USUMANU DANFODIYO UNIVERSITY, SOKOTO

(POSTGRADUATE SCHOOL)

USE OF *GLIRICIDIA SEPIUM* AQUEOUS LEAF EXTRACT AS AN ANTISICKLING AGENT: THYROID HORMONES AND LIPID PROFILE IN WISTAR RATS EXPOSED TO THE EXTRACT.

A Dissertation

Submitted to the

Postgraduate School

USMANU DANFODIYO UNIVERSITY, SOKOTO, NIGERIA.

In Partial Fulfillment of the Requirements

For the Award of the Degree of

MASTER OF SCIENCE IN MEDICAL LABORATORY SCIENCE (CHEMICAL PATHOLOGY).

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APRIL, 2017

DEDICATION

Dedicated to my beloved parents, wife, daughter, brothers and sisters, who have been praying for me in order to see the difficult and impossible tasks become easier and possible. May the Almighty Allah reward them abundantly, ameen.

CERTIFICATION

This Dissertation by **MUSA** Kasim (Adm. Number 14211226008), has met the requirements for the award of the Degree of Master of Science in Medical Laboratory Science (Chemical Pathology) of the Usmanu Danfodiyo University Sokoto, and is approved for its contributions to knowledge.

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AKNOWLEDGEMENTS

All praise be to Allah (S.W.T) and may His peace blessing be on the most noble of His servant, Muhammad (S.A.W) may the same be upon His relatives, companions and all those that follow his foot prints. I remain highly grateful and indebted to my supervisors Dr Taofeeq Oduola, Prof. B.M. Agaie and AA Ngaski who have been my mentors, role model and invaluable source of encouragement through their constructive and expertise suggestion in this research work. May Allah the Almighty reward, assist and guide them in whatever endeavor they find themselves. Appreciation also goes to Dr. M.K. Dallatu PG Co-ordinator, Dr. A.S. Mainasara and other senior colleagues within and outside the Faculty of Medical Laboratory Sciences for their professional advice and support. Special appreciation goes to my parents, Alhaji Musa Gwani, Hajiya Aishatu Musa, brothers and sisters particularly my elder brother Muhammad Musa for their constant prayers throughout the period of my studies. I would like to express my warmest appreciation to my wife Aisha and daughter Nafisat, who have been very supportive during the course of my study. I must acknowledge the assistance of my H.O.D, Infectious Diseases Hospital, Zambuk, Gombe in person of Tanimu Umar and other staff of the department who help me in one way or the other during the period of this study.

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ACRONYMS/DEFINITION OF TERMS

ABS	Absorbance
ADP	Adenosine Diphosphate
ATP	Adenosine Triphosphate
CVD	Cardiovascular Disease
ELISA	Enzyme Link Immuno Sorbent Assay
H_2O_2	Hydrogen Peroxide
H_a	Alternate Hypothesis
HDL	High Density Lipoprotein
HDL-C	High Density Lipoprotein Cholesterol
H_O	Null Hypothesis
IASP	International Association for the study of pain
KG	Kilogram
LD_{50}	Lethal dose in 50
LDL	Low Density Lipoprotein
LDL-C	Low Density Lipoprotein Cholesterol
Ml	Milliliter
OECD	Organisation of Economic Cooperation and Development
SPSS	Statistical Package for Social Science
T_3	Triiodothyronine
T_4	Thyroxine
TC	Total Cholesterol
TG	Triglyceride

TSH	Thyroid Stimulating Hormone
UDUS	Usmanu Danfodiyo University, Sokoto
VLDL	Very Low Density Lipoprotein
VLDL-C	Very Low Density Lipoprotein Cholesterol
WHO	World Health Organization

ABSTRACT

Gliricidia sepium aqueous leaf extract is used in the management of patients with different diseases including sickle cell disease in some parts of Nigeria, without considering its safety. The present study determined the effect of ingestion of *Gliricidia sepium* aqueous leaf extract on thyroid hormones and lipid profile in wistar rats. The oral acute (LD_{50}) and sub-chronic toxicity studies ware performed according to OECD guidelines 425 in Wistar rats. Thyroid function tests (T_3, T_4 and TSH) were carried out using ELISA. TC, TG, HDL, LDL and VLDL were carried out using spectrophotometric techniques. No mortality or signs of toxicity were recorded in the acute oral toxicity study in the rats.. In acute and sub-chronic toxicity studies, there was no significant difference in lipid profiles of control and experimental rats. T_3 was significantly higher ($p< 0.05$), and T_4 and TSH significantly lower ($p< 0.05$) in test than in control groups in acute toxicity study. In sub-chronic toxicity study, T_3 and TSH revealed no significant difference in test and control groups; T_4 was significantly higher ($p< 0.05$) in test than the control group but not dose response related. The thyroid gland tissues of both control and experimental groups showed normal thyroid follicles and follicular epithelial cells. *Gliricidia sepium* leaf extract had no adverse effect on thyroid and lipid profiles in Wistar rats.

CHAPTER ONE

1.0 INTRODUCTION

Gliricidia sepium is a medium size leguminous tree belonging to the family Fabaceae, scientific name *Gliricidia sepium* (Jacq.) Kunth ex Walp, common name mother of cocoa, international common name English: gliricidia, and local common name Nigeria: (Yoruba) Agunmaniye (Nyoka *et al*., 2012). It is considered as the second most important multi-purpose legume tree (*Rani, 2007).* It is a fast-growing, nitrogen-fixing tree used throughout the tropics for the many environmental services. Spanish colonists adapted the local vernacular in naming the species 'madre de cacao' (mother of cocoa) to describe its use as a cocoa shade tree. The toxic properties of the seeds and bark of *G. sepium* give rise to the generic epithet of this species (*Gliricidia* = mouse killer) as well as a number of common names (e.g. Mata-Raton). Present day uses of this species throughout the native range (e.g. firewood, living fences, shade, construction and as an ornamental) are likely extensions of early utilization and popularity (Rico-Gray *et al*., 1991). *Gliricidia sepium* has also been used extensively outside its native range in places which include the Caribbean, the Philippines, India, Sri Lanka and West Africa. These landrace populations are largely remnants of colonial introductions used to shade plantation crops although more recently they have been integrated into indigenous farming practices being used for fuelwood, living fences, animal forage, green manure and soil stabilisation. After *Leucaena leucocephala*, *G. sepium* is believed to be the most widely cultivated multipurpose tree. In many cases, gliricidia will yield as much as or more biomass than *L. leucocephala* (Stewart *et al.,* 1992). One of the reasons for its recent popularity is its complete resistance to the defoliating

psyllid (*Heteropsylla cubana*) which has devastated *L. leucocephala* in many parts of the tropics.

MEDICINAL USES

- *Gliricidia sepium* has been reported to possesses antisickling properties (Oduola *et al*., 2016)
- G. *sepium* is a folk remedy for some of inflammation related diseases, fractures, gangrene, headache, fever, itch, prickly heat, rheumatism, urticaria, and wounds (Abulude and Adebote, 2009).
- Different parts of the plants are reported to have antimicrobial (Nazli *et al*., 2011), nematicidal (Nazli *et al*., 2008), larvicidal (Kaliyamoorthy *et al*., 2012), and antioxidant property (Akharaiyi *et al*., 2012).
- The tree is also an important source of several phytochemicals like flavonoids (Rastrelli *et al*., 1999), triterpenoid, saponins, stigmastanol glucoside, rhamnogalactoside of kaempferol, coumarin, coumaric acid and melilotic acid, 12a-hydroxy retenoids, green manure, fodder, and fuel wood which were reported in various parts of the plant and bark, medicine uses (Beena and Joji, 2010)
- *G. sepium* is widely used for its medicinal and insect repellent properties. Farmers in Latin America often wash their livestock with a paste made of crushed *G. sepium* leaves to ward off torsalos. In the Philippines, the extract obtained from its leaves is used to remove external parasites (*Stuttle, 2015).*
- G. *sepium* is also used to provide crop shade for cacao, coffee, and other shade loving crops (Chadhokar, 2010; Csurhes and Edwards, 1998).

NUTRITIONAL VALUES

It has a high nutritive value. Crude protein content is 18-30% and in vitro digestibility ranges from 60-65%, but palatability is poor (Stewart *et al*., 1996).

SICKLE CELL DISEASE

Sickle cell disease (SCD) is a hereditary blood disorder caused by a single amino acid substitution (Glu→Val) at the sixth position of the beta-globin chains of haemoglobin. This single amino acid substitution causes a significant reduction in the solubility of the deoxy form of sickle haemoglobin (deoxy-Hb S), causing polymer formation inside the red blood cells (Iyamu *et al*., 2003; Gutsaeva *et al*., 2014). Through a complex interplay of adhesive events among blood cells, these altered erythrocytes can obstruct the vasculature, producing episodes of pain, haemolytic anaemia, organ injury, and early mortality. Although the molecular basis of SCD is well characterized, the complex mechanisms underlying vasoocclusion have not been fully elucidated (Manwani and Frenette, 2013).

THYROID GLAND

The thyroid gland is a butterfly-shaped organ and is composed of two cone-like lobes or wings, *lobus dexter* (right lobe) and *lobus sinister* (left lobe), connected via the isthmus. Each lobe is about 5 cm long, 3 cm wide and 2 cm thick. The organ is situated on the anterior side of the neck, lying against and around the larynx and trachea, reaching posteriorly the oesophagus and carotid sheath. It starts cranially at the oblique line on the thyroid cartilage (just below the laryngeal prominence), and extends inferiorly to approximately the fifth or sixth tracheal ring. The thyroid gland controls rate of use of oxygen, energy sources, protein synthesis, and controls the body's sensitivity to other

hormones. It participates in these processes by producing thyroid hormones, the principal ones being thyroxine (T_4) and triiodothyronine (T_3), which is more active (*Kim et al., 2013).*

THYROID HORMONES

These are chemical substances made by the thyroid gland. This gland uses iodine to make thyroid hormones, which are essential for the function of every cell in the body. They help regulate growth and the rate of chemical reactions (metabolism), and are involved in the circadian rhythms that govern sleep, among other essential functions. The two most important thyroid hormones are thyroxine (T4) and triiodothyronine (T3). Thyroid stimulating hormone (TSH), which is produced by the anterior pituitary gland, acts to stimulate hormone production by the thyroid gland. The pituitary gland is stimulated to make TSH by the hypothalamic hormone, thyrotropin releasing hormone (Kim *et al*., 2013).

LIPID PROFILE

Lipid profile is a panel of blood lipid tests that serves as an initial broad medical screening tool for abnormalities in lipids, such as cholesterol and triglycerides. Lipid profile are commonly ordered as part of a medical examination, along with other profiles such as the complete blood count (CBC) and basic metabolic profile (BMP). This test is used to identify hyperlipidemia (various disturbances of cholesterol and triglyceride levels), many forms of which are recognized risk factors for cardiovascular disease and rarely pancreatitis. A total cholesterol reading can be used to assess an individual's risk for heart

diseases; however, it should not be relied upon as the only indicator. The individual components that make up total cholesterol reading—LDL, HDL, and VLDL—are also important in measuring risk *(Sidhu and Naugler, 2012).*

COMPONENTS OF LIPID FROFILE

Low-density lipoprotein (LDL)

High-density lipoprotein (HDL)

Triglycerides

Total cholesterol

Very low-density lipoprotein (VLDL)

Cholesterol:HDL ratio *(NCEP, 2002).*

EFFECTS OF THYROID HORMONES ON LIPID METABOLISM

Thyroid function regulates a wide array of metabolic parameters. Thyroid function significantly affects lipoprotein metabolism as well as some cardiovascular disease (CVD) risk factors, thus influencing overall (CVD) risk (Duntas, 2002). Indeed, even within the normal range of thyroid-stimulating hormone (TSH) values, a linear increase in total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C) and triglycerides (TGs) and

a linear decrease in high-density lipoprotein cholesterol (HDL-C) levels has been observed with increasing TSH (Asvold *et al*., 2007).

1.1 STATEMENT OF RESEARCH PROBLEM

Traditionally, herbs have been considered to be non-toxic and have been used for the treatment of various diseases by the general public and traditional medicine doctors worldwide (O'Hara *et al*., 1998). Therefore, people every year turn to herbal medicine because they believe plant remedies are free from undesirable side effects (Philomena, 2011). Although medicinal plants are widely used and assumed to be safe, they can potentially be toxic and can affect the vital organs such as kidney, liver, thyroid gland and heart.

1.2 JUSTIFICATION OF THE STUDY

World Health Organization (WHO) documented that 80% of the world populations used a large variety of medicinal plant (Cloudio, 2007). Aqueous leaf extract of G. *sepium* is being used by herbalists in Northern parts of Nigeria in the treatment of patients with sickle cell disease (Oduola *et al*., 2016).. *Gliricidia sepium* aqueous leaf extract has been reported to possess antisickling property (Oduola *et al*., 2016). Toxic effects of some medicinal plants on thyroid gland and lipid metabolism have been well documented (Wazida *et al*., 2013) but not for *Gliricidia sepium* Thyroid hormones play important roles in metabolic processes in the body such as development, growth and metabolism (Bowen, 2010). And any disruption of its production and functions could produce fatal effect in man and animals.

1.3 RESEARCH HYPOTHESIS

Null Hypothesis (H_0)

There will be no significant effect on thyroid hormones and lipid profiles of wistar rats treated with G. *sepium* aqueous leaf extract.

Alternate Hypothesis (H_a)

There will be a significant effect on thyroid hormones and lipid profiles of wistar rats treated with G. *sepium* aqueous leaf extract.

1.4 RESEARCH QUESTION

1. What is the median lethal dose (LD_{50}) of G. *sepium* aqueous leaf extract in wistar rats?

2. What is the effect of ingestion of G. *Sepium* aqueous leaf extract on thyroid hormones (T_3, T_4, TSH) in wistar rats ?

3. What is the effect of ingestion of G. *Sepium* aqueous leaf extract on lipid profile in wistar rats?

4. What is the effect of ingestion of G. *Sepium* aqueous leaf extract on thyroid gland tissue in wistar rats ?

1.5 AIM AND OBJECTIVES OF THE STUDY

1.5.1 AIM

The aim of this study is to determine the effect of ingestion of *Gliricidia sepium* aqueous leaf extract on thyroid hormones and lipid profiles in wistar rats.

1.5.2 OBJECTIVES

1. To determine the median lethal dose (LD_{50}) of aqueous leaf extract of G. *sepium* in wistar

rats.

2. To determine the effect of oral administration of aqueous leaf extract of G. *sepium* on thyroid hormones (T3, T4 and TSH) in wistar rats.

3. To determine the effect of oral administration of aqueous leaf extract of G. *sepium* on lipid profile (total cholesterol, triglyceride, high density lipoprotein cholesterol, low density lipoprotein cholesterol and very low density lipoprotein cholesterol) in wistar rats.

4. To investigate the gross and histopathological effect of G. *sepium* aqueous leaf extract on thyroid gland.

5. To determine the thyroid and lipid profiles of rats administered with G. *sepium* leave extract.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 MEDICINAL PLANTS

Medicinal plants have been identified and used throughout human history. Plants have been used as remedies for diseases from time immemorial. There is a tremendous increase in the consumption of herbs as an alternative source of medicine to maintain health and improve the quality of life (Nostro *et al*., 2000). Plants have the ability to synthesize a wide variety of chemical compounds that are used to perform important biological functions. At least 12,000 such compounds have been isolated so far; a number estimated to be less than 10% of the total available (*Tapsell et al., 2006).* Chemical compounds in plants mediate their effect on the human body through processes identical to those already well understood for the chemical compounds in conventional drugs; thus herbal medicines do not differ greatly from conventional drugs in terms of how they work. This enables herbal medicines to have beneficial pharmacological effect, but also gives them the same potential as conventional pharmaceutical drugs to cause harmful side effects (*Lai and Roy, 2004)*. The use of plants as medicines predates written human history. Ethnobotany, the study of traditional human uses of plants, is recognized as an effective way to discover future medicines. In 2001, researchers identified 122 compounds used in modern medicine which were derived from "ethnomedical" plant sources; 80% of these have had an ethnomedical use identical or related to the current use of the active elements of the plant (*Fabricant and Farnsworth , 2001).* Some of the pharmaceuticals currently available to physicians are derived from plants that have a long history of use as herbal remedies (*Swain and Tony, 1968).*

2.1.1 Medicinal Use

A survey released in May 2004 by the National Center for Complementary and Integrative Health in USA, focused on the use of complementary and alternative medicines (CAM), what was used, why it was used. The survey was limited to adults, aged 18 years in 2002. According to this survey, herbal therapy, or use of natural products other than vitamins and minerals, was the most commonly used CAM therapy (18.9%) when all use of prayer was excluded (*Barnes et al., 2004).* Herbal remedies are very common in Europe. In Germany, herbal medications are dispensed by apothecaries (e.g., Apotheke). Prescription drugs are sold alongside essential oils, herbal extracts, or herbal teas. Herbal remedies are seen by some as a treatment to be preferred to pure medical compounds which have been industrially produced (*James and Duke, 2000).* In India, the herbal remedy is so popular that the Government of India has created a separate department - AYUSH - under the Ministry of Health & Family Welfare. The National Medicinal Plants Board was also established in 2000 by the Government of India in order to deal with the herbal medical system (*Kala and Prakash, 2007).* The use of herbs to treat disease is almost universal among non-industrialized societies and is often more affordable than purchasing modern pharmaceuticals. The World Health Organization (WHO) estimates that 80 percent of the population of some Asian and African countries presently use herbal medicine for some aspect of primary health care. Studies in the United States and Europe have shown that their use is less common in clinical settings, but has become increasingly more common in recent years as scientific evidence about the effectiveness of herbal medicine has become more widely available (*Jacquart, 2008).*

According to Okoli *et al*., (2007) medicinal herbs and used for managing some common ailments

in Esanland, Edo State, Nigeria. The Esan people have a rich cultural heritage, which is reflected in the well developed herbal medicine used to cure and manage various disease conditions. Herbal medicine offered remedies to common ailments ranging from common cold to complex pathological disorders including those relating to the respiratory, circulatory and genito-urinary systems (*Jacquart, 2008).* Seventy herbal plants used in 115 different methods were identified. About 83% of the herbal preparations were given orally, while 17% were applied topically. The aerial parts of the plants (84%) were most frequently administered in the form of decoctions (*Jacquart, 2008).* Some of the herbs also form part of their diets and include: *Talinum trangulare, Boerhavia diffusa, Euphorbia hirta, Gongronema latifolium* and *Aframomum melegueta.* It has been concluded that medicinal herbs have played and will continue to play major roles in the management of common diseases in these communities (Okoli *et al*., 2007). According to Gbile and Adesina (1986), the Nigerian flora has made and would continue to make great contributions to health care of Nigerians. In fact the indigenous medicinal plants form an important component of the natural wealth and culture of Nigeria.

2.1.2 Safety

A number of herbs are thought to cause adverse effects (*Talalay and Talalay, 2001).* Furthermore, "adulteration, inappropriate formulation, or lack of understanding of plant and drug interactions have led to adverse reactions that are sometimes life threatening or lethal (*Elvin-Lewis, 2001).* Proper double-blind clinical trials are needed to determine the safety and efficacy of each plant before it can be recommended for medical use (*Vickers, 2007).* Although many consumers believe that herbal medicines are safe because they are "natural", herbal medicines and synthetic drugs may interact, causing toxicity to the patient. Herbal remedies can also be

dangerously contaminated, and herbal medicines without established efficacy may unknowingly be used to replace medicines that do have corroborated efficacy (*Ernst, 2007).* Standardization of purity and dosage is not mandated in the United States, but even products made to the same specification may differ as a result of biochemical variations within a species of plant (*Ernst, 2007).* Plants have chemical defense mechanisms against predators that can have adverse or lethal effects on humans. Examples of highly toxic herbs include poison hemlock and nightshade (*Müller, 1998).* They are not marketed to the public as herbs, because the risks are well known, partly due to a long and colorful history in Europe, associated with "sorcery", "magic" and intrigue (*Lee, 2006).* Although not frequent, adverse reactions have been reported for herbs in widespread use (*Pinn, 2001).* On occasion, serious untoward outcomes have been linked to herb consumption. A case of major potassium depletion has been attributed to chronic licorice ingestion (*Lin et al., 2003),* and consequently professional herbalists avoid the use of licorice where they recognize that this may be a risk. Black cohosh has been implicated in a case of liver failure (*Lynch et al., 2006).* Examples of herbal treatments with likely cause-effect relationships with adverse events include aconite, which is often a legally restricted herb, ayurvedic remedies, broom, chaparral, Chinese herb mixtures, comfrey, herbs containing certain flavonoids, germander, guar gum, liquorices root, and pennyroyal (*Ernst, 2010).* There is also concern with respect to the numerous well-established interactions of herbs and drugs (*Elvin-Lewis, 2001).* Some herbs may amplify the effects of anticoagulants (*Spolarich and Andrews, 2007).* Certain herbs as well as common fruit interfere with cytochrome P450, an enzyme critical to much drug metabolism (*Nekvindová and Anzenbacher, 2007). A prospective study shows that 25% of the childhood blindness in Nigeria were associated with the use of traditional eye medicine (Harries and Cullinan, 1994). Perhaps, the biggest problem in Nigeria with herbal medicine are a lack of*

standardization and of safety regulations (Ekeanyanwu, 2011).

2.2 *GLIRICIDIA SEPIUM*

Gliricidia sepium is a medium-sized leguminous tree which occurs in abundance throughout its native range in Mesoamerica. Domestication of gliricidia has been in progress for several millennia and the multitude of indigenous common names from Mayan and Quiche peoples (Pertchik and Pertchik, 1951) reveals the importance of this species to early occupants of the region.

DESCRIPTION

Gliricidia sepium is a medium-sized tree and can grow from 10 to 12 meters high. The bark is smooth and its color can range from a whitish gray to deep red-brown. It has composite leaves that can be 30 cm long. Each leaf is composed of leaflets that are about 2 to 7 cm long and 1 to 3 cm wide. The flowers are located on the end of branches that have no leaves. These flowers have a bright pink to lilac color that is tinged with white. A pale yellow spot is usually at the flower's base. The tree's fruit is a pod which is about 10 to 15 cm in length. It is green when unripe and becomes yellow-brown when it reaches maturity. The pod produces 4 to 10 round brown seeds. *G. sepium* is native to tropical dry forest in Mexico and Central America. The tree grows well in acidic soils with a pH of 4.5 - 6.2. The tree is found on volcanic soils in its native range in Central America and Mexico. However, it can also grow on sandy, clay and limestone soils (*Stuttle, 2015).*

DISTRIBUTION

Gliricidia sepium is native to the seasonally dry Pacific coast of Central America and is now widespread throughout the tropics within 6°S and 19°N of the equator. It grows well from sea

level to an altitude of 1600 m, in areas where the mean temperature ranges from 20°C to 29°C, and annual rainfall is between 900 and 1500 mm, with a five-month dry period. It does not withstand frost and night temperatures below 15°C. It is tolerant to water logging and to a wide range of poorly fertile soils (Ecocrop database, 2009). The distribution of G. *sepium* within Mexico, Central and South America has undoubtedly been greatly altered and extended by a long history of local use, cultivation, incipient domestication, translocation and subsequent naturalization. This has been promoted by massive habitat disturbance, now making it difficult to discern the true extent of the native distribution of this species (Hughes, 1987; Simons, 1996). In addition to its native range it is cultivated in many tropical and subtropical regions including the Caribbean, northern parts of South America, central Africa, parts of India and Southeast Asia (Hughes, 1987). *G. sepium* is also found in Africa as well as Nigeria (ILDIS, 2002; WAC, 2005).

TOXICITY

Gliricidia means "mouse killer" in Latin. In Central America, leaves mixed with cooked maize are used as a rodenticide (FAO, 2009). Leaves are also reported to be toxic to horses (Ecocrop, 2009) and many animals cannot tolerate the consumption of large quantities of gliricidia. Ruminants do not seem to be affected under normal feeding. Gliricidia toxicity could be due to the conversion by bacteria of coumarin to dicoumerol during fermentation (Cook *et al*., 2005). Cyanogens, HCN (up to 4 mg/kg), unidentified alkaloids and tannins may be present in the plant. *Gliricidia sepium* can be a nitrate accumulator (Bennison *et al*., 1993).

Figure 2.1: Pictures of Leaves of *Gliricidia sepium* (Stewart *et al*., 1996).

2.2.1 Properties of *Gliricidia Sepium*

Antisickling properties of the aqueous leaf extract of *Gliricidia sepium* was reported by Oduola *et al*., (2016). The findings revealed strong antisickling activity in humans; 20% antisckling at 5 mg, 50% at 10 mg, 80% at 15 mg and 100% antisickling from 20 mg upward for the leaf extract. The results showed that aqueous extract of *Gliricidia sepium* has strong antisickling properties justifying its use by the herbalists in the management of sickle cell disease, (Oduola *et al*., 2016).

According to report of Akharaiyi *et al.,* (2012), *G. sepium* possess antimicrobial properties. On the basis of the results obtained, *Gliricidia sepium* leaf extracts therefore seemed reliable and could be used for prevention, alleviation and curing of diseases of microbial and non microbial origins (Akharaiyi *et al.,* 2012).

Phytochemical profile and antioxidant activity of *Gliricidia sepium* were reported by Sankar (2013), the results showed that *Gliricidia sepium* exhibited free radical scavenging, reduce power and natural chelating property. The plant contained considerable amount of saponin, phenol, alkaloids and flavonoids. Phytochemical analysis of *Gliricidia sepium* extract indicated that phenol component had the highest concentration with a value of 1.5 mg/ml while flavonoids content with 0.45mg/ml. It was therefore suggested that *Gliricidia sepium* leaf extracts may be considered as good source of natural antioxidants for medicinal uses such as against aging and other ailments relating to radical mechanisms (Sankar, 2013).

Kola *et al.,* (2014) reported the *in vitro* and *in vivo* anti-inflammatory activity of *Gliricidia sepium* aqueous extract. The results obtained provide a scientific basis for the use of *Gliricidia sepium* as an anti-inflammatory agent. The extract showed anti-inflammatory activity in later phases in dose dependent manner and the inhibitory effect of aqueous extract may be due to the inhibition of cyclooxygenase induced prostaglandin synthesis and neutrophil mobilization. This

anti-inflammatory effect of the extract was attributed to the presence of flavonoids and saponins in the plant (Kola *et al.*, 2014).

2.3 SICKLE CELL DISEASE

The term *sickle cell disease* (SCD) describes a group of inherited red blood cell disorders. People with SCD have abnormal hemoglobin, called *hemoglobin S or* sickle hemoglobin, in their red blood cells (Gary, *2015).* The most common type is known as sickle-cell anaemia (SCA). It results in an abnormality in the oxygen-carrying protein haemoglobin found in red blood cells. This leads to a rigid, sickle-like shape under certain circumstances (Gary, *2015).* Problems in sickle cell disease typically begin around 5 to 6 months of age. A number of health problems may develop, such as attacks of pain ("sickle-cell crisis"), anemia, bacterial infections, and stroke (Gary, *2015).* Long term pain may develop as people get older. The average life expectancy in the developed world is 40 to 60 years (Gary, *2015).* Sickle-cell disease occurs when a person inherits two abnormal copies of the haemoglobin gene, one from each parent (Gary, *2015).* Several subtypes exist, depending on the exact mutation in each haemoglobin gene (Gary, *2015).* An attack can be set off by temperature changes, stress, dehydration, and high altitude (Gary, *2015).* A person with a single abnormal copy does not usually have symptoms and is said to have sickle-cell trait (Gary, *2015).* Such people are also referred to as carriers. The greatest burden of sickle cell anaemia (SCA) is in sub-Saharan Africa (SSA), where 75% of the 300,000 global births of affected children live, and estimates suggest that 50–80% of these patients will die before adulthood (Makani *et al*., 2011). The WHO estimates that 70% of SCA deaths in Africa are preventable with simple, cost-effective interventions such as early identification of SCA patients by newborn screening (NBS) and the subsequent provision of comprehensive care

(WHO, 2006). A lot of efforts had been made and are still being made to get treatment for sickle cell disease, especially drugs that will prevent sickling of red cells that usually precipitate crisis and hence ameliorate the excruciating pathological complications of the disease (Oduola *et al*., 2006; Nwaoguikpe *et al*., 2013). *Adansonia digitata* bark extract was documented to possess antisickling activities (Mpiana *et al*., 2014). Antisickling activities of *Zanthoxyllum heitzii* aqueous fruit extract (Pauline *et al*., 2013) and aqueous leaf extract of *Ocimum basilicum* (Tshilanda *et al*., 2014) were also been reported. As of 2013 about 3.2 million people have sickle-cell disease while an additional 43 million have sickle-cell trait (GBDS, 2013). About 80% of sickle-cell disease cases are believed to occur in sub-Saharan Africa (*Rees et al., 2010)*. It also occurs in parts of India, the Arabian peninsula, and among people of African origin living in other parts of the world. In 2013, it resulted in 176,000 deaths, up from 113,000 deaths in 1990 (*GBD, 2013)*. The condition was first described in the medical literature by the American physician James B. Herrick in 1910 (*Serjeant, 2010)*. In 1949 the genetic transmission was determined by E. A. Beet and J. V. Neel. In 1954 the protective effect against malaria of sickle-cell trait was described (*Serjeant, 2010)*.

2.3.1. Sign and Symptoms

1. Sickle-cell crisis

The terms "sickle-cell crisis" or "sickling crisis" may be used to describe several independent acute conditions occurring in patients with SCD. SCD results in anemia and crises that could be of many types including the vaso-occlusive crisis, aplastic crisis, sequestration crisis, haemolytic crisis, and Most episodes of sickle-cell crises last between five and seven days. Although infection, dehydration, and acidosis (all of which favor sickling) can act as triggers, in most

instances, no predisposing cause is identified **(***Pearson, 1977).*

2. Vaso-occlusive crisis

The vaso-occlusive crisis is caused by sickle-shaped red blood cells that obstruct capillaries and restrict blood flow to an organ resulting in ischaemia, pain, necrosis, and often organ damage. The frequency, severity, and duration of these crises vary considerably. Painful crises are treated with hydration, analgesics, and blood transfusion; pain management requires opioid administration at regular intervals until the crisis has settled. For milder crises, a subgroup of patients are managed on Nonsteroidal anti-inflammatory drugs (NSAIDs) such as diclofenac or naproxen. For more severe crises, most patients require inpatient management for intravenous opioids; patient-controlled analgesia devices are commonly used in this setting. Vaso-occlusive crisis involving organs such as the penis (*Olujohungbe and Burnett, 2013)* or lungs are considered an emergency and treated with red-blood cell transfusions. Incentive spirometry, a technique to encourage deep breathing to minimise the development of atelectasis, is recommended **(***Glassberg, 2011).*

3. Splenic sequestration crisis

Because of its narrow vessels and function in clearing defective red blood cells, the spleen is frequently affected **(***Anie and Green, 2012).* It is usually infarcted before the end of childhood in individuals suffering from sickle-cell anemia. This spleen damage increases the risk of infection from encapsulated organisms **(***Wong et al., 1992),* preventive antibiotics and vaccinations are recommended for those lacking proper spleen function. Splenic sequestration crises are acute, painful enlargements of the spleen, caused by intrasplenic trapping of red cells and resulting in a precipitous fall in hemoglobin levels with the potential for hypovolemic shock. Sequestration

crises are considered an emergency. If not treated, patients may die within 1–2 hours due to circulatory failure. Management is supportive, sometimes with blood transfusion. These crises are transient, they continue for 3–4 hours and may last for one day **(***Khatib et al., 2009).*

4. Acute chest syndrome

Acute chest syndrome (ACS) is defined by at least two of the following signs or symptoms: chest pain, fever, pulmonary infiltrate or focal abnormality, respiratory symptoms, or hypoxemia **(***Glassberg, 2011).* It is the second-most common complication and it accounts for about 25% of deaths in patients with SCD, majority of cases present with vaso-occlusive crises then they develop ACS **(***Paul et al., 2011).* Nevertheless, about 80% of patients have vaso-occlusive crises during ACS.

5. Aplastic crisis

Aplastic crises are acute worsenings of the patient's baseline anaemia, producing pale appearance, fast heart rate, and fatigue. This crisis is normally triggered by parvovirus B19, which directly affects production of red blood cells by invading the red cell precursors and multiplying in and destroying them. Parvovirus infection almost completely prevents red blood cell production for two to three days. In normal individuals, this is of little consequence, but the shortened red cell life of SCD patients results in an abrupt, life-threatening situation. Reticulocyte counts drop dramatically during the disease (causing reticulocytopenia), and the rapid turnover of red cells leads to the drop in haemoglobin. This crisis takes 4 days to one week to disappear. Most patients can be managed supportively; some need blood transfusion (*Slavov et al., 2011).*

6. Haemolytic crