significant different between days 0 and 3 ($p < 0.05$). Mean ± SE, n = 20.

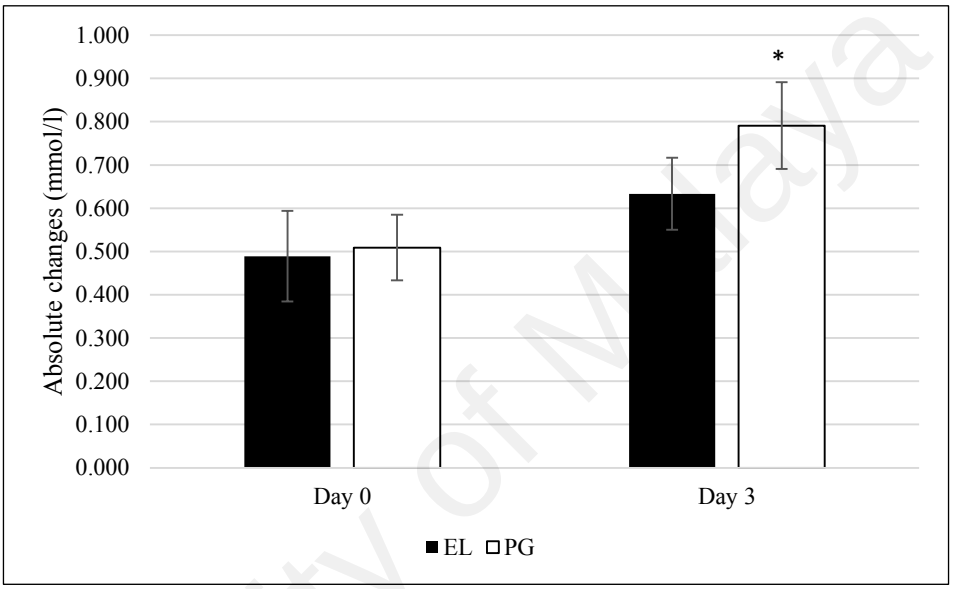


Figure 4. 8: Plasma concentration of glucose (mmol/l) in *E. longifolia* - treated group (EL) and placebo group (PG). Data represented as black bar – EL and white bar – PG. *Significant different between days 0 and 3 ($p < 0.05$). Mean ± SE, n = 20.

Regarding the hormones, serum testosterone concentrations showed a significant treatment x duration interaction ($p < 0.05$) (Table 4.5 and Figure 4.9), where a significant increase in *E. longifolia* group on day 3 compared to day 0 ($p < 0.05$). In addition, a significant difference was observed between treatments on day 3, whereby serum testosterone in *E. longifolia* group was higher compared to PG group ($p < 0.05$).

As for serum cortisol concentrations, the results showed significant treatment x duration interactions ($p < 0.01$) (Table 4.5 and Figure 4.10) where an increment of serum cortisol concentrations in *E. longifolia* group on day 3 was observed compared to day 0 ($p < 0.05$). In addition, there was a significant difference between treatments on day 3, whereby, serum cortisol in *E. longifolia* group was increased compared to PG group ($p < 0.01$).

Serum insulin and plasma TC, HDL and LDL concentrations resulted in no significant treatment x duration interactions. Thus, there were also no changes for insulin, TC, HDL and LDL for either group.

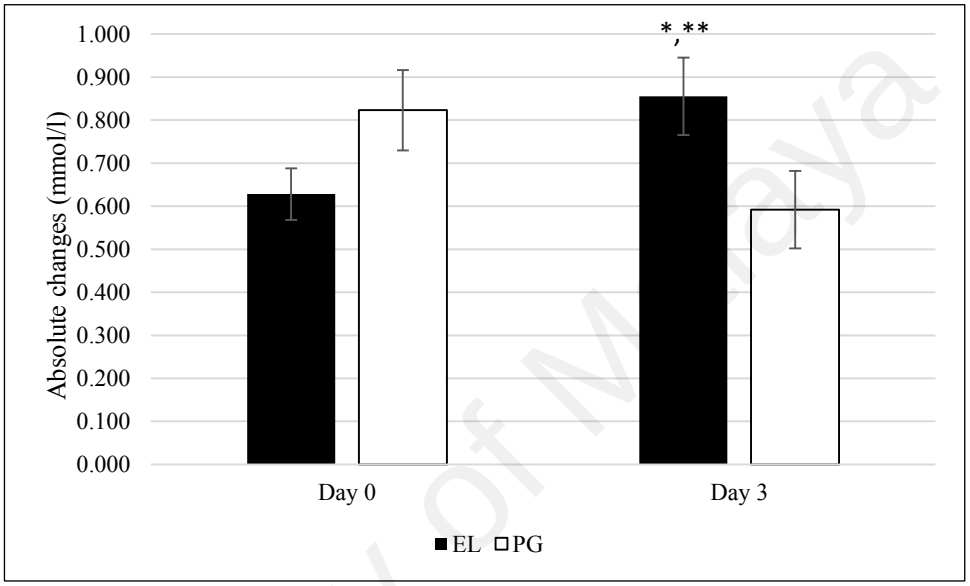


Figure 4. 9: Serum concentration of testosterone (mmol/l) in *E. longifolia* - treated group (EL) and placebo group (PG). Data represented as black bar – EL and white bar – PG. *Significant different between days 0 and 3 ($p < 0.05$). ** Significant different between EL and PG on day 3 ($p < 0.05$). Mean ± SE, n = 20.

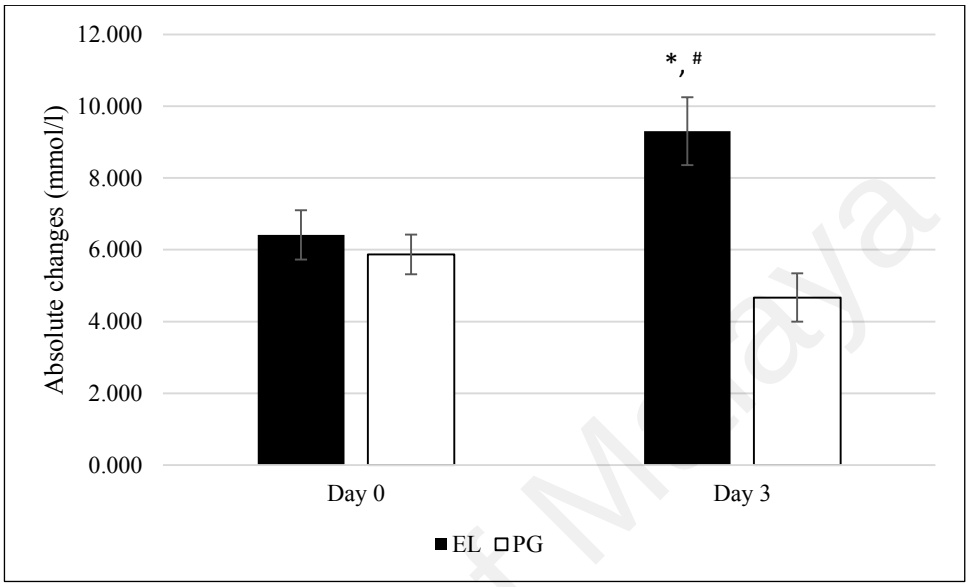


Figure 4. 10: Serum concentration of cortisol (mmol/l) in *E. longifolia* - treated group (EL) and placebo group (PG). Data represented as black bar – EL and white bar – PG. *Significant different between days 0 and 3 ($p < 0.05$). # Significant different between EL and PG on day 3 ($p < 0.01$). Mean ± SE, n = 20.

4.4 Discussion

In the present study, the ergogenic effects of *E. longifolia* supplementation on substrate utilisation and blood metabolites were evaluated. The study found that 1.7 mg/kg of body weight of *E. longifolia* supplementation for 3 days demonstrates significant changes in lipid metabolisms. Additionally, the present study also showed that *E. longifolia* supplementation elicits significant changes in plasma FFA, glycerol, TG and serum cortisol concentrations. To the best of our knowledge, this was the first acute study to demonstrate the effect of *E. longifolia* on lipolysis.

Increased utilisation of FA during exercise is known to improve endurance exercise capacity and performances (Azevedo *et al*. 1998, Yeh *et al*. 2011). The present study demonstrated that *E. longifolia* supplementation increased lipid utilisation as energy substrate on day 3 compared to PG group. Increase lipid utilisation is believed to be of vital importance in aerobic performances as it is associated with enhanced lipolysis thus, delaying the complete glycogen depletion and increase circulation of FFAs in the body (Ikeuchi *et al*. 2006). The increased levels of fatty acid in circulation and active muscle contribute to an increase in fat metabolism, which in turn decreases CHO utilisation resultingin an increase in aerobic performances (Coggan *et al*. 2000). According to Yeh and co-researchers, reliance on CHO utilisation leads to lactate production which decreases the pH. This in turn inhibits muscle contractions and depletes glycogen which could affect aerobic capacity and performances (Yeh *et al*. 2011). Therefore, utilisation of lipid as energy substrate would lead to improved aerobic capacity.

Theoretically, during a prolonged exercise, lipolysis takes place by releasing FFA and glycerol from TG (fat) into the blood and therefore, contributing to increases in plasma FFA and glycerol concentrations and decrease in TG concentration in blood plasma after exercise (Arner *et al*. 1990, Romijn *et al*. 1993). However, the present study demonstrated a significant reduction in plasma FFA in *E. longifolia* group compared to

PG group. As the present study showed higher fat oxidation rates on day 3 in *E. longifolia* group compared to PG, this suggests that the plasma FFA in *E. longifolia* group has been catabolised to synthesise to ATP, resulting lower plasma FFA concentration in the blood. Aerobic training is known to have an impact on substrate metabolism as well as increasing muscle mitochondria density (Holloszy and Coyle 1984, Onanong Kulaputana 2007). Therefore, increased aerobic training increases FA oxidation to synthesised ATP thus, resulting in a decrease in plasma FFA through lipolysis.

Plasma glycerol and TG were also explored in the present study. An increase in plasma glycerol and decrease in plasma TG concentrations confirm the positive effect of *E. longifolia* supplementation on lipid mobilisation and utilisation. This could be due to fat replacing the role of glucose as the primary energy source as exercise continues (Bursztyn 1990, Ayu-Suzailiana *et al*. 2010). According to Romijn and co-researchers, glycerol in the blood is an indication of lipolysis and as glycerol increases, total FFA from lipolysis made available for oxidation at the rate of two-threefold greater than the rate of oxidation to be converted as energy source (Romijn *et al*. 1993). As a result, this clearly showw that *E. longifolia* can stimulate lipid mobilisation and utilisation to prevent fatigue in athletes.

Plasma glucose is an important energy source in exercising human, supplying 20 - 50% of total oxidative energy production during exercise (Coggan 1991). A significant increase in plasma glucose was observed in PG group. As the present study showed higher CHO oxidation rates on day 3 in PG group compared to *E. longifolia* group, this suggests that the plasma glucose in PG group has been catabolised to synthesise ATP, resulting in an increased hepatic glucose production and rate of muscle glycogenolysis to produce more glucose to sustain the exercise training (Ayu-Suzailiana *et al*. 2010).

Previous studies have proven that endurance exercise has an impact on serum testosterone and cortisol concentrations (Daly *et al*. 2005, Fahrner and Hackney 1998).

Endurance exercises have been shown to increase the circulating testosterone and cortisol concentrations depending on the training intensity, mode or duration of the exercise (Maresh *et al*. 2006). The present study demonstrated a significant increase in serum testosterone after 3 days of *E. longifolia* supplementation. This finding is in line with other studies suggesting that consumption of *E. longifolia* potentially restores normal testosterone levels (Talbott *et al*. 2006, Talbott *et al*. 2013, Tambi 2003). Previous studies suggest that the increase in testosterone level could be due to eurypeptides, a group of small peptides in *E. longifolia* which influences the release rate of free testosterone from SHBG (Chaing *et al*. 1994, Talbott *et al*. 2013, Tambi 2003). This study also shows significant increase in cortisol levels in *E. longifolia* group. Cortisol has been suggested to act with other hormones to potentiate the sensitivity of adipose tissue lipolysis to cyclic AMP (Askew *et al*. 1975). Previous studies show that the increase in cortisol led to concomitant increase in FFA and glycerol concentrations, which are indicators of lipolysis in human (Djurhuus *et al*. 2002, Samra *et al*. 1998). In addition, experiments using radioisotope labelled-palmitate infusion *in vivo* also demonstrated increase in FFA concentrations along with high level of cortisol (Divertie, Jensen, and Miles 1991). These findings indicate that the *E. longifolia* supplementation would influence lipolytic metabolism in response to hormone stimulation and consequently providing alternative resources, i.e. FFA and glycerol, for higher yield of energy generation.

The results of the present study showed that there were no significant changes in body mass, skeletal muscle mass, body fat mass and percentage body fat in *E. longifolia* and PG groups. It has been suggested that *E. longifolia* supplementation may affect body composition and body weight. Hamzah & Yusof reported that 100 mg/day of *E. longifolia* for 5 weeks increased body lean mass and decreased percentage body fat in young healthy men (Hamzah and Yusof 2003). The reason of the results obtained might be attributed to

the acute supplementation as the significant effect was observed in longer period of *E. longifolia* supplementation.

4.5 Conclusion

Acute supplementation of 1.7 mg/kg of body weight of *E. longifolia* imposes a significant effect on lipolysis in collegiate athletes. These findings suggest that *E. longifolia* can be broadly used as an ergogenic aids to boost energy yield, prevent fatigue and hopefully, to enhance aerobic performances. Although the findings from this study demonstrate the efficacy of traditional herb *E. longifolia* in promoting lipolysis, it warrants further investigations effects of longer duration of consumption.

CHAPTER 5: FIVE WEEKS OF *EURYCOMA LONGIFOLIA* JACK INCREASES FAT UTILISATION IN COLLEGIATE ATHLETES

5.1 Introduction

Eurycoma longifolia Jack (*E. longifolia*) or commonly known as *Tongkat Ali* is one of the most widely consumed herbs in Malaysia and has been widely used as an ergogenic aid for many athletes (Ayu-Suzailiana *et al*. 2010, Chen, Muhamad, and Ooi 2012). Some of the proposed exercise related effect of *E. longifolia* include increase in muscle strength, anxiolytic effect and increase fat-free mass (Chen, Muhamad, and Ooi 2012, Hamzah and Yusof 2003, Henkel *et al*. 2014, Talbott *et al*. 2013, Yusof *et al*. 2016).

