1.1 Indole, Schiff Base and Organometallic Compounds

Alkaloids are heterocycles with nitrogen atom included in the aromatic system, of which the presence contributes many important needs to plants, animals and human beings. Alkaloids have a variety of skeletons such as imidazole, indole, quinoline, piperazine and many others but indole serves the most common alkaloid. Indole, which is also called benzopyrrole is well known as the dominant alkaloid. It is a planar heteroaromatic molecule with a ten-electron π system. The nitrogen is very weakly basic because of delocalization of its unshared electrons on the nitrogen atom into the π system. The total net atomic electron densities are shown in Figure 1 (Badger, 1961).

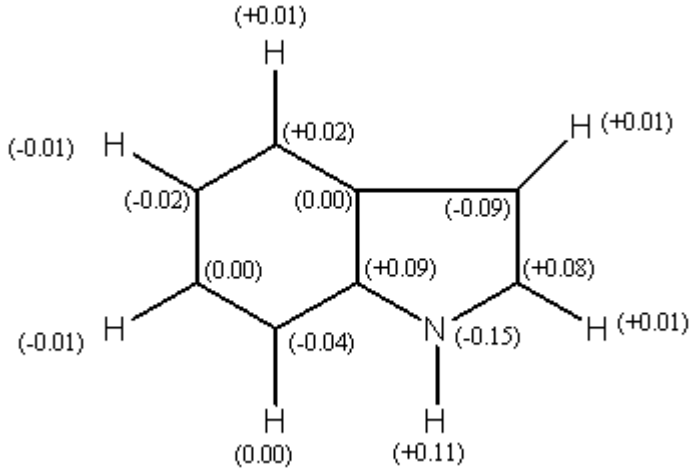

Figure 1: Structure of indole with its electronegativity values (Badger, 1961).

There are several examples of naturally occurring indoles of physiological and medicinal significance. Physiologically significant indole derivatives include L-tryptophan, serotonin and melatonin. L-tryptophan is an essential amino acid while serotonin is a hormone that is derived from L-tryptophan and serves as a vasoconstrictor and regulates gastric secretion

and intestinal peristalsis. Melatonin is an indole derivative which is synthesized naturally from N-acetylserotonin in the pineal gland.

Indole derivatives have also been used in medicinal treatment and are medicinal significant for clinical use. Reserpine is an important tranquilizer. Vincristine and vinblastine are antineoplastic agents used to treat choriocarcinoma and Hodgkin's disease (Badger, 1961). Indole alkaloids from marine natural products found by Hamann *et al*. (2005) showed interesting biological activities including cytotoxic activity on several cancer cell lines. They are shown to be anti-viral, anti-microbial, anti-parasitic, anti-inflammatory, anti-serotonin, Ca^{2+} releasing agents with calmodulin-antagonist and anti-topoisomerase-I activity.

Schiff bases display good activities and are found to be remarkably effective in pharmaceuticals. Schiff base compounds are synthesized from aldehydes or ketones and primary amines by condensation to give an imine, where the absorption band is found at 1635 cm^{-1} (Akitsu *et al*., 2005) in the IR spectrum. Much research has been done on imino compounds in order to study their medicinal and pharmaceutical applications. When alkaloid compounds are included in the structure of Schiff bases, the biological activities will increase significantly.

Pandeya *et al*. (1999) synthesized some Schiff and Mannich bases of isatin derivatives in order to investigate their efficacy as antibacterial, antifungal and anti-HIV agents. It is also well known that compounds containing N and S donors serve as good chelating agents and their key role in coordination to the metals has been increasingly explored (Singh *et al.*, 2007).

Coordination chemistry have broaden the perspectives of studying metal based compounds as alternatives towards the effort of seeking new and more potent therapeutic agents. Drugs available on the market today have satisfactorily treated various chronic diseases such as diabetes, peptic ulcer and cancer but their use in therapy is often associated with undesirable side effects. Organometallic compounds are now being recognized as important and potentially useful tools for various diagnoses and therapies.

Some metals, especially transition elements are essential for biological functions and are found in enzymes and cofactors required for various processes (Ahmad *et al.*, 2006). Some metal ions involved in these processes requires specific oxidation states to fulfill the necessary catalytic or structural requirement, while other processes are much less specific and is possible to replace one metal ion by another (Hughes, 1972). Because of this, there are several organometallo drugs used as therapeutic agents to treat a few diseases. Research in coordination chemistry has opened a vast and valuable opportunity in pharmaceuticals. Non-essential metals like platinum, gold, silver, vanadium and bismuth are being used as agents to treat cancer, rheumatoid, bacterial infection, diabetes, peptic ulcer and gastritis (Ahmad *et al.*, 2006). Gallium(III) nitrate has also used to reduce calcium loss from bones of cancer patients (Shriver, 1999).

Metal ions are needed for many critical functions in human being though they can induce toxicity if taken excessively. Deficiency can lead to disease such as pernicious anemia resulting from iron deficiency, growth retardation arising from insufficient dietary zinc and heart disease in infants owing to copper deficiency (Bertini, 2004). Metals in biological systems function in a few different ways. Transition metals having numbers of oxidation states are required for certain functions to enable the body to undergo biological processes.

Nickel(II) and copper(II) complexes having square planar molecular geometry involved in enzymatic catalysis for hydrogenation and oxidation (Roat-malone, 2007).

Copper and nickel were chosen for this experiment because of several factors. Copper is the third largest transition metal required after iron and zinc (Lee, 1996; Cotton, 1999) and it is essential in smaller amount though larger amount is toxic. Copper deficiency results in the inability to use iron stored in the liver and resulting in anaemia (Lee, 1996). Copper ions can undergo various oxidation processes that are carried out by amine oxidase, ascorbate oxidase, cytochrome oxidase and galactose oxidase (Lee, 1996). It also affects the elasticity of aortic walls, brain function, skin pigmentation and holds a role in iron metabolisme (Lee, 1996). Although the function of nickel in biological systems is less known, its importance has been found in at least four types of enzymes such as urease, carbon monoxide dehydrogenase (CODH, or acetyl coenzyme A synthase), hydrogenase and methyl-*S*-coenzyme M reductase (Cotton, 1999).

As reported by Sinha *et al*., (2007) in their study, radio-labelled bio-molecules that used radiometals such as ^{99m}Tc(VII), ^{186}Re(VII) and ^{188}Re(VII) have been useful in tumor imaging and can also be exploited by antibody-directed tumor targeting. Sinha *et al*., (2007) also prepared Schiff base complexes from indole-3-carboxaldehyde with some amino acids in order to investigate potential bactericidal, fungicidal and anti-tumor activities. Other heavy metals have been applied in pharmaceutical applications and the need in using metals is obviously becoming important because the pharmacological activities of certain compounds is actually depending on the metal ions, its chelating ligands and the structure of the compounds (Ahmad *et al*., 2006).

Einaga *et al*. in their work in 2005 stated that Cu-N vibrational bands could be seen at higher wavenumber than that seen for Cu-O vibrational bands. On the other hand, Kasumov *et al*. (2005) and Masoud *et al*. (2004) reported that the vibrational bands for Cu-N and Ni-N were found at lower wavenumber than that found for Cu-O and Ni-O respectively. Metal - nitrogen (M-N) and metal - oxygen (M-O) vibrational bands observed by Akitsu *et al*. (2005) and Kasumov *et al*. (2005) were identified between 550 and 400cm^{-1}.

Some organic compounds which are biologically inactive may become biologically active or less biologically active compounds may increase their activity when coordinated to a metal ion (Singh *et al*., 2007). Singh *et al*. synthesized several Schiff base complexes of transition metals which were cobalt(II), nickel(II), copper(II) and zinc(II) and they found that cobalt(II) complexes displayed better results against a few bacterial strains as compared to their ligand. Ahmad and co-workers (2006) had explored the function of metal in complexes used as drugs so to work out the possible mechanism of action played by the metal in order to treat diseases. Ahmad *et al*., (2006) stated that the cytotoxicity of gold(III) is associated with the presence of gold(III) center and some gold-based drugs were found to be more cytotoxic than the corresponding platinum complexes.

1.2 Neuroprotective Effect

Free radicals are chemical species that have an odd number of electrons (Jensen, 2003) and they can cause several severe diseases such as diabetes, neurodegenaration, aging (Yazdanparast *et al*., 2007), cancers, cardiovascular and cell degenerations (Weecharangsan *et al*., 2006). The free radicals can be considered as a subset of reactive oxygen species

(ROS) (Jensen, 2003). Some ROS play important role in energy production, phagocytosis, regulation of cell growth and intracellular signaling although ROS are also capable of damaging essential biomolecules such as proteins, DNA and lipids (Yazdanparast *et al*., 2007).

Oxidative stress can be defined as the imbalance between the oxidants and the antioxidants (Jensen, 2003) or the excess formation or incomplete removal of ROS such as superoxide ($\cdot O_2^-$), hydroxyl radical ($\cdot OH$) and peroxyl radical ($RO\cdot_2^-$) (Yazdanparast *et al*., 2007). The central nervous system is susceptible to oxidative stress and the oxidative damage is made worse because neurons do not renew themselves (Jensen, 2003). Hydrogen peroxide (H_2O_2) is an oxidant (Jensen, 2003) and is able to cross the cell membrane to the inside of the cells (Matés *et al*., 2000) thus, it may produce the highly reactive hydroxyl radical through Fenton reaction, as described in Equation 1 in the presence of a transition metal (Jensen, 2003; Mates *et al*., 2000) which actually kills nerve cells (Weecharangsan *et al*., 2006).

$$H_2O_2 + Fe^{2+} \rightarrow Fe^{3+} + HO^- + HO\cdot \qquad \text{[Equation 1]}$$

Various natural extracts has been reported to exhibit neuroprotective activity. The water extract of *Curcuma longa* reduces PC12 cell death induced by pyrogallol and hydrogen peroxide. Hwang *et al*. (2004) discovered the neuroprotective effect of the ethanolic extract of the grape (*Vitis vinifera*) seed on Mongolian gerbil forebrain. Resveratrol was found in the grape skin extract and has been considered responsible for the protective effect against coronary heart disease (Hwang *et al*., 2004). The proanthocyanidins found in the grape seeds possess cardioprotective and nephroprotective activities by scavenging hydroxyl and peroxyl radicals (Hwang *et al*., 2004).

1.3 Peptic Ulcers and their Complications

Peptic ulcers occur in the parts of the digestive tract that are exposed to gastric juice containing acid and pepsin secreted by the H^+/K^+ATPase or proton pump on the parietal cells (oxyntic cells) of the stomach (Grossman, 1981). Gastric ulcer is one type of peptic ulcer after duodenal ulcer (Patrick, 2005; Grossman, 1981) and it occurs on the stomach wall. It can be defined as red hemorrhagic bands of erosion on the stomach lining (Patrick, 2005) and this disease is caused by endogenous noxious agents such as hydrochloric acid, proteolytic enzymes and bile. The exogenous peptic ulcer agents however, include the infection of *Helicobacter Pylori* bacteria, consumption of non-steroidal anti-inflammatory drugs such as nalfon, butazolidin, ibuprofen and indomethacin (Yamada, 1999, Grossman, 1981), cigarette smoking (Yamada, 1999; Yuan *et al*., 2007), steroid ingestion (Grossman, 1981), alcohol consumption, psychological stress, oral biphosphonates, potassium chloride, immunosuppressive medications and decline in prostaglandin levels amongst older people (Yuan *et al*., 2007). Development of gastric lesions also increment in gastric acid and pepsin secretion, decrease in gastric blood flow, the suppression of endogenous generation of prostaglandin, inhibition of mucosal growth and cell proliferation and alteration of gastric mobility (Toma *et al*., 2003). The pathogenesis of gastric ulcer formation has come to a crossroad where the mechanism of ulcer formation is actually unclear.

Gastric mucosa provides protection on the wall of the stomach from erosion by gastric acid. Gastric acid is released by cells known as parietal cells or oxyntic cells in the stomach. These parietal cells are innervated with nerves from the autonomic nervous system (Patrick, 2005). When the autonomic nervous system is stimulated, a signal is sent to the parietal

cells culminating in the release of the neurotransmitter acetylcholine at the nerve termini. Acetylcholine crosses the gap between the nerve and parietal cell and activates the cholinergic receptors of the parietal cells leading to the release of gastric acid into the stomach. Gastric acid will be released even before food has entered the stomach (Patrick, 2005). Other than acetylcholine, gastrin and histamine too play a role in stimulating the secretion of gastric acid. Gastrin is a polypeptide hormone which is built up by 17 amino acids in the G cells and travels to the parietal cells through blood supply, thus further stimulating parietal cells in the pyloric region of the stomach to secrete hydrochloric acid. (Gringauz, 1997).

A hormone, histamine also interacts with H_2 receptor to release gastric acid. Hence, in order to reduce or inhibit gastric acid secretion, a drug should be able to block the activity of either neurotransmitter or hormones (Patrick, 2005). For instance, cimetidine inhibits H_2 receptor as well as pentagastrin. Blocking the pump in the canalicular membranes of the parietal cells can also inhibit acid from being pumped out into the stomach. There are several drugs of various classes that are useful in treating gastric ulcers such as antacids (aluminium hydroxide, magnesium hydroxide and calcium carbonate), proton pump inhibitors (omeprazole, esomeprazole and pantoprazole), H_2 antagonists (cimetidine, ranitidine and famotidine), antibiotics (amoxicillin, clarithromycin and metronidazole), misoprostol and bismuth subsalicylate (Beers, 2004).

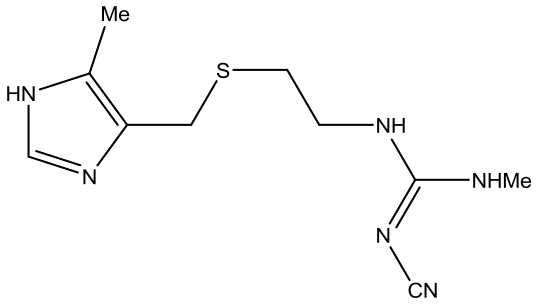

Figure 2: The molecular structure of cimetidine, a H2-receptor antagonist (Patrick, 2005).

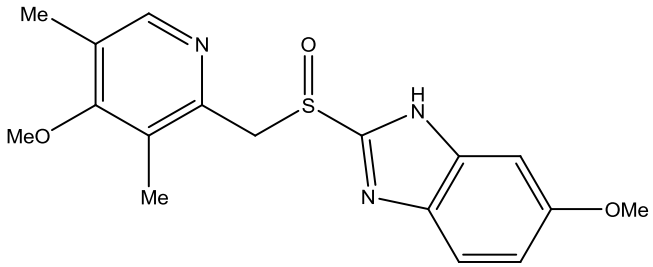

Figure 3: The molecular structure of omeprazole, a proton pump inhibitor (Patrick, 2005).

Ulcerations can be induced in many ways in order to seek gastroprotective effect on gastric ulcer. Some researchers induce ulcerations by using indomethacin which belongs to the NSAID group. Indomethacin is used for analgesic purposes and to treat inflammation but its side effect causes a person to have stomach ulcer due to its pharmacological action in inhibiting cyclooxygenase enzyme from synthesizing prostaglandin. Most research focused on medicinal plant extracts such as *Mammea americana* L. bark/latex extract (Toma *et al*., 2005), *Nigella sativa* oil (El Abhar *et al*., 2003) and *Urtica dioica* L. (Gülcin *et al*., 2004). Gharzouli *et al*., (1999) reported that natural honey appeared to be preventive against ethanol-induced ulcers where high concentrations of sugars in honey were able to prevent the formation of gastric ulcer by inducing an adaptive cytoprotective mechanism. Jamal *et*

al. (2006) in their research reported the effect of *Elettaria cardamomum* Maton. fruits as being gastroprotective on ethanol-induced gastric ulcers. It is believed that high content of antioxidants in plants extracts scavenged free radical which is also believed to associate with the formation of gastric ulcers (Gülcin *et al*., 2004; Jamal *et al*., 2006 and Gharzouli *et al*., 1999).

There are some organometallic compounds showed gastroprotective effects other than plant extracts. For instance, diphenyl diselenide was tested for its preventive effect on ethanol- and indomethacin-induced gastric lesions as an anti-ulcer agent. The study was carried out by Savegnago and his co-workers (2007) and demonstrated excellent results *in vitro*. Diphenyl selenide was found to inhibit the activity of K^+ATPase and possessed as an anti-secretory agent. This mechanism is therefore enhancing the treatment of gastric ulcer.

Ahmad *et al*. (2006) reported that bismuth anti-ulcer agents which are commonly used to treat gastrointestinal disorders due to the effectiveness attributed to bactericidal action against gram negative bacterium *Helicobacter Pylori* could actually cause neurological dysfunction. Bismuth is detected in blood, plasma or serum when this drug is consumed excessively. Low serum testosterone level in experimental animals indicated that the reproductive organs might be affected due to the toxicity effect shown when bismuth is being taken. Nevertheless, the toxicity will be reversed if one has stopped consuming it for weeks or months (Ahmad *et al*., 2006).

1.4 Physical and Physiological Properties of Alcohol (Ethanol)

Ethanol is a poisonous drug like many other notorious drugs such as heroin and cocaine and it has both pharmacological and toxic effects. It is now classified as a carcinogen as it is associated to liver cancer and parts of the gastrointestinal tract. The liver will be the main target for ethanol although the brain may suffer when one is chronically exposed to ethanol (Timbrell, 2002). The pharmacological effect of ethanol can be observed due to its presence in the central nervous system (CNS). The effect is not only observed in CNS but also in other organs such as the brain, skin, gastrointestinal tract, heart, liver and kidney (Stewart, 1961).

Consumption of alcoholic drinks was found to be associated to gastric ulcer when ethanol induced gastric mucosal damage in experimental animals (Yamada, 1999). Disturbance of mucosal microcirculation, ischemia and appearance of free radicals, endotelin release, degranulation of mast cells, inhibition of prostaglandins and decrease in mucus production are responsible for ethanol-induced ulcer (Samonina *et al*., 2004). Ethanol-induced gastric mucosal damage is characterized by microcirculatory changes such as statis and plasma leakage (Peskar *et al*., 1986). Sluggish blood flow and statis have also been observed after administration of exogenous leukotriene (LT) C_4 (Peskar *et al*., 1986). Leukotriene exerts its damaging effect on the brain, stomach, sex organs, liver and pancreas.

In a previous study, however, ethanol-induced gastric lesions were thought to cause direct damage of gastric mucosal cells and generated free radicals and hyperoxidation of lipids (Yuan *et al*., 2007). Ethanol treated rats showed a significant increase in plasma

concentrations of the gastric hormone, gastrin, and an increase in the gastric mucosal H^+/K^+ATPase activity. H^+/K^+ATPase is the dimeric enzyme responsible for H^+ secretion by the gastric parietal cells (Yuan *et al*., 2007). Those who ingest alcoholic drinks experience hangover which includes tiredness, nausea, headache and dizziness and it tends to get worse with drinks that contain more congeners such as red wine, brandy and blended whiskeys (Mustafa, 2001).

However, drinks that contain 40% or more ethanol cause inflammation of the stomach lining (Mustafa, 2001). If alcoholic drinks are frequently consumed, the defensive barrier of the stomach will be destroyed and this will cause the stomach wall to expose to endogenous harmful agents such as hydrochloric acid. Gastric ulcers induced by ethanol were found to be developed when ethanol produced gastric damage by impairing gastric defensive factors such as mucus and mucosa circulation (Szabo *et al*., 1998). The protection barrier will be destroyed in the stomach of a person who consumes alcoholic drinks very often in large quantities. Copper(II) complexes of amino acids has been discovered to show potent activity in preventing the formation of gastric ulcers induced by ethanol (Franco *et al*., 1997).

1.5 Diabetes Mellitus and Its Complications

Diabetes is a systemic disease characterized by hyperglycemia, hyperlipidemia and hyperaminoacidemia. It is caused by a decrease in the secretion or activity of insulin and is also frequently associated with neuropathic disorders and a predisposition to atherosclerosis (Galloway, 1988). There are 2 types of diabetes, diabetes insipidus and diabetes mellitus.

Diabetes insipidus occurs rarely and is not related to sugar content in blood. It is a disease of lack of arginine vasopressin (AVP), also known as anti-diuretic hormone (ADH) that causes excessive production of very dilute urine (Beers, 2004).

On the other hand, diabetes mellitus is a disorder in which blood sugar (glucose) levels are abnormally high because the body does not produce enough insulin (Beers, 2004) and can be subclassified as insulin-dependent diabetes mellitus (IDDM) or Type 1 (formerly known as juvenile – onset or ketosis – prone diabetes) and non-insulin-dependent diabetes mellitus (NIDDM) or Type 2 (formerly known as maturity – onset or adult – onset). Type 1 diabetes usually happens to youth and is also known as juvenile diabetes where 90% of the insulin producing cells of the pancreas are permanently destroyed, thus little or no insulin is being produced. Insulin is a hormone that is produced by beta cells, which covers the largest proportion of islet cells in the pancreas (Gringauz, 1997). Only about 10% of all people with diabetes have Type 1 disease and most of them develop the disease before they reach the age of 30 (Beers, 2004). However, in Type 2 diabetes, insulin is secreted continuously, sometimes even at higher than normal levels. The body however, develops resistance to the effects of insulin and this unable the blood glucose to be carried into the cells (Beers, 2004). A few classes of drugs had been developed and are currently used in treatment of diabetes mellitus and these drugs are classified into different groups which are of biguanide (metformin), sulfonylurea (glibenclamide, glipizide and tolbutamide), meglitinide (nateglinide and repaglinide), glucosidase inhibitors (acarbose and miglitol) and thiazolidinedione (rosiglitazone) groups (Beers, 2004). Drugs for treatment of hyperglycemic patients associated with diabetes mellitus fall into four classes of mechanism of action which are insulin and its analogs, insulinotropic agents, insulin – sensitizing agents and α – glucosidase inhibitors (Abraham, 2003). Sulfonylureas and

meglitinides enhance the stimulation of insulin secretion in the pancreas while thiazolidinediones and biguanides do not affect the release of insulin but increase the body's response to insulin (Beers, 2004).

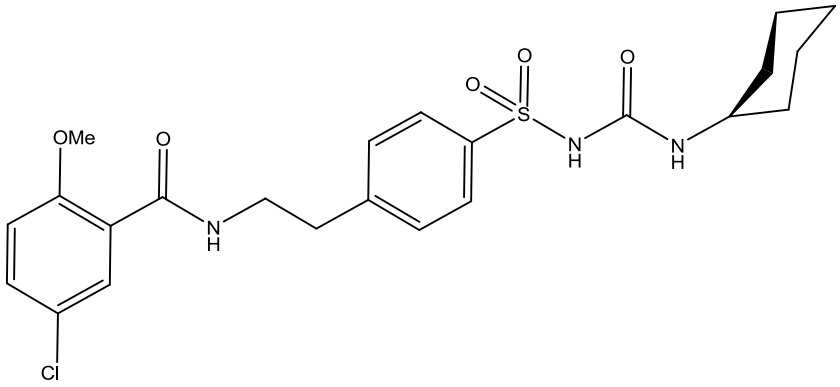

Figure 4: The molecular structure of glibenclamide (Patrick 2005).

For instance, Bahekar *et al*. (2007) had synthesized several heterocyclic compounds mainly of imidazole substituted on the pyrrole ring. His research group has replaced the benzene ring of 5-chloro-3-imidazole-2-methylindole (BL11282) with pyridine so to study the effect of these new analogues having pyrrolopyridine on anti-diabetic effect instead of indole. 5-chloro-3-imidazole-2-methylindole has been discovered to be effective as therapeutic agent for the treatment of Type 2 diabetes where it did not stimulate basal insulin secretion and did not affect the activity of KATP channel but was remarkably potentiate glucose-induced insulin secretion. Other than this, there are also studies on medicinal plants that were already proven to reveal anti-diabetic effect in rats. Arambewela *et al*. (2005) studied the ability of betel leaves in lowering blood glucose level and found that the leaf was not only efficient for reducing blood glucose level, it also served as a highly good anti-oxidant agent as compared to the standard anti-oxidant agent, butylated hydroxytoluene (BHT).

1.6 Present studies

According to those reviews, there has been no study done on the anti-ulcer and anti-diabetic properties for halogenated indole Schiff base compounds and their copper(II) and nickel(II) complexes though many research has been done for other biological activities such as anti-bacterial, anti-cancer and anti-oxidant. Fewer studies are carried out to investigate the bioactivities of synthetic compounds thus, this phenomena has afforded the idea to attempt a study of some synthetic compounds of indole Schiff base which are derived from halogenated indole-3-carboxaldehyde and indole-3-acetichydrazide. The copper(II) and nickel(II) complexes of the ligands will be synthesized.

The compounds are to be studied for their efficacy in gastroprotective effect as well as in reducing blood glucose level. *In vivo* and *in vitro* toxicological studies were carried out in order to clarify their toxic effect to at least witness any abnormal behaviours that might arise after consuming the compounds for at least 24 hours. Since copper(II) ion is one of the essential mineral in our body, the toxicology and effectiveness of copper(II) complex of halogenated indole Schiff base will be investigated. The study will also include the effect of molecular geometry of the complexes, the role of copper(II) and nickel(II) ions and the effect of halogen atoms in the ligands as well as in the complexes.