Although the exact mechanisms have not been identified, it has been suggested that *E. longifolia* contains small peptides known as 'eurypeptides' that help in improving energy level using rodents (Zanoli *et al*. 2009). It acts in the body by increasing the release rate of free testosterone from its binding hormone SHBG thus, regulating the normal level of testosterone rather than stimulating the production of testosterone. It is well known that testosterone contributes to the changes in physical function as declining testosterone is linked to loss of muscle mass and muscle strength, reciprocally, raised serum testosterone would increase muscle mass, which then results in greater potential for generating greater force in the muscle (Delhez, Hansenne, and Legros 2003, Henkel *et al*. 2014, Tambi 2003).

In previous investigations, the acute effect of *E. longifolia* on endurance performance such as treadmill running and cycling ergometer indicate that *E. longifolia* might not be an effective ergogenic aid for improving endurance performances (Ayu-Suzailiana *et al*. 2010, Ooi *et al*. 2001). However, in strength training, *E. longifolia* demonstrated positive results which indicate *E. longifolia* could been effective ergogenic aid for improving strength performances. For example, Hamzah & Yusof reported that *E.*

longifolia supplementation (100 mg/day) for 5 weeks with resistant training on healthy adult males (average age 25 years) resulted in significant improvement in fat-free mass, fat mass, maximal strength (1-RM) and muscle circumferences compared to a PG group (Hamzah and Yusof 2003). In addition, treatment using 400 mg/day of *E. longifolia* for 5 weeks on physically active male and female seniors (aged 57 - 72 years) resulted in significant increases in total and free testosterone concentrations and muscular force in both men and women (Henkel *et al*. 2014). Another study by Yusof and co-researchers demonstrated that ingestion of *E. longifolia* supplementation (100 mg/day) for 12 weeks showed significant changes in muscle strength in middle-aged women (Yusof *et al*. 2016). Thus, the effect of *E. longifolia* may depend on the type of exercise, the dosage used and the characteristic of the participants.

As *E. longifolia* is shown to improve testosterone level, thus far, it's *in vivo* activity does not exhibit doping like effect. George and co-researchers conducted a study on healthy male aged 30 - 55 years old with 300 mg/day of *E. longifolia* extract for 12 weeks and found that the ratio of testosterone to epitestosterone did not have significant changes instead, muscular strength on the back and leg has improved significantly (George *et al*. 2013). This indicates that *E. longifolia* supplementation has an ergogenic effect and can be used by competitive and recreational athletes prior to their competition or training.

Therefore, this study was carried out to investigate the effects of *E. longifolia* root extract supplementation at the dosage of 1.7 mg/kg of body weight as recommended by Li and co-researchers on substrate utilisation and blood metabolites (Li *et al*. 2013). Collegiate athletes were supplemented with *E. longifolia* for 5 weeks before the experimental trial. It was hypothesised that the *E. longifolia* supplementation would trigger metabolic responses in favour of lipid utilisation.

5.2 Methods

5.2.1 Experimental approach to the problem

This study used a randomised, double-blinded, placebo-controlled design. All participants participated in an endurance training program that consisted of 60 min of treadmill running at 65% of VO_2max on weeks 0, 3 and 5. The dependable variables assessed in this study were FFA, glycerol, TG, TC, HDL, LDL, glucose, insulin, testosterone and cortisol. The participants were assigned randomly to either 1.7 mg/kg of body weight of supplement *E. longifolia* (Physta®, MY), batch number TA150101, from Biotropics Malaysia Berhad or PG group. The certificate of analysis of *E. longifolia* is appended in appendix. The participants ingested 1 capsule of supplement or PG 60 mins prior to the exercise session on training days and in the morning after breakfast on non-training days. In addition, the participants are asked to maintain their normal diet during the 5-week period.

5.2.2 Participants

Twenty (n = 20) collegiate athletes (Mean ± SD; Age: 25.7 ± 3.8, Height: 169.8 ± 4.3 cm, Weight: 63.8 ± 9.2 kg, VO_2max: 54.9 ± 8.1 ml/min/kg) who have been regularly exercising at least 3 times per week, aged 18 - 30 years old, body mass index of between 18.5 – 25.0 kg/m^2 and a minimum VO_2max of 40 ml/kg/min volunteered as participants for this investigation. The recommended sample size, 14, was calculated using PS: Power and Sample Size Calculations software (W.D. Dupont & W.D Plummer. Nashville, TN). Furthermore, each participant completed a ParQ to determine their safety and possible risk of training prior to the 5-week study. All procedures were approved by the University Malaya Research Ethics Committee (Reference Number: UM.TNC2/RC/H&E/UMREC-42 and each participant signed an informed consent prior to any testing.

5.2.3 Body composition determination

Body composition was assessed at pre-training stage using a body composition analyser (Inbody 360, Korea). The participants were instructed to avoid exercise for at least 24 hours and fast at least 10 hours prior to the training. Body weight and percentage body fat were determined using the body composition analyser.

5.2.4 Determination of submaximal and VO_2max

Each participant performed a submaximal test and incremental test to exhaustion on a motorised treadmill (h/p/cosmos quasar, Germany) for the determination of speed and VO_2max. Each participant wore a face mask and the expired gas samples were collected and were analysed using a calibrated Quark CPET metabolic cart (COSMED, Italy). The metabolic cart was calibrated prior to each test. Each participant was fitted with a Polar heart rate monitor (Polar Electro Inc., USA) to monitor heart rate throughout the test. Following a 5 mins warm up at 5 km/h, the test began with the participants walking at 6 km/h 0% grade. The velocity was increased 1 km/h every 4 mins to 10 km/h. At 10 km/h, the exercise intensity was increased by raising the treadmill grade 3% every 3 mins until voluntary exhaustion. VO_2max were defined as the highest values recorded during the last 30 sec of the test. Assessment of VO_2max will only be accepted if i) despite increasing workload, a plateau in oxygen uptake is obtained; ii) Age-predicted maximum HR with ± 10 beats/min and; iii) respiratory exchange ratio of > 1.1.

5.2.5 Endurance training test

All participants visited the laboratory once every two weeks for 5 weeks. Body composition was analysed and fasting blood was collected prior to the test. Each participant was given standardise breakfast consisted of a piece of bread (Gardenia®) and a glass (250 ml) of water 1 hour prior to the test. Following a 5 mins warm up at 5 km/h and 0% grade, the participants ran for 60 min at 65% of their VO_2max based on the submaximal and VO_2max test. The participants wore heart rate monitors and were supervised to assure the participants maintained their fitness level and appropriate training intensity was maintained throughout the 60 mins test. Blood was collected after the 60 mins test whereas expired gas are collected at 15 mins interval. The participants were instructed not to perform any additional exercise training after the 60 mins test. In addition, participants were advised to consume water after the 60 mins test to avoid dehydration.

5.2.6 Blood analysis

Blood collected were stored in appropriate tubes and centrifuged at 3000 rpm for 15 mins at 4 °C (Heraus™ Multifuge™ X1 Centrifuge Series, USA). Aliquots of samples were transferred into Eppendorf tubes and stored in the freezer at -80 °C for analysis of FFA, glycerol, TG, TC, HDL, LDL, glucose, insulin, testosterone and cortisol. The parameters were analysed using spectrophotometer (Epoch, BioTek, USA) at wavelength according to the assay kit manufacturer's protocol.

5.2.7 Statistical analyses

All statistical analyses were performed using SPSS 20.0 (IBM Corp. Armonk, NY). Normality of the data was examined using Kolmogorov-Smirnov test and a two-way analysis of variance (ANOVA). Post-hoc Bonferroni test was used to show pair-wise difference and independent *t*-test was used to determine the effect of measured parameters and time and the changes of parameters over the time. Statistical significant was set and accepted at $p < 0.05$. All data are presented as mean values and standard deviation (mean ± SD).

5.3 Results

5.3.1 Body composition analyses

The present study showed that there was a small decrement observed in *E. longifolia* group on body mass, skeletal muscle mass, body fat mass and percentage body fat however, it was not statistically different compared to PG.

On average, the *E. longifolia* group have a decrement in body mass ($d = 0.1$, small), skeletal muscle mass ($d = 0.0$, small), body mass index ($d = 0.0$, small) and percentage body fat ($d = 0.1$, small).

5.3.2 Fat and carbohydrate metabolism

There was a general interaction in fat oxidation rates during the experimental trials between the effects of treatments and duration ($p < 0.01$) (Table 5.1 and Figure 5.1). There was also significant difference observed in fat oxidation rates between treatments ($p < 0.01$). The treatment effects was observed on week 3 ($p < 0.01$) at min 30 ($p < 0.05$) and week 5 ($p < 0.01$) at min 30 ($p < 0.05$), 45 ($p < 0.05$) and 60 ($p < 0.05$). Within *E. longifolia* groups, no significant changes was observed in duration effects and time trial effects. As for PG group, there was a duration effects where changes was observed between weeks 0 and 5 ($p < 0.01$) and weeks 3 and 5 ($p < 0.01$) and time trial effects where changes was observed on week 0 between mins 15 and 60 ($p < 0.05$) and week 3 between mins 15 and 45 ($p < 0.05$), mins 15 and 60 ($p < 0.01$), 30 and 60 ($p < 0.05$).

There was a general interaction in CHO oxidation rates during the experimental trials between the effects of treatments and duration ($p < 0.01$) (Table 5.2 and Figure 5.2). There was also significant difference observed in CHO oxidation

Table 5. 1: Rates of fat oxidation (g/min) in *E. longifolia*- treated group (EL) and placebo group (PG) on weeks 0, 3 and 5.

	E. longifolia				**PG**			
Week \ **Min**	**15**	**30**	**45**	**60**	**15**	**30**	**45**	**60**
Week 0 (g/min)	0.53	0.57	0.61	0.64	0.52	0.55	0.61	0.65
Week 3 (g/min)	0.58	0.69	0.70	0.73	0.50	0.54	0.57	0.61
Week 5 (g/min)	0.54	0.61	0.69	0.71	0.44	0.40	0.43	0.50

Table 5. 2: Rates of carbohydrate (CHO) oxidation (g/min) in *E. longifolia*- treated group (EL) and placebo group (PG) on weeks 0, 3 and 5.

	E. longifolia				**PG**			
Week \ **Min**	**15**	**30**	**45**	**60**	**15**	**30**	**45**	**60**
Week 0 (g/min)	1.49	1.40	1.27	1.15	1.42	1.35	1.15	1.06
Week 3 (g/min)	1.34	1.15	1.04	0.99	1.44	1.35	1.30	1.24
Week 5 (g/min)	1.48	1.31	1.14	1.06	1.44	1.61	1.57	1.40

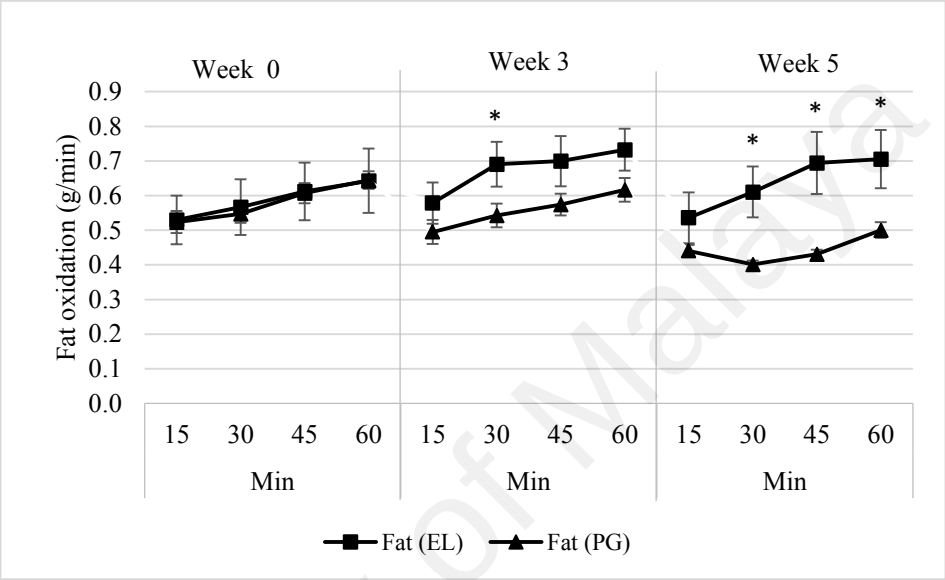


Figure 5. 1: Rates of fat oxidation (g/min) in *E. longifolia*- treated group (EL) and placebo group (PG). Data represented as (●) – EL and (♦) – PG at 65% VO_2max on weeks 0, 3 and 5. * EL significantly different from PG ($p < 0.05$). Mean ± SE, n = 20.

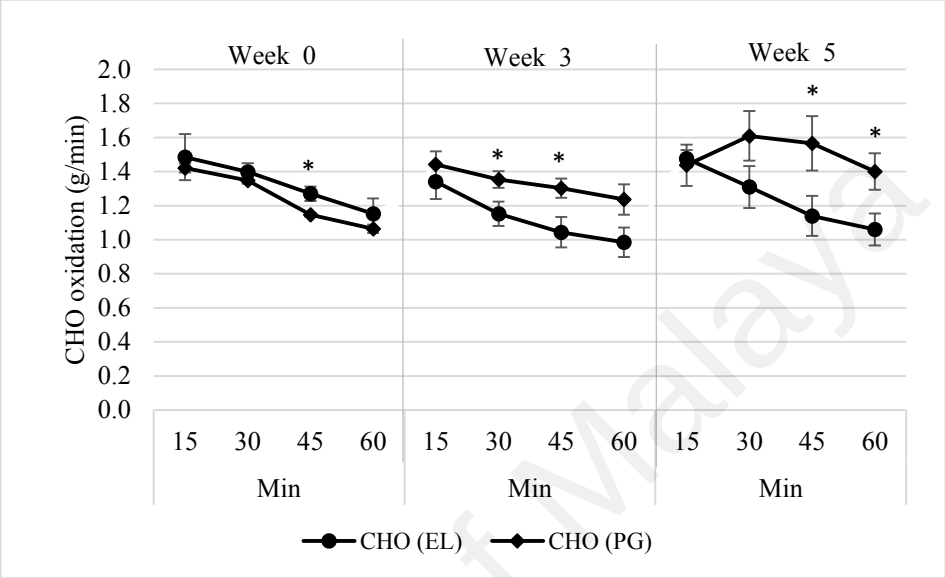


Figure 5. 2: Rates of CHO oxidation (g/min) in *E. longifolia*- treated group (EL) and placebo group (PG). Data represented as (●) – EL and (♦) – PG at 65% VO_2max on weeks 0, 3 and 5. * EL significantly different from PG ($p < 0.05$). Mean ± SE, n = 20.

rates between treatments ($p < 0.01$). The treatment effects was observed on week 0 at min 45 ($p < 0.05$), week 3 ($p < 0.01$) at mins 15 ($p < 0.05$) and 45 ($p < 0.05$) and week 5 ($p < 0.01$) at mins 45 ($p < 0.05$) and 60 ($p < 0.05$). Within *E. longifolia* groups, there was a duration effects where changes was observed between weeks 0 and 3 ($p < 0.05$) and time trial effects where changes was observed on week 5 between mins 15 and 60 ($p < 0.05$). As for PG group, there was a duration effects where changes was observed between weeks 0 and 5 ($p < 0.01$) and weeks 3 and 5 ($p < 0.05$) and time trial effects where changes was observed on week 0 between mins 15 and 60 ($p < 0.01$).

5.3.3 Energy expenditure

The contribution of fat and CHO to total energy expenditure during experimental trials was significantly different between *E. longifolia* and PG groups. There was a general interaction between the effects of treatment x duration on energy expenditure from fat ($p < 0.01$) (Table 5.3 and Figure 5.3). Energy expenditure from fat in *E. longifolia* group increase significantly on weeks 3 ($p < 0.01$) and 5 ($p < 0.01$) compared to PG. Among *E. longifolia* group, a significant increase in energy from fat was observed from weeks 0 to 3 ($p < 0.01$) and decrease from weeks 3 to 5 ($p < 0.01$) whereas in PG group, a significant decrease was observed from weeks 0 to 5 ($p < 0.01$). As for contribution of energy from CHO, there was a general interaction between the effects of treatment x duration ($p < 0.01$) (Table 5.3 and Figure 5.4). Energy expenditure from CHO in *E. longifolia* group decrease significantly on weeks 3 ($p < 0.01$) and 5 ($p < 0.01$) compared to PG. Among *E. longifolia* group, a significant decrease in energy from CHO was observed from weeks 0 to 3 ($p < 0.01$) and increase from weeks 3 to 5 ($p < 0.01$) whereas in PG group, a significant increase was observed from weeks 0 to 5 ($p < 0.01$).

Table 5. 3: Energy expenditure from fat and carbohydrate (CHO) (%) in *E. longifolia*-treated group (EL) and placebo group (PG) on weeks 0, 3 and 5.

	Fat		**Carbohydrate (CHO)**	
Energy Expenditure	***E. longifolia***	**PG**	***E. longifolia***	**PG**
Week 0 (%)	48.8	48.1	48.4	49.8
Week 3 (%)	64.9	45.9	34.2	54.6
Week 5 (%)	51.0	34.8	49.5	65.7

Table 5. 4: Plasma and serum metabolites concentrations (mmol/l) in *E. longifolia* - treated group (EL) and placebo group (PG) on weeks 0, 3 and 5.

Variables	***E. longifolia***			**PG**		
	Week 0	**Week 3**	**Week 5**	**Week 0**	**Week 3**	**Week 5**
FFA (mmol/l)	0.21	0.33	0.14	0.14	0.08	0.08
Glycerol (mmol/l)	0.66	0.98	0.88	0.59	0.55	0.51
TG (mmol/l)	0.31	0.25	0.22	0.29	0.24	0.16
LDL (mmol/l)	0.17	0.11	0.06	0.16	0.13	0.12
Glucose (mmol/l)	0.49	1.18	0.62	0.51	1.27	0.95
Insulin (mmol/l)	0.75	1.09	0.49	0.67	0.59	0.70
Testosterone (mmol/l)	0.63	1.37	1.26	0.67	0.93	0.83

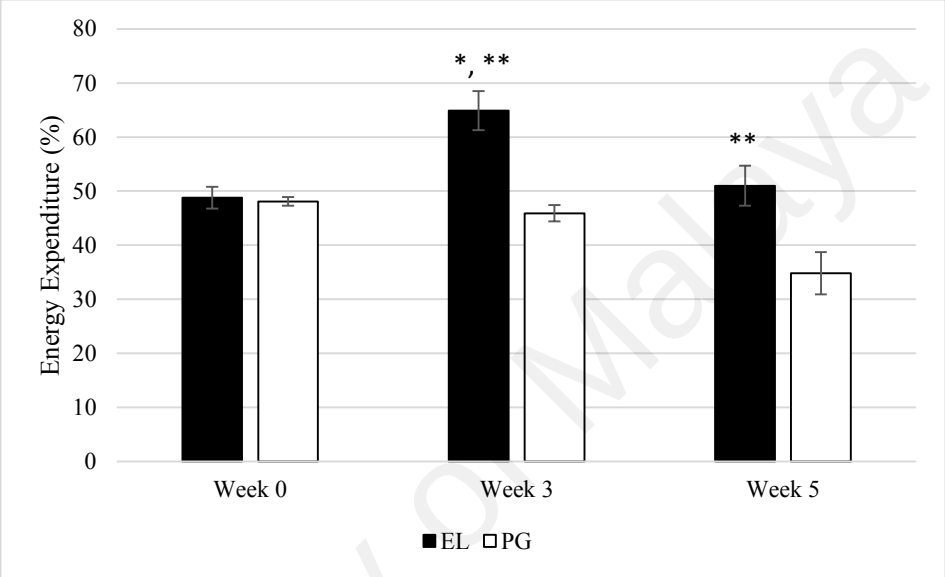


Figure 5. 3: Energy expenditure from fat (%) in *E. longifolia*-treated group (EL) and placebo group (PG). Data represented as black bar – EL and white bar – PG. *Week 3 significant different between weeks 0 and 5 ($p < 0.01$) ** Significant different between EL and PG on weeks 3 and 5 ($p < 0.01$). Mean ± SE, n = 20.

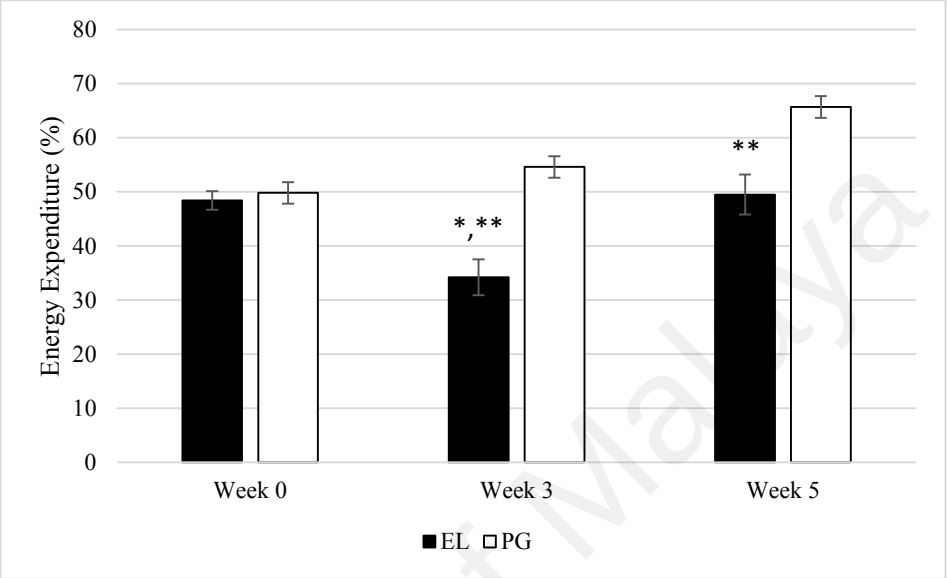


Figure 5. 4: Energy expenditure from CHO (%) in *E. longifolia*- treated group (EL) and placebo group (PG). Data represented as black bar – EL and white bar – PG. *Week 3 significant different between weeks 0 and 5 ($p < 0.01$) ** Significant different between EL and PG on weeks 3 and 5 ($p < 0.01$). Mean ± SE, n = 20.

5.3.4 Plasma and serum metabolites concentrations

In the present study, the plasma and serum metabolites analysed were FFA, glycerol, TG, TC, HDL, LDL, glucose, insulin, testosterone and cortisol. Plasma FFA concentrations resulted in significant treatment x duration interactions ($p < 0.01$), between duration and between treatment effects (Table 5.4 and Figure 5.5). Plasma FFA concentrations in *E. longifolia* group on week 3 was significantly higher compared to weeks 0 ($p < 0.05$) and 5 ($p < 0.01$). In addition, differences were observed between treatments on week 3 whereby, plasma FFA concentrations in *E. longifolia* group was significantly higher compared to PG group ($p < 0.01$).

Plasma glycerol concentrations resulted in significant treatment x duration interactions ($p < 0.05$), between duration and between treatments effects (Table 5.4 and Figure 5.6). Differences were observed between treatments whereby plasma glycerol concentrations in *E. longifolia* group is significantly higher compared to PG group on weeks 3 ($p < 0.01$) and 5 ($p < 0.05$). Plasma TG concentrations resulted no significant treatment x duration interactions and between treatment effects but there were significant effects between duration (Table 5.4 and Figure 5.7). A significant reduction of plasma TG concentrations in *E. longifolia* group were observed on week 5 compared to week 0 ($p < 0.01$).

Plasma LDL concentrations resulted no significant treatment x duration interactions but there were significant effects between treatment and between duration (Table 5.4 and Figure 5.8). In *E. longifolia* group, a significant decrease was observed from weeks 0 to 3 ($p < 0.01$) and 3 to 5 ($p < 0.01$). In addition, differences were observed between treatments whereby plasma LDL concentrations in *E. longifolia* group is significantly lower compared to PG group on week 5 ($p < 0.01$).

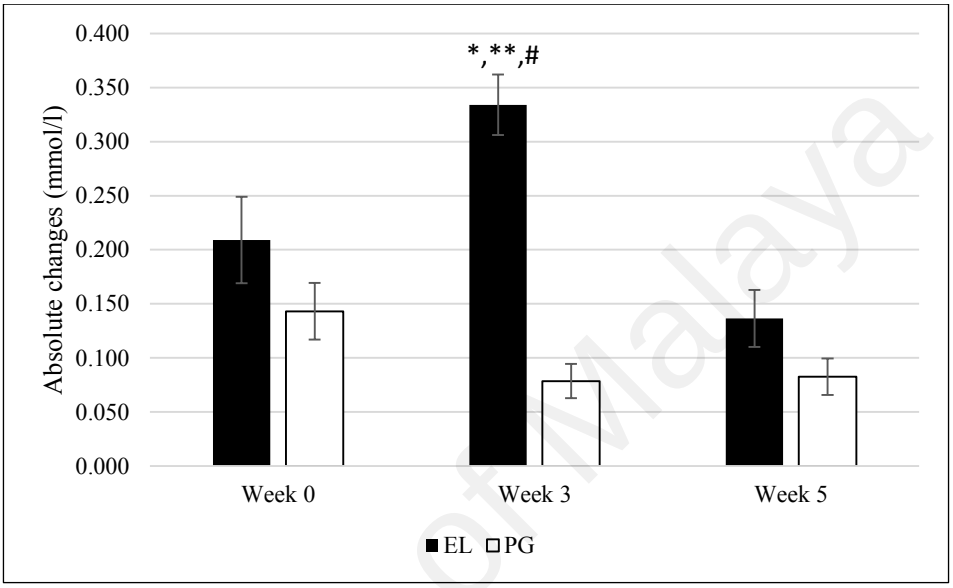


Figure 5. 5: Plasma concentration of FFA (mmol/l) in *E. longifolia*-treated group (EL) and placebo group (PG). Data represented as black bar – EL and white bar – PG. *Week 3 significant different between week 0 ($p < 0.05$) **Week 3 significant different between week 5 ($p < 0.01$) #Significant different between EL and PG ($p < 0.05$). Mean ± SE, n = 20.

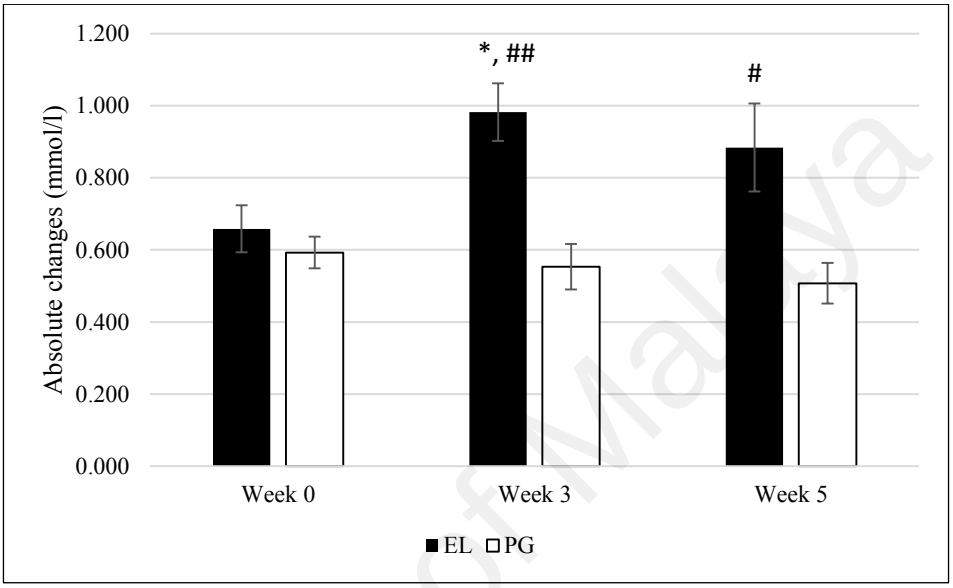


Figure 5. 6: Plasma concentration of glycerol (mmol/l) in *E. longifolia*-treated group (EL) and placebo group (PG). Data represented as black bar –EL and white bar –PG. *Week 3 significant different between week 0 ($p < 0.05$) #Significant different between EL and PG ($p < 0.05$) ##Significant different between EL and PG ($p < 0.01$). Mean ± SE, n = 20.

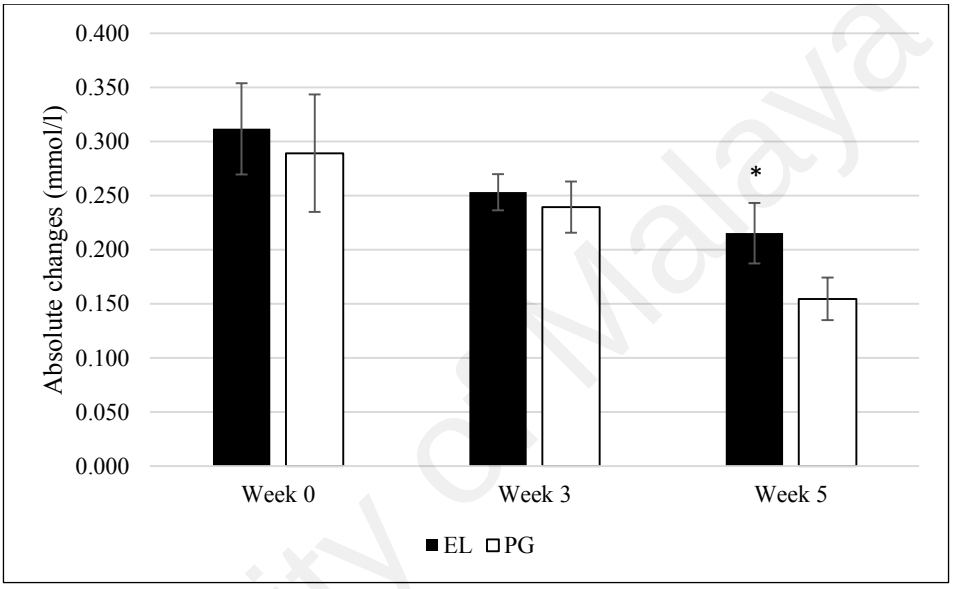


Figure 5. 7: Plasma concentration of TG (mmol/l) in *E. longifolia*-treated group (EL) and placebo group (PG). Data represented as black bar – EL and white bar – PG. * Significant different between weeks 0 and 5 ($p < 0.01$). Mean ± SE, n = 20.

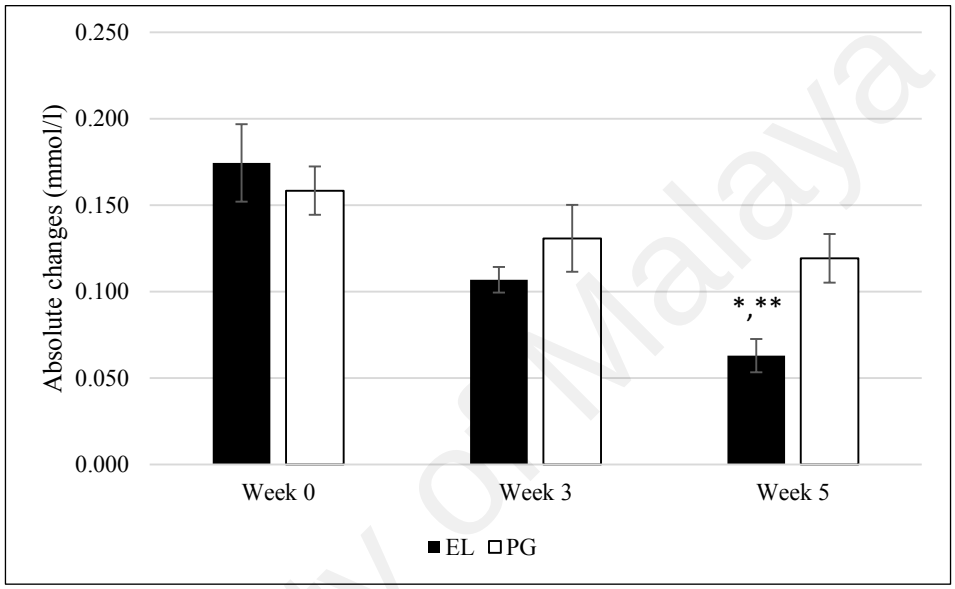


Figure 5. 8: Plasma concentration of LDL (mmol/l) in *E. longifolia*-treated group (EL) and placebo group (PG). Data represented as black bar – EL and white bar – PG. * Significant different between weeks 0 and 5 ($p < 0.01$) ** Significant different between EL and PG on week 5 ($p < 0.01$). Mean ± SE, n = 20.

As for plasma glucose concentrations, the result showed no significant treatment x duration interactions and between treatment effects but there were significant effects between duration (Table 5.4 and Figure 5.9). Thus, a significant increment of plasma glucose concentrations in *E. longifolia* ($p < 0.01$) and PG group ($p < 0.01$) were observed on week 3 compared to week 0. As for plasma insulin concentrations, the result showed significant treatment x duration interactions ($p < 0.05$) and between treatment effects but not in between duration effects (Table 5.4 and Figure 5.10). Differences were observed between treatments whereby plasma insulin concentrations in *E. longifolia* group is significantly higher compared to PG group on week 3 ($p < 0.01$).

Serum testosterone concentrations resulted in significant treatment x duration interactions ($p < 0.01$), between duration and between treatments effects (Table 5.4 and Figure 5.11). Serum testosterone concentrations in *E. longifolia* group on weeks 3 ($p < 0.01$) and 5 ($p < 0.01$) was significantly higher compared to weeks 0. In addition, differences were observed between treatments on weeks 3 ($p < 0.01$) and 5 ($p < 0.01$) whereby, serum testosterone concentrations in *E. longifolia* group was significantly higher compared to PG group.

Serum cortisol and plasma TC and HDL concentrations resulted in no significant treatment x duration interactions, duration effects and treatment effects. Thus, there were no significant changes for cortisol, TC and HDL for either group.

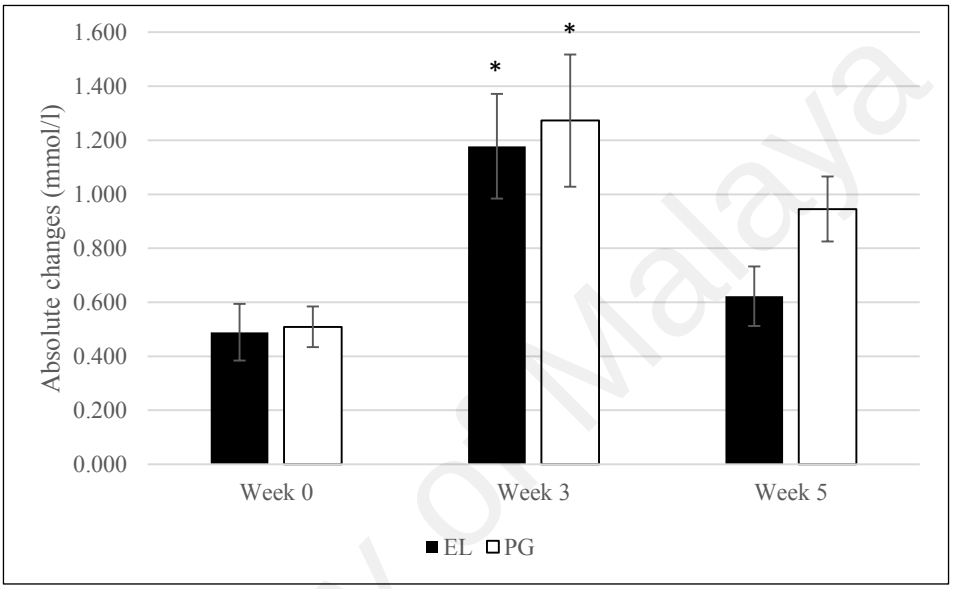


Figure 5. 9: Plasma concentration of glucose (mmol/l) in *E. longifolia*-treated group (EL) and placebo group (PG). Data represented as black bar –EL and white bar – PG. * Significant different between weeks 0 and 3 ($p < 0.01$). Mean ± SE, n = 20.

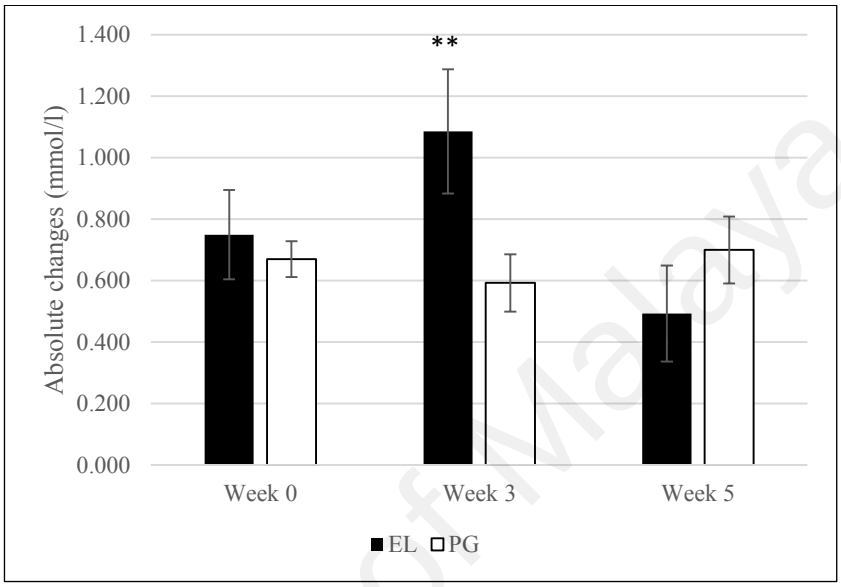


Figure 5. 10: Plasma concentration of insulin (mmol/l) in *E. longifolia*-treated group (EL) and placebo group (PG). Data represented as black bar – EL and white bar – PG. ** Significant different between EL and PG on week 3 ($p < 0.01$). Mean ± SE, n = 20.

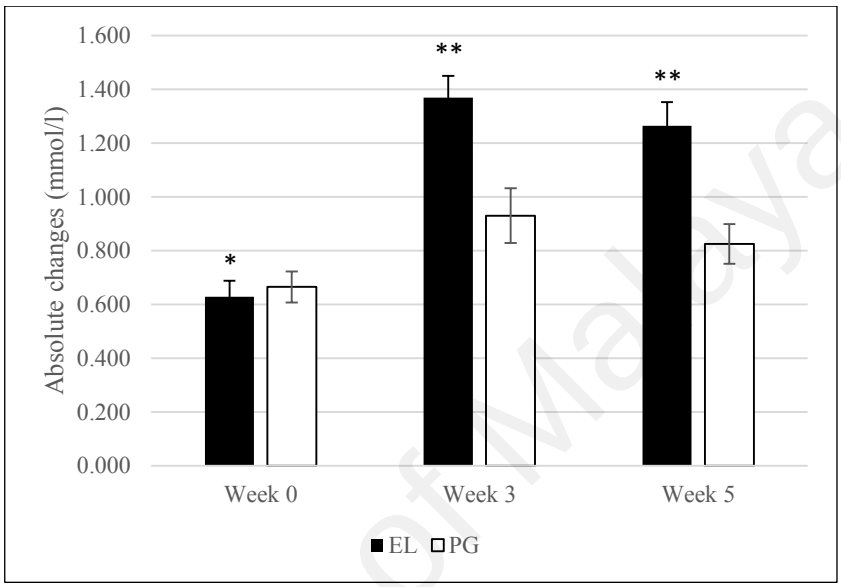


Figure 5. 11: Serum concentration of testosterone (mmol/l) in *E. longifolia*-treated group (EL) and placebo group (PG). Data represented as black bar – EL and white bar – PG. *Week 0 significant different between weeks 3 and 5 ($p < 0.01$) ** Significant different between EL and PG on weeks 3 and 5 ($p < 0.01$). Mean ± SE, n = 20.

5.4 Discussion

The present study examined the effect of 5 weeks of *E. longifolia* supplementation (1.7 mg/kg body weight) on the substrate utilisation and blood metabolites during steady state exercise at 65% of the participant's respective VO_2max. In Chapter 4, the data have shown the efficacy of acute *E. longifolia* in increasing fat utilisation and promoting lipolysis. In agreement with Chapter 4, the present study also found significant increment in fat utilisation in *E. longifolia* when compared to PG. The current study also showed that daily supplementation of *E. longifolia* root extracts (1.7 mg/kg of body weight) for 5 weeks elicits significant changes in plasma FFA, glycerol, TG, LDL, glucose, insulin and serum testosterone concentration when compared to PG.

Fat fuel is supplied by the circulating FFAs for ATP production during exercise. During exercise from 15 to 60 mins on weeks 3 and 5 in *E. longifolia*, fat oxidation rates were significantly increased compared to PG, and considerable fat utilisation was evident. It is likely that the body favours fat over CHO as a source of fuel. This is also consistent with the energy expenditure and observed FFA and plasma glycerol measured. Although the exact mechanism has not been revealed, previous studies suggest that the rise in fat oxidation could be linked with the increased testosterone concentrations stimulated by the eurypeptides in the *E. longifolia* extract. Those studies have reported that increased in testosterone concentrations could increase lipolysis in abdominal subcutaneous adipose tissue by stimulating the hormone sensitive lipase (Frederiksen *et al*. 2012, Rebuffe-Scrive, Mårin, and Björntorp 1991). Fat fuel would serve as an important energy source for endurance athletes to sustain longer and prevent glycogen depletion which would lead to lactate production (Yeh *et al*. 2011). Thus, the results suggest that *E. longifolia* extract could serve as an ergogenic aid by stimulating lipolysis and promoting fat utilisation, benefiting those who participate in sports that require high energy demand.

Compared to Chapter 4, *E. longifolia* consumptions for 5 weeks showed a significant increase in plasma FFA concentration, especially week 3. This finding was in agreement with the previous study on endurance athletes (Ayu-Suzailiana *et al*. 2010). The improvement in aerobic metabolisms in this study is best explained by alterations to fat lipolysis as an increase in plasma FFA concentrations can be seen from week 0 to 5 (Ayu-Suzailiana *et al*. 2010, Bursztyn 1990). As the body starts to favour fat as the source of energy, this stimulated the lipolysis process to produce a great amount of free FFA to sustain the body needs (Romijn *et al*. 1993). The increase of FFA could also indicate that there is a greater fat mobilisation but not necessarily as the consequences of increased utilisation (Friedberg *et al*. 1963). With the increase mobilisation, the utilisation turnover rate can be increased as there are available FFAs for the body to be catabolised to synthesis ATP. This showed that *E. longifolia* can stimulate lipid mobilisation and the body does not have to rely on glucose as an immediate source of energy. Therefore, this clearly showed that *E. longifolia* can stimulate lipid mobilisation and increase utilisation to prevent fatigue in athletes.

Previous studies have shown that the increase of plasma FFA concentration is accompanied by an increase of plasma glycerol and decrease of plasma TG (Arner *et al*. 1990, Romijn *et al*. 1993). This study showed that plasma glycerol increases significantly in weeks 3 and 5 compared to 0 and plasma TG decreased significantly on week 5 compared to week 0. A decreasing trend was also observed in plasma TG. During exercise, the plasma glycerol concentrations in the blood reflect the total rate of body lipolysis. This can be proven as adipose tissue does not contain glycerol kinase. Therefore, all glycerol released from lipolysis will appear in blood plasma. In addition, glycerol cannot be produced metabolically by any process other than hydrolysis of TG. Thus, the changes observed suggest that *E. longifolia* stimulates lipolysis.

As LDL particles are small enough to pass through the endothelium layer, the LDL particles and their content are more prone to being oxidised. Foam cells formed by macrophages during ingestion will not be able to process the oxidised LDL thus, leaving it to rupture and release the oxidised materials and fat on the arterial wall. Over the time, this would trigger a cascade of immune responses leading to atheroma (Al-Joufi *et al*. 2016, Soloperto and Casciaro 2012). During the experimental trial, the results demonstrated a gradual decrease of LDL concentrations between weeks 0 to 5 in *E. longifolia* group. Although no studies have been conducted on the hypolipemiatic effect of *E. longifolia* so far, some epidemiological studies demonstrated that increased testosterone concentrations are associated with the decreased of LDL concentrations (Monroe and Dobs 2013, Shabsigh *et al*. 2005). As testosterone will be aromatised to 17β-estradiol in the body, a study conducted by Giri and co-researcher found that administration of 2 mg of 17 β-estradiol on elderly men demonstrated a significant reduction in LDL concentrations and number of particles (Giri *et al*. 1998). Aside from that, testosterone is also reduced to 5α-dihydrotestosterone (DHT), which are associated with lipid metabolism. A study conducted by Ly and co-workers shows that application of 70 mg of DHT gel on elderly men for 3 months decreased LDL concentrations after 1 month (Ly *et al*. 2001). Therefore, the results of the study suggest that consumption of *E. longifolia* could lower LDL concentrations and prevent cardiovascular diseases especially, congenital cardiac defects among competitive athletes.

In the present study, plasma glucose concentrations significantly increased in both groups at week 3 compared to weeks 0 and 5. This could be due to glycogenolysis in muscle cells and liver tissues to meet the energy demand during exercise (Ayu-Suzailiana *et al*. 2010). Similarly, on the same week, an increase in insulin concentrations was observed in *E. longifolia* group rather in PG group. This shows that *E. longifolia* extract possesses antihyperglycemic properties. Although no studies have been done on humans,

a significant reduction (38 - 47%) in blood glucose was observed in streptozotocin-induced hyperglycaemic adult rats using 150 mg/kg of body weight of *E. longifolia* extract (Husen, Pihie, and Nallappan 2004). Apart from that, 50 µg/mL of *E. longifolia* extract was showed to increase insulin sensitivity by increasing the uptake of glucose by more than 200% in 3T3-L1 adipocytes (Lahrita, Kato, and Kawabata 2015). These findings suggesting that *E. longifolia* extract improves insulin sensitivity thus, allowing glucose to be used as energy. Besides that, increased insulin sensitivity also promotes lipolysis and therefore, increasing energy yield to meet the energy demand during endurance training for a longer period of time.

Several studies have reported consumption of *E. longifolia* contributes significantly in increasing testosterone concentrations (Henkel *et al*. 2014, Talbott *et al*. 2013, Tambi 2003). In this study, the results showed a significant increase in weeks 3 and 5 compared to week 0. The rise in testosterone concentrations could be due to the release of free testosterone from SHBG influenced by the eurypeptides in *E. longifolia* (Chaing *et al*. 1994, Talbott *et al*. 2013, Tambi 2003). A study of men aged 51 years and above with low testosterone found that daily supplementation of 200 mg *E. longifolia* extract for one month significantly improved serum testosterone levels as well as Aging Males' Symptoms (Tambi, Imran, and Henkel 2012). Another study on physically active male and female seniors aged 57 – 72 years found that 400 mg/day of *E. longifolia* extract significantly increases total and free testosterone in both genders (Henkel *et al*. 2014). Aside from that, a study on endurance cyclist also found that 100 mg/day of *E. longifolia* extract increases testosterone level 16% higher compared to PG group (Talbott *et al*. 2006). These results suggest that *E. longifolia* extract could increase testosterone concentrations which in turn, acting as ergogenic aid by enhancing muscle mass and promoting lipid reduction.

5.5 Conclusion

In conclusion, *E. longifolia* supplementation of 1.7 mg/kg of body weight for 5 days impose a significant effect on substrate utilisation and blood parameters measured in collegiate athletes. The present study supports the ergogenic property of *E. longifolia* extract in terms of favouring lipid as an energy source to boost energy yield and prevent fatigue among the collegiate athletes in the present study. Therefore, *E. longifolia* can be considered as an ergogenic aid to enhance lipid utilisation among endurance athletes.

CHAPTER 6: LIPID LOWERING EFFECT OF *EURYCOMA LONGIFOLIA* JACK AQUEOUS ROOT EXTRACT IN HEPATOCYTES

6.1 Introduction

The root of *E. longifolia* has recorded a long ethnobotanical history as health concoctions. The extract of *E. longifolia* root has been shown to possess a myriad of inhibitory activities such as anti-malarial, anti-ulcer, anti-pyretic, anti-cancer, and anti-proliferation (Kuo *et al*., 2003, Nurhanan *et al*., 2005, Park *et al*., 2014, Zakaria *et al*., 2009, Solomon *et al*., 2014). Nevertheless, *E. longifolia* root extract is more renowned for its potentials in enhancing male sexuality (Chan *et al*., 2009, Solomon *et al*., 2014, Kotirum *et al*., 2015). *In vivo* experiments report increased in testosterone concentrations following 14 days-administration of aqueous extract of *E. longifolia* (Solomon *et al*., 2014, Chan *et al*., 2009). Alongside, sperm concentration, total and progressive mortality and vitality were also shown to be increased. Additionally, improvement in sexual behaviours has also been reported when rats were subjected to treatment with *E. longifolia* root extract (Ang and Sim, 1997, Ang and Sim, 1998a, Ang and Sim, 1998b).

Since modulation of testosterone concentration in men is correlated to the supplementation of *E. longifolia* root extract, Hamzah and Yusof have previously studied its effect on body composition, and muscle strength and size in men (Hamzah and Yusof, 2003). With 5 weeks regime of intense strength training, the results demonstrated augmentation of lean body mass and strength in treated group than those in the PG group. Interestingly, a decrease of percentage body fat was also been observed within the two test groups. Similarly, reductions in body weight and omentum fat when administered with *E. longifolia* root extract have also been reported by Solomon and co-workers (Solomon *et al*., 2014). Therefore, this study was designed to investigate whether *E. longifolia* root extract possesses lipid-lowering potential and/or absorption inhibitory potentials of the supplementation.

6.2 Methods

6.2.1 Cell culture

The human hepatic cell line WRL-68 was obtained from the American Type Culture Collection (ATCC, USA). The cells were maintained by regular sub-cultivating in 1X growth media of Dulbecco's modified Eagle medium, DMEM (Gibco, USA), supplemented with 10% heat-inactivated fetal bovine serum (FBS), 100 u/ml of penicillin, 100 u/ml of streptomycin and 20 mM of HEPES buffer at 37 ˚C in a humidified incubator in the presence of 5% CO_2 (Gan *et al*., 2015).

6.2.2 Cell viability assay

Cytotoxicity of *E. longifolia* root extract and palmitic acid against WRL-68 cells were determined using cell viability assay in a 96-well cell culture plate. Briefly, culture media containing increasing concentrations of *E. longifolia* root extract and palmitic acid were provided to the preformed WRL-68 cell monolayer, independently. Fresh media containing MTS solution (Promega, USA), at the concentration recommended by the manufacture, was replaced after 24 h post addition of *E. longifolia* root extract or palmitic acid. The cell culture plate was maintained at culture condition for additional two hours prior to quantitation of the formazan level converted in each well with Epoch spectrophotometer (BioTek Instruments, USA). The toxicity of the *E. longifolia* root extract and palmitic acid were derived from the comparison made with the viability of cells provided with only culture media, respectively.

6.2.3 Fatty acid treatment

A starting solution of 200 mM palmitic acid was prepared by dissolving the palmitate (Sigma, USA) in absolute ethanol. The starting solution was subsequently mixed with 10% bovine serum albumin (BSA) (Sigma, USA) by shaking overnight at room temperature to generate 4 mM palmitic acid stock solution. A sub-confluent monolayer of WRL-68 cells was provided with 150 µM BSA-bound-palmitate in 1X serum free growth medium over 24 h to induce cellular lipid uptake (Luo *et al*., 2012).

6.2.4 Preparation of *E. longifolia* root extract

The *E. longifolia* standardised extract (Physta®, MY), batch number TA150101, was obtained from Biotropics Malaysia Berhad. The certificate of analysis is appended in appendix. Powder (2 g) was allowed to dissolve in 35 ml of double distilled water overnight at room temperature and the solution was then centrifuged at 13,000 rpm for 30 min at 4 ˚C (Satorious, DE). The centrifugation was performed to remove any undissolved particles and amorphous polysaccharides. The clear supernatant was separated and subjected to freeze drying. The extraction yield was measured and expressed in percentage. The extract was then reconstituted with double distilled water to a 50 mg/ml stock and stored at −20 ˚C for further analyses. Additionally, the stock extract was passed through a 0.2 µm syringe filter (Satorious AG, DE) for sterilisation prior to cell culture treatments.

6.2.5 Treatment with *E. longifolia* root extract

Generally, *E. longifolia* concoction with final concentrations of 1, 2, 4 and 8 µg/ml of *E. longifolia* root extract were prepared from *E. longifolia* stock in 1X serum free growth media. Control sets were provided with 1X serum free growth media without the

fortification of *E. longifolia* root extract. To measure the lipid lowering effect of *E. longifolia*, studies were performed on induced fatty liver cells. Meanwhile, different concentrations of *E. longifolia* concoctions were introduced to the liver cells 24 h before or during the palmitic acid induction to investigate if the extract possesses prophylactic and/or anti-(fatty acid)absorption activities, respectively.

6.2.6 Oil-Red-O staining

Control and treated WRL-68 cells were stained with Oil-Red-O at the end of every assay to examine the amount of intracellular lipid accumulation. Briefly, cells were washed with cold phosphate-buffered saline and fixed with 10% paraformaldehyde (Sigma, USA) for 1 h. One hundred microliters of 0.5% of Oil-Red-O (Sigma, USA) in 60% ethanol was added to the cells for 1 h after the removal of fixing solution (Yao *et al*., 2011). The cells were then rinsed with distilled water and images were photographed (Olympus DP73, JP). Finally, the intensity of stain was quantified by dissolving the lipid stain with dimethyl sulfoxide (DMSO), a universal solvent (Sigma, USA) and the intensity of the red coloured dye were read at 510 nm on Epoch spectrophotometer (BioTek Instruments, USA).

6.2.7 Statistical analyses

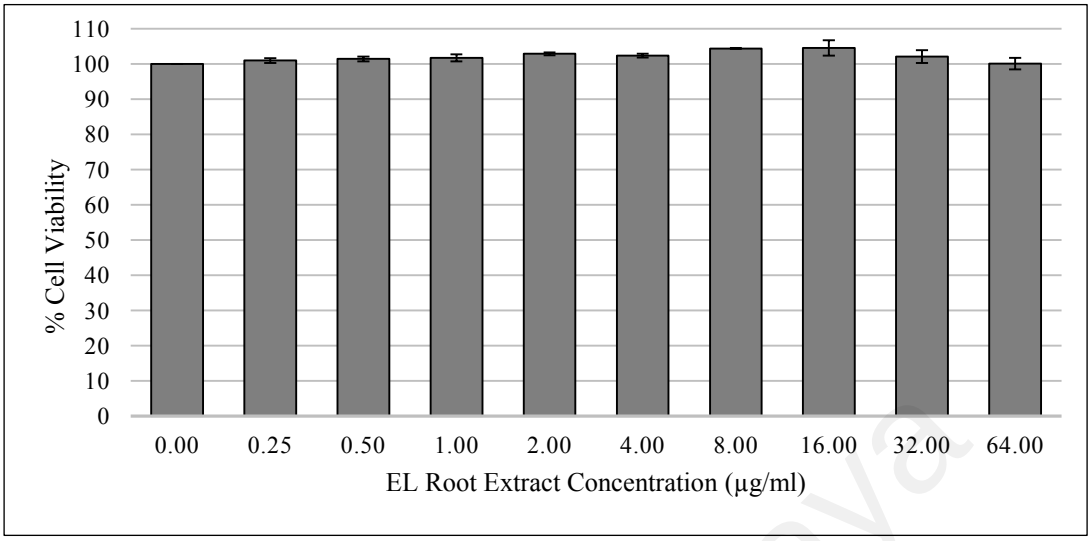
Histograms are expressed as mean ± standard error of the mean. The comparisons between groups were made using Student's *t*-test with statistical computer package SPSS version 17.0 software for Windows (IBM, USA) (Dashti *et al*., 2014).

6.3 Results and discussion

A clear aqueous extract from *E. longifolia* root was prepared as mentioned in the methods section. The total extract yield resulting from the centrifugation was 91.98 ± 0.12%. Subsequently, the *E. longifolia* root extract was investigated for its potential toxicity. Increasing concentrations of *E. longifolia* root extract starting from 0.25 up to 64 μg/ml were mixed in the cell culture media. The media were then used to culture the human hepatic cell line WRL-68 and cell viability assay was conducted 24 h after. Since the viability of WRL-68 cells cultured in EL root extract up to 64 μg/ml demonstrated ~100% viability as compared to those without (Figure 6.1), the results revealed no toxicity effect of the *E. longifolia* root extract for the concentrations tested.

On the other hand, increasing concentrations of palmitic acid starting from 50 μM up to 800 μM in culture media have resulted in gradual loss of WRL-68 cell viability (Figure 6.1). When cultured with media containing 50 μM of palmitic acid, the cell viability of WRL-68 was ~95% when compared to those cells cultured in palmitic acid-free media (control sets). Culturing the cell line with media containing 100 and 200 μM of palmitic acid have decreased the cell viability to ~90% when compared to the control sets. The plummeting trend of cell viability continues with the presence of higher concentration of palmitic acids; reduction of another 5% of cell viability was observed in culture with the presence of 400 μM of palmitic acid and the cell viability was reduced to ~80% when cells were cultured in media containing 800 μM of palmitic acid. Similarly, significant changes in hepatic cell morphology and even cell death have also been observed by others when media containing high concentration of palmitic acid was used (Feldstein *et al*., 2004). Therefore, culture media containing 150 μM palmitic acid was used to induce WRL-68 intracellular fatty acid uptake.

(A)



(B)

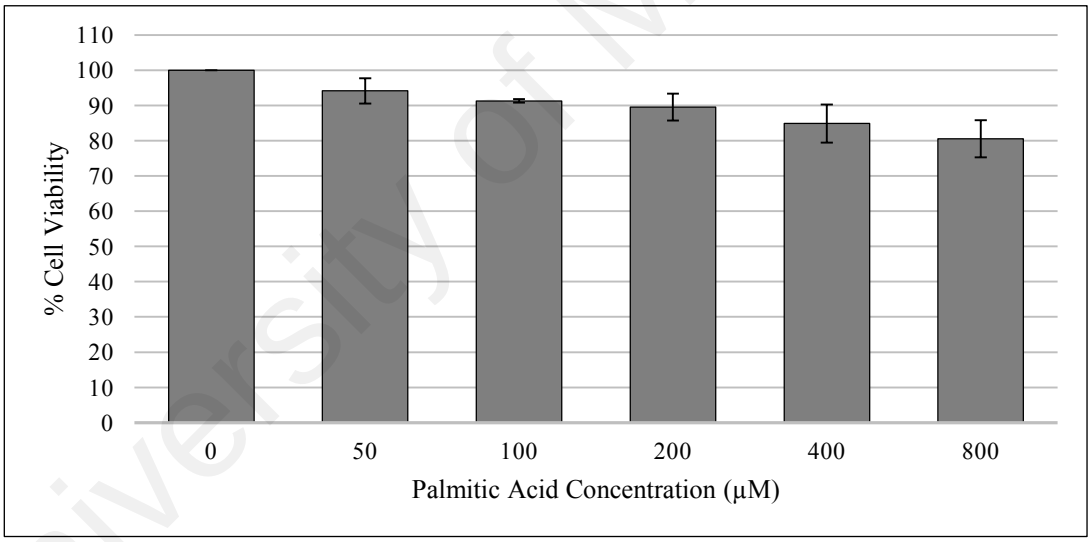


Figure 6. 1: Cell viability human hepatic cell line WRL-68 when cultured with (A) *E. longifolia* (EL) root extract and (B) Palmitic acid. Cell viability assay was used to evaluate the cytotoxicity of *E. longifolia* root extract and palmitic acid concentrations used in this study. Data showed the percentage viability of the cells after 24 h when compared to same cells cultured without *E. longifolia* root extract or palmitic acid, respectively. Results were mean of six experiments (n = 6) and the error bars are expressed as standard error of the mean.

In order to investigate the lipid-lowering potential of the *E. longifolia* root extract, WRL-68 was first induced to store fatty acid in the cell. Cells with increased fatty acids build up were then treated with 1, 2, 4, or 8 μg/ml of *E. longifolia* root extracts. The concentration of the cellular fatty acids was assessed 24 h post-treatment and comparison were made with those without *E. longifolia* root extract as control (Figure 6.2). From the images, markedly reduced amount of cells with intracellular lipid droplets (stained red) were observed in those cells treated with *E. longifolia* root extract. Moreover, reduced intensity of the red dye has also been observed in cells especially in those treated with 2 and 4 μg/ml of *E. longifolia* root extract. Accordingly, intracellular fatty acids were extracted with DMSO and subjected to colour intensity measurement. It is worth mentioning, that the average amount of fatty acids quantified in the control sets was regarded as 100% build up. Results revealed that treatment with *E. longifolia* root extract at concentration of 1 to 8 μg/ml diminished the fatty acids build up when compared to control sets (Figure 6.3). These observations have therefore, demonstrated the lipid-lowering potential of *E. longifolia* root extract.

To the best of our knowledge, this is the first observation which demonstrates the intriguing lipid lowering effect of this medicinal plant without the presence of testosterone in cell milieu. It was observed that with 24 h treatment of 1 μg/ml of *E. longifolia* root extract resulted in 18.2% ($p > 0.05$) lesser fatty acids detected compared to control cells. Meanwhile, treatment with 2 and 4 μg/ml of *E. longifolia* root extracts resulted in 71.2% ($p < 0.01$) and 63% ($p < 0.01$) dropped in cellular fatty acids, respectively. Lastly, treatment with 8 μg/ml of the same extract resulted in 33.7% ($p > 0.05$) when compared with the control cells. The basis for the reduced lipid-lowering potential at 8 μg/ml of *E. longifolia* root extract remains unclear and requires further investigation.

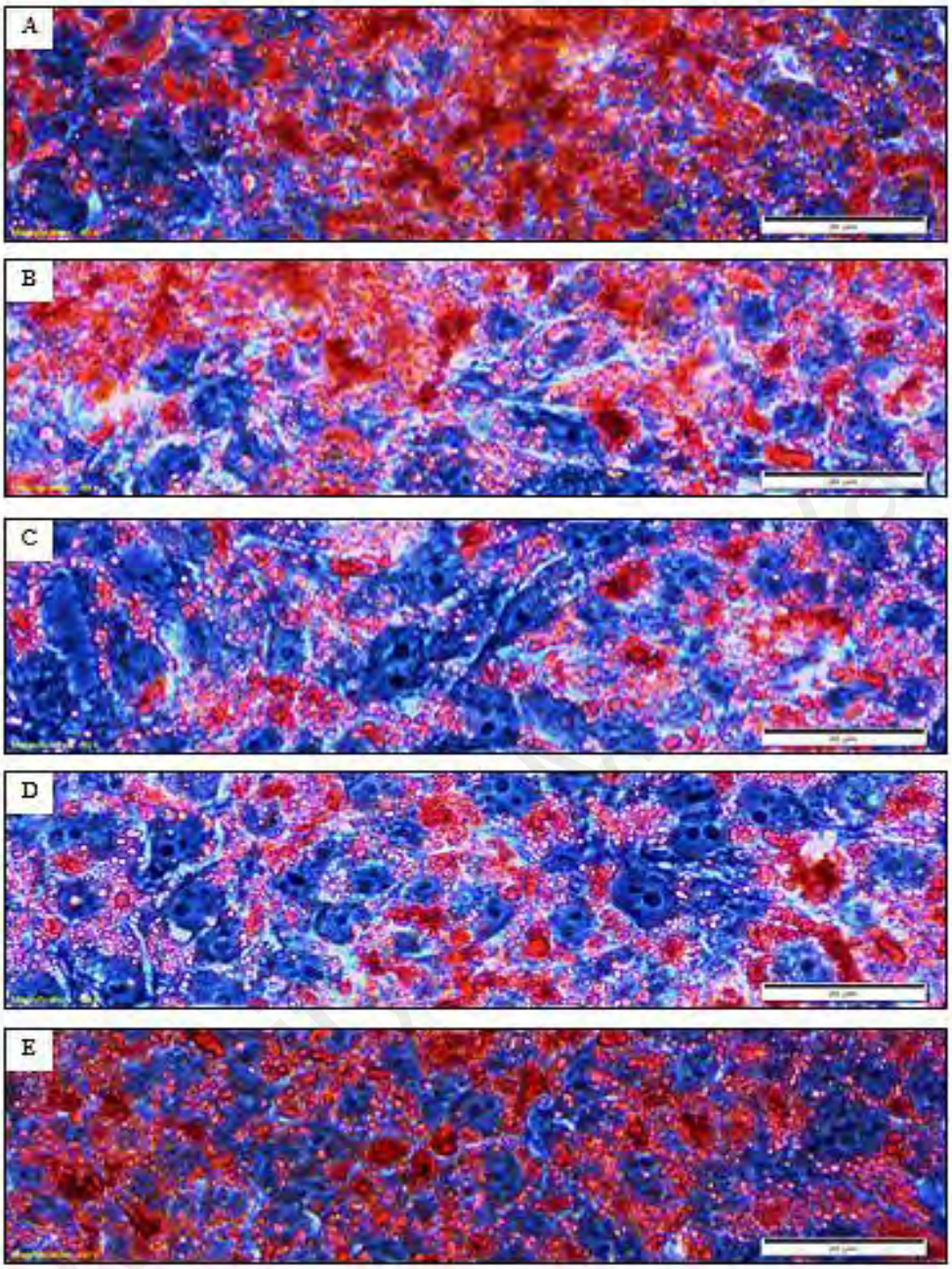


Figure 6. 2: Lipid lowering effect of *E. longifolia* root extract treatment on fat induced-human hepatic cell line WRL-68. The intracellular lipids were stained red with Oil Red O. Images were captured at 60 X magnification and scale at 20 µm. (A) Control set. Cells without the supplementation of *E. longifolia* root extract. (B) Cells with supplementation of 1 µg/ml of *E. longifolia* root extract. (C) Cells with supplementation of 2 µg/ml of *E. longifolia* root extract. (D) Cells with supplementation of 4 µg/ml of *E. longifolia* root extract. (E) Cells with supplementation of 8 µg/ml of *E. longifolia* root extract.

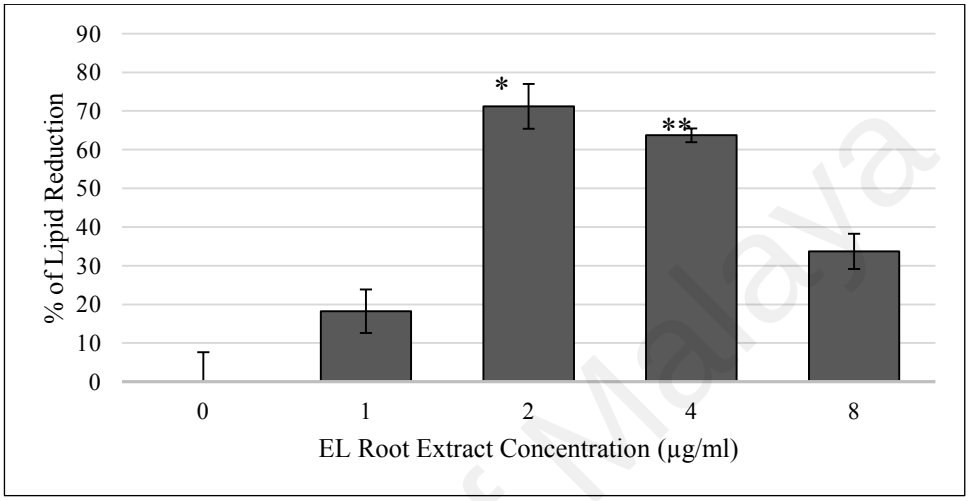


Figure 6. 3: Percentage reduction of fatty acids in human hepatic cell line WRL-68 upon supplement with *E. longifolia* root extract at various concentrations. Colour intensity measurement after dissolving the stained cells with DMSO from three (n = 3) independent experiments. Results are presented with average value of control sets (with 0 µg/ml of *E. longifolia* root extract) normalized to 0% of lipid reduction. Error bars are expressed as standard error of the mean and statistical significance as compared to the control sets ($p < 0.05$) is indicated by single asterisk and ($p < 0.01$) is indicated by double asterisks above the respective error bars.

The study extends to further investigate the capability of *E. longifolia* root extract to inhibit fatty acids uptake by the cells. Firstly, the hepatic cell line WRL-68 was treated with the same set of *E. longifolia* root concoctions for 24 h prior to the induction of intracellular fatty acids accumulation. This was performed to investigate whether the *E. longifolia* root extract has any prophylactic inhibitory effect on fatty acids absorption. Similarly, all treated cells were compared with a control set, which was cultured in media free of *E. longifolia* root extract. The cells were stained for intracellular lipid droplets accumulated after 24 h of induction. Interestingly, the cell images suggest a slight reduction of the red dye in those cells treated with different concentrations of *E. longifolia* root extract when compared to the control sets (Figure 6.4). Furthermore, spectrophotometric measurement of the colour intensity also resulted up to 22% reduction of the colour intensity, which associated to lesser amount of fatty acids absorption in those cells treated with 2 µg/ml of *E. longifolia* root extract onwards (Figure 6.5). However, the reductions were statistically insignificant ($p > 0.08$). Therefore, the results showed weak inhibition efficiency of fatty acids adsorption activity by *E. longifolia* root extract when WRL-68 cells were prophylactically treated.

In order to examine if the *E. longifolia* root extract would interfere with the fatty acids adsorption during the induction of intracellular fatty acids accumulation itself, different concentrations of *E. longifolia* root extract were included during the induction with 150 µM palmitic acid. Similarly, all treated cells were compared with a control set, which was induced without the addition of *E. longifolia* root extract in the induction media. Again, the cells were stained for intracellular lipid droplets accumulated after 24 h of induction. The images of the cells (Figure 6.6) and the spectrophotometric readings (Figure 6.7) showed no significant difference in both cellular morphology and colour intensity between those induced WRL-68 cells with or without the presence of *E. longifolia* root extract. The results therefore, indicate no interference to fatty acids

adsorption during the induction of intracellular fatty acid uptake in the presence of *E. longifolia* root extract.

Administration of *E. longifolia* root extract was shown to regulate the testosterone level (Hamzah and Yusof, 2003, Chan *et al*., 2009, Kotirum *et al*., 2015). In turn, testosterone regulates many physiological processes such as muscle protein metabolism, sexual and cognitive functions, secondary sex characteristics and plasma lipids (Bhasin *et al*., 2001). Studies involving testosterone replacement in healthy young men have demonstrated significant increase in overall muscle mass and reduced body fat (Bhasin *et al*., 2001, Woodhouse *et al*., 2004, Hamzah and Yusof, 2003). Interestingly, supplementation with complimentary medicine *E. longifolia* root extract has demonstrated similar outcome to that of testosterone replacement regime.

Moreover, as it was well characterised, increased total fat is associated with increased risk of atherosclerotic heart disease, hypertension, and dyslipidemia (Woodhouse *et al*., 2004). Therefore, interventions that decrease accumulation of fat in the intraabdominal and intermuscular depots would be expected to decrease cardiovascular risk. It is noteworthy, that this study has unravelled additional benefit of aqueous *E. longifolia* root extract to independently reduce intracellular lipids. This phenomenon is most desirable as the *E. longifolia* root extract may possess higher fat burning efficacy by testosterone-induced pathway and *E. longifolia* pathway. Work is in progress to further clarify the lipid-lowering potential of *E. longifolia* root extract and to further elucidate the cellular signalling mechanisms that explain the phenomenon.

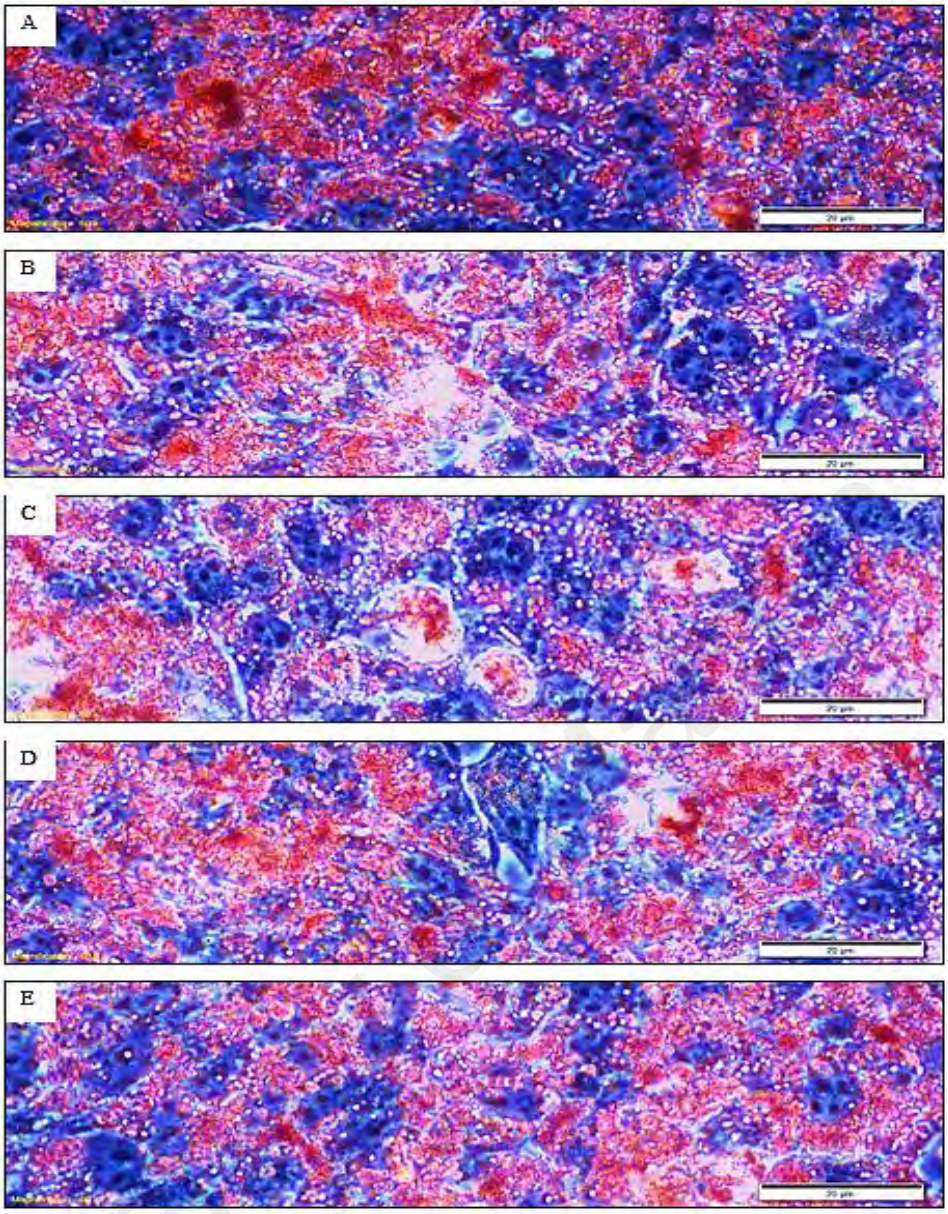


Figure 6. 4: Prophylactic effect of *E. longifolia* root extract treatment on fat induction of human hepatic cell line WRL-68. The different concentrations of *E. longifolia* root extract were supplied 24 h prior to induction of intracellular fatty acid uptake by palmitic acid. The intracellular lipids were stained red with Oil Red O. Images were captured at 60X magnification and scale at 20 µm. (A) Control set. Cells without the supplementation of *E. longifolia* root extract. (B) Cells with supplementation of 1 µg/ml of *E. longifolia* root extract. (C) Cells with supplementation of 2 µg/ml of *E. longifolia* root extract. (D) Cells with supplementation of 4 µg/ml of *E. longifolia* root extract. (E) Cells with supplementation of 8 µg/ml of *E. longifolia* root extract.

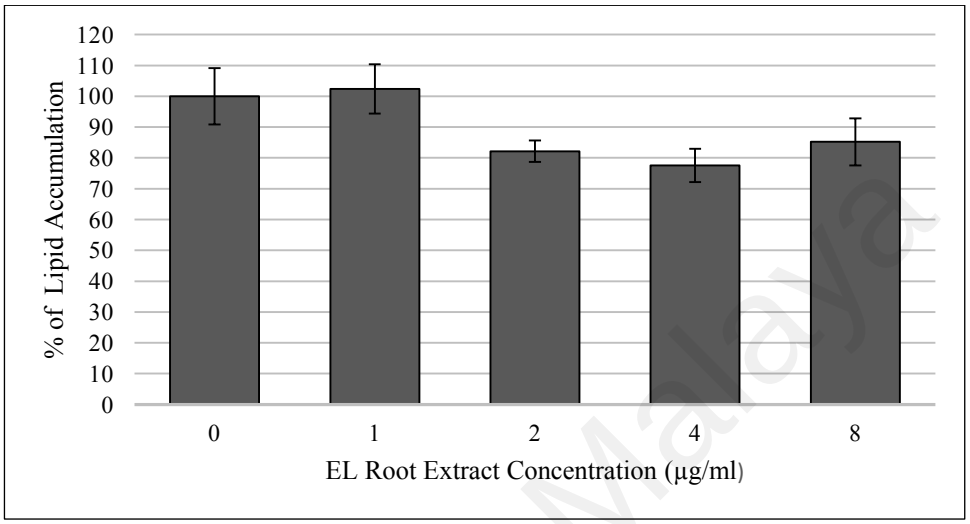


Figure 6. 5: Percentage accumulation of fatty acids in human hepatic cell line WRL-68 after prophylactic supplementation of *E. longifolia* (EL) root extract at various concentrations. Colour intensity measured after dissolving the stained cells with DMSO from three (n = 3) independent experiments. Results are presented with average value of control sets (with 0 µg/ml of *E. longifolia* root extract) normalized to 100% of lipid accumulation. Error bars are expressed as standard error of the mean. The variance shown in the histograms were not significantly different from the control ($P > 0.05$).

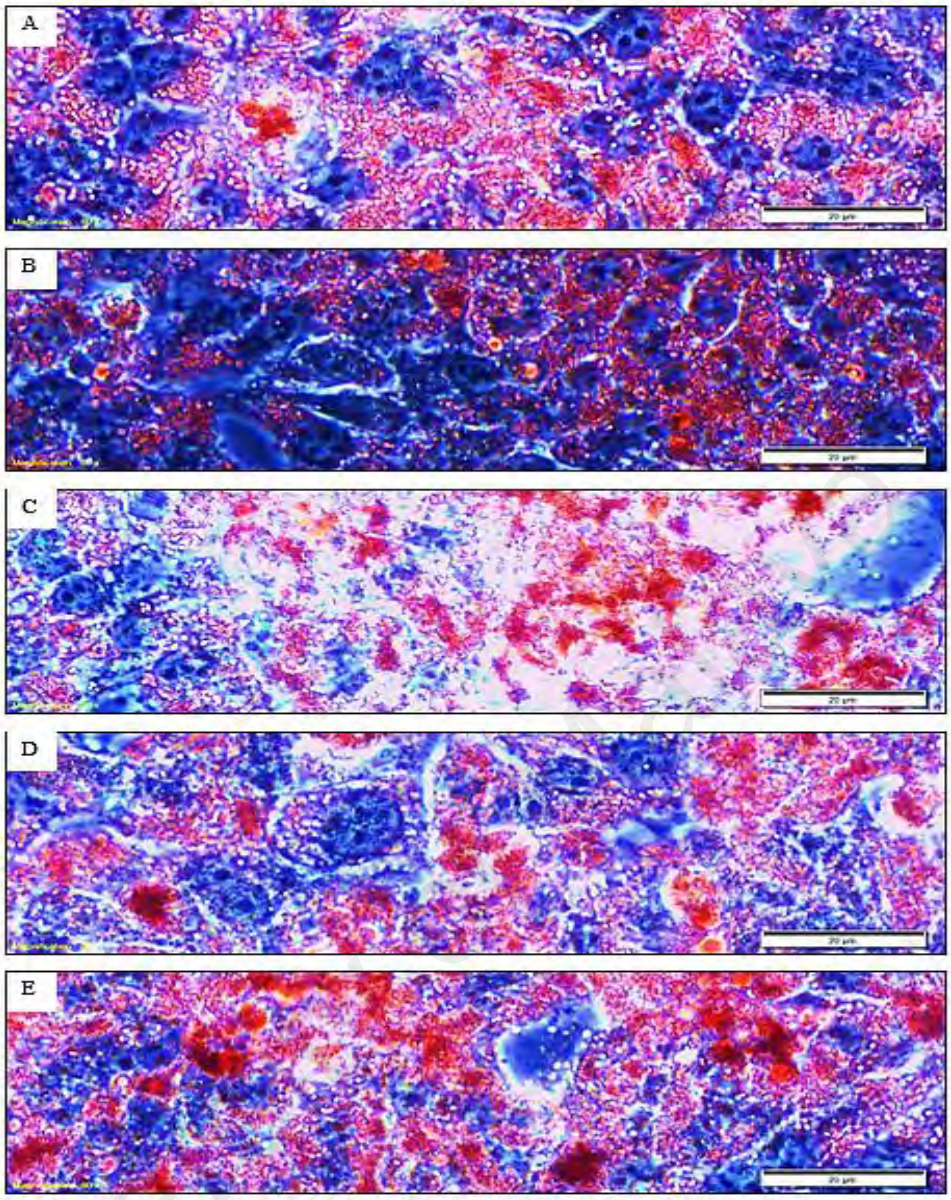


Figure 6. 6: Effect of *E. longifolia* root extract treatment on fat induction of human hepatic cell line WRL-68. The different concentrations of *E. longifolia* root extract were supplied during the induction of intracellular fatty acid uptake by palmitic acid. The intracellular lipids were stained red with Oil Red O. Images were captured at 60X magnification and scale at 20 µm. (A) Control set. Cells without the supplementation of *E. longifolia* root extract. (B) Cells with supplementation of 1 µg/ml of *E. longifolia* root extract. (C) Cells with supplementation of 2 µg/ml of *E. longifolia* root extract. (D) Cells with supplementation of 4 µg/ml of *E. longifolia* root extract. (E) Cells with supplementation of 8 µg/ml of *E. longifolia* root extract.

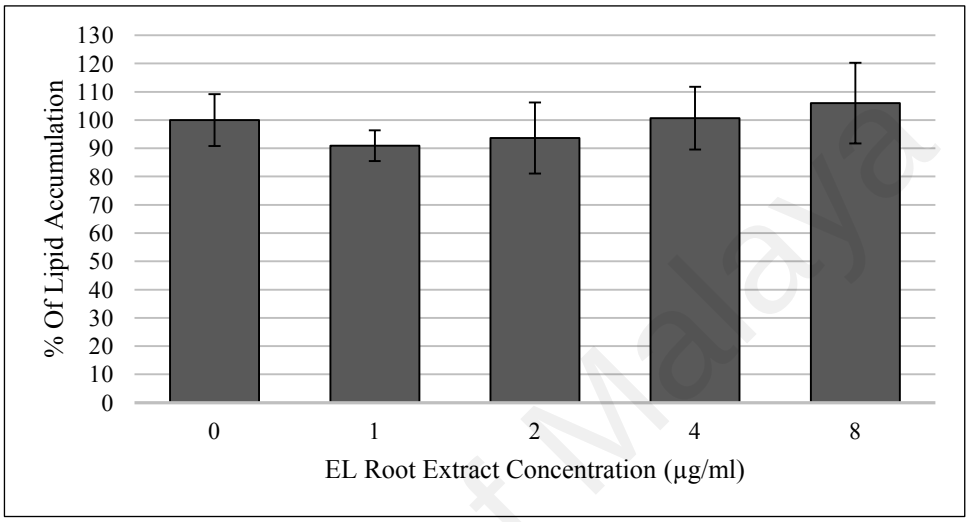


Figure 6. 7: Percentage accumulation of fatty acids in human hepatic cell line WRL-68 after supplementation of *E. longifolia (*EL) root extract at various concentrations during intracellular induction of fatty acid accumulation. Colour intensity measured after dissolving the stained cells with DMSO from three (n = 3) independent experiments. Results are presented with average value of control sets (with 0 µg/ml of *E. longifolia* root extract) normalized to 100% of lipid accumulation. Error bars are expressed as standard error of the mean. The variance shown in the histograms were not significantly different from the control ($P > 0.05$).

6.4 Conclusion

In conclusion, this study provides evidence of intracellular lipid metabolism by *E. longifolia* root extract. The reduction in intracellular lipid droplets was best shown when the induced WRL-68 cells were treated with 2 µg/ml *E. longifolia* root extract. Moreover, treating the cells prior to or during the induction of intracellular fatty acid accumulation with 150 µM of palmitic acid showed no efficacious inhibition of fatty acid adsorption activity, albeit the marginal decrease in fatty acid build-up observed in those WRL-68 cells prophylactically treated with 2 µg/ml *E. longifolia* root extract or higher. Nevertheless, this study demonstrates the lipid reduction potential of *E. longifolia* root extract as viable alternative medicine, especially for those of testosterone and fat-related ailments.

CHAPTER 7: GENERAL DISCUSSION AND CONCLUSION

This thesis investigated the CAM usage by the collegiate athletes, particularly on the herb *E. longifolia* that is often cited as ergogenic aid to improve stamina in endurance exercises.

More specifically, the aim of the thesis was:

1. to investigate the prevalence of CAM usage among collegiate athletes in Malaysia.
2. to investigate the acute consumption effect of *E. longifolia* Jack on aerobic metabolisms during prolonged exercise.
3. to investigate the chronic consumption effect of *E. longifolia* Jack on aerobic metabolisms during prolonged exercise.
4. to investigate the lipid-lowering effect of *E. longifolia* Jack in an isolated cell culture.

In this chapter, the findings of all the studies are discussed in two parts; Part A and Part B. Part A discusses on the prevalence of CAM usage among collegiate athletes and the future research questions. While Part B focuses the on the lipid-lowering effects of *E. longifolia* during prolonged exercise and isolated cell culture as well as their future research questions.

7.1 Part A: Prevalence of CAM among collegiate athletes

The usage of CAM varies widely among population and countries (Furnham 2002). Many people today choose herbal supplements rather than prescription medicine as the supplements are natural, safe, and affordable (Debas, Laxminarayan, and Straus 2006). It

has also been use as a remedy for health related issue and maintenance, particularly in improving stamina and increasing muscle mass (Atkinson 2007). Previous studies have showed that CAM are frequently used as self-medication or energy booster among (Theberge 2008a, Kong *et al*. 2007). Although this evidence suggesting that CAM could have been an important role in determining athlete's health and exercise performances however, little is known about the prevalence of CAM usage in collegiate athletes in Malaysia.

Chapter 3 examined the prevalence of CAM usage among national collegiate athletes in Malaysia using the questionnaire I-CAM-Q. The findings showed that 86% of the collegiate athletes used at least one kind of CAM in the past 12 months and the usage was significantly higher in women than men. The high prevalence of CAM usage among athletes this study was not surprising and in line with previous survey from other countries. Nichols and Harrigan reported that 56% of the intercollegiate athletes in Hawaii used at least one kind of CAM in the past 12 months and significantly higher in women than men as well (Nichols and Harrigan 2006). The high prevalence of CAM usage which attracted the athletes in using CAM could be speeding up recovery from injuries and illness that may interfere their training session or competition, improve body images, recommendation from family and peers and health maintenance (Nicholl, Coleman, and Williams 1995, Spigelblatt 1995). As the athletes are constantly competing among each other, they would rather take the risk and leave no option untried to get back to the field as soon as they can (Ernst 2000, Nichols and Harrigan 2006).

Aside from that, it was also found that athletes preferred to visit physician who practise both conventional and alternative medicine. This was seen not only in athletes but in other studies which involved diseases as well. It seems that physicians who have knowledge and experiences on CAM are likely to respond positively to athletes/patients and offering them with wider choices in maintaining their health (Winslow and Shapiro

2002). Among the CAM therapies, acupuncture seems to be the popular choice among the collegiate athletes as well as in general populations and those with diseases (AlBedah *et al*. 2013, Opheim *et al*. 2012). Previous studies have found that acupuncture could benefits in both quality life and clinical symptom as it may accelerate the release of specific neuropeptides in the central nervous system and therefore, activating the self-healing mechanism (Schneider, Streitberger, and Joos 2007, Kong *et al*. 2007).

Previous studies have shown a high prevalence of CAM usage among general population in Malaysia, particularly herb-based therapies (Siti *et al*. 2009). Yet, the findings from Chapter 3 showed that the collegiate athletes commonly used Vitamin C to for health maintenance. Vitamin C is well known to influence physical performance such as synthesis of carnitine which convey long adipose acid chain into the mitochondria, facilitates the convey and uptake of non-heme iron at the mucosa, potent antioxidant that regenerates oxidized by-products and enhance immune function (Lukaski 2004, Kotze *et al*. 1977, Sen and Hanninen 1994). Previous studies have demonstrated that usage of Vitamin C could reduce lipid peroxidation and muscle damage during high exercise intensity and prevent infections among competitive athletes (Zoppi *et al*. 2006, Peters *et al*. 1993). This could therefore, attracts the interest of athletes in taking Vitamin C supplementations as they cannot afford to have any infections and muscle damage which could delay their training session and affect their performances while in competitions.

Other than that, Chapter 3 also found that praying for health also seems to be popular among the collegiate athletes in Malaysia. As praying is a part of religion practise, there is controversy whether praying for own health should be included in CAM therapies (Opheim *et al*. 2012, AlBedah *et al*. 2013). If praying for health is disregarded, relaxation technique would be the most common CAM therapies used among the collegiate athletes due to stress during competitions and trainings. As relaxation techniques are associated

with stress management, many athletes have claimed that practising relaxation help them cope with stress and play a part in relapse prevention (Opheim *et al*. 2012).

The usage of CAM among athletes as an ergogenic aid seems to gain much popularity in Malaysia. In order to gain future insight on the potential benefits of the CAM supplementations and therapies for athletes, analytical and intervention studies are required as different countries would be practising CAM based on their culture and availability (i.e. herbs). Therefore, CAM could play an important role by speeding up recoveries, releasing stress and optimising their health when undergoing exercise trainings and competitions in athletes.

7.1.1 Future research questions: CAM

Although Chapter 3 have investigated the prevalence of CAM usage among collegiate athletes in Malaysia, there are still a number of key questions remain to be answered:

1. Do athletes have any knowledge on the CAM that they are practising?
2. Do athletes decide on the CAM therapies based on their belief and attitude?
3. Do athletes income affect the type of CAM they are practising?

7.2 Part B: Lipid lowering potential of *E. longifolia* in human and cellular model.

Chapter 4 has examined the acute effects of *E. longifolia* on aerobic metabolism among collegiate athletes recruited from Chapter 3. The study was designed using as a randomised, double-blinded, and placebo-controlled study model. Twenty male athletes were randomly assigned either to *E. longifolia* group (n = 9) or PG group (n = 11) and ingested 1.7 mg/kg of body weight of either *E. longifolia* or PG for three days prior to the treadmill exercise test (65% of VO_2max) for an hour. Expired air, blood plasma and serum metabolites were being evaluated in this study. Chapter 4 demonstrated significant changes in lipid metabolisms, plasma FFA, glycerol, TG as well as serum testosterone and cortisol concentrations. Utilisation of fatty acid have been shown to improve endurance exercise capacity and performances (Azevedo *et al*. 1998, Yeh *et al*. 2011). As seen in Chapter 4, significant increase in lipid oxidation was observed in *E. longifolia* group compared to PG group. This indicates that the increase demand of energy during the exercise trial was met by the enhanced rate of lipolysis rather than CHO utilisation. By reducing CHO utilisation, glycogen depletion and lactate production could be prevented thus, enable the endurance athletes to perform for a longer duration (Coggan *et al*. 2000). The lipolytic effect observed in was further supported by the decrease in TG and increase of glycerol concentration in the blood. According to Romijn and co-researchers, glycerol in the blood is an indication of lipolysis and as glycerol increases, total FFA from lipolysis made available for oxidation at the rate of two-threefold greater than the rate of oxidation to be converted as energy source (Romijn *et al*. 1993, Romijn *et al*. 1995). Although significant reduction of FFA was observed in, this could suggests that the plasma FFA in *E. longifolia* group has been catabolised to synthesise to ATP to meet the increased energy demand during exercise trial. Aside from that, Chapter 4 also showed significant increase in testosterone concentrations in *E. longifolia* group. This finding was in line with other studies suggesting that consumption of *E. longifolia* is able

to restore normal testosterone levels may be due to eurypeptides, a group of small peptides in *E. longifolia*, influences the release rate of free testosterone from SHBG (Talbott *et al*. 2006, Talbott *et al*. 2013, Tambi 2003, Chaing *et al*. 1994). In addition, significant increase in cortisol concentration was also observed. Previous studies have demonstrated that with the increase in cortisol concentrations, this could stimulate the sensitivity of adipose tissue lipolysis thus, increasing the FFA and glycerol concentrations, which are indicators of lipolysis in human (Askew *et al*. 1975, Djurhuus *et al*. 2002, Samra *et al*. 1998). These findings indicate that the *E. longifolia* supplementation would influence lipolytic metabolism which in turn, providing alternative resources, i.e. FFA and glycerol, for higher yield of energy production.

In Chapter 5, the chronic effects of *E. longifolia* on aerobic metabolism among collegiate athletes were examined. Using similar methodological approach, the experiment was conducted for a prolonged period. As expected the findings in Chapter 5 also demonstrated significant changes in lipid oxidation, plasma FFA, glycerol, TG and serum testosterone concentrations in *E. longifolia* group. In addition to that, insulin and LDL also showed significant changes in *E. longifolia* group. The significant increment in testosterone concentration observed is in line with previous studies after the consumption of *E. longifolia* supplementation (Talbott *et al*. 2013, Tambi, Imran, and Henkel 2012, Henkel *et al*. 2014). As mentioned, this could be due to the release of free testosterone from SHBG influenced by the eurypeptides in *E. longifolia*. With the increase of testosterone concentrations seen in Chapters 4 and 5, this could give a rise in fat oxidation as suggested by previous studies (Frederiksen *et al*. 2012, Rebuffe-Scrive, Mårin, and Björntorp 1991). Testosterone could stimulate the lipid hormone, HSL which is required for the hydrolysis of TG to plasma FFA and glycerol for energy production (Rebuffe-Scrive, Mårin, and Björntorp 1991). Therefore, consumption of *E. longifolia* can serve as an ergogenic aid by stimulating lipolysis and promoting fat utilisation, benefiting those

who participate in sports that require high energy demand. In contrast to preceding chapter, Chapter 5 demonstrated significant increase in plasma FFA. As the body favours fat over CHO as an energy source, this stimulates the lipolysis process which results in greater proportion of FFA and glycerol released into the circulation and skeletal muscle for oxidation (Horowitz 2003, Romijn *et al*. 1993). Furthermore, as adipose tissue does not contain glycerol kinase, glycerol released from lipolysis will appear in blood plasma. Thus, a significant decrease in TG and a significant increase in plasma FFA and glycerol showed that lipolysis was evident in *E. longifolia* group. In addition, a significant decrease in LDL concentrations observed in *E. longifolia* group showed that *E. longifolia* could prevent cardiovascular diseases especially, congenital cardiac defects among competitive athletes. Although there are no direct link between *E. longifolia* and LDL, the decrease in LDL concentrations could be linked to the increase of testosterone concentrations. Previous studies have shown that 17 β-estradiol and DHT by testosterone could reduce LDL concentrations and are associated with lipid metabolism (Giri *et al*. 1998, Ly *et al*. 2001). Lipolysis are very sensitive to the changes in insulin concentration. An increase in insulin during exercise decreases lipolysis (Horowitz 2003). However, despite the increase in insulin concentration, there is still lipolysis occurring suggesting that *E. longifolia* possesses both lipid-lowering and antihyperglycaemic properties. Although no studies have been carried out on humans, administration of 150 mg/kg of body weight of *E. longifolia* extract on hyperglycaemic rats showed significant reduction (38 - 47%) in blood glucose (Husen, Pihie, and Nallappan 2004). This shows that *E. longifolia* extract improves insulin sensitivity thus, allowing both glucose and fat to be used as energy to meet the energy demand during endurance training for a longer period of time.

In Chapter 6, the study adopts an *in vitro* model to investigate whether *E. longifolia* extract possesses lipid lowering and/or absorption inhibitory potentials. The

human hepatic cell line WRL-68, both normal and fatty liver cells were treated with various concentrations (1, 2, 4 and 8 µg/ml) of the *E. longifolia* extract. The fatty cells was assessed 24 h post-treatment and compared with those without *E. longifolia* extract as control. Results showed that the intensity of the red dye in those treated with 2 and 4 µg/ml of *E. longifolia* extract were reduced up to ~72%, demonstrating *E. longifolia* possesses lipid-lowering effect. The lipid lowering-effect of *E. longifolia* can also be seen in previous work conducted by Lahrita and co-researcher in 3T3-L1 adipocytes (Lahrita, Kato, and Kawabata 2015). The study extends to further investigate the capability of *E. longifolia* extract in inhibiting fatty acids uptake by the cells and interfering with the fatty acids absorption during the induction of intracellular fatty acids accumulation itself. However, the investigation did not elicit any significant inhibition or interfering with the fatty acids absorption when compared to the respective control cells. This study, therefore, unravelled the novel potential of intracellular lipid lowering effect of *E. longifolia*.

7.2.1 Future research questions

Although Chapters 4, 5 and 6 have investigated the lipid-lowering effects of *E. longifolia* on humans and cells, there are still a number of key questions remain to be answered:

1. Does longer term (< 5 weeks) *E. longifolia* supplementation augment fat metabolism during exercise?
2. Does *E. longifolia* supplementation augment fat metabolism at rest?
3. What are the mechanism to describe the lipid-lowering effect of *E. longifolia* supplementation?
4. Which of the eurypeptides in the *E. longifolia* are responsible for the lipid-lowering effect?

5. Does the lipid-lowering effect of *E. longifolia* work in female athletes?
6. Can *E. longifolia* supplementation be used as an ergogenic aid to improve endurance exercises at a higher dosage (2 – 4 mg/kg body weight)?

7.3 Conclusion

This study has shown that there is a high prevalence of CAM usage among national collegiate athletes in Malaysia, especially female athletes. Athletes' prefer to seek physicians who have knowledge on both conventional medicine and CAM. In addition, both male and female athletes prefer using CAM to promote recovery, especially by using supplementation such as Vitamin C, and relaxation technique as self-help practices. This is because usage of supplements for recovery and performance booster are readily available and affordable. Therefore, it is shown that Malaysian athletes similar from those from other countries, rely on CAM usage to manage their well-being. Experiments using human subjects carried out in this study revealed that *E. longifolia* could promote lipolysis which in turn, benefiting athletes by disproportioning the metabolism towards fat burning for better energy yield. While the *in vitro* study showed that the reduction of lipid still occurs despite the absence of testosterone suggesting that the lipid-lowering effect of *E. longifolia* was independent of testosterone, and the optimal effective dose is 2 µg/ml *E. longifolia* extract.

With the data from this study, the knowledge on epidemiology of CAM use among national athletes is revealed. These findings will serve as one of the foundations in policy making for sports management. As a growing country in sports, *E. longifolia* can be widely used as ergogenic aids to improve performance and achieved the desired body composition for competition. Interestingly, results from this study also unravelled the novel usage of *E. longifolia* in weight management and metabolic diseases such as

obesity, which poses a great burden on both developed and developing countries, Malaysia included.

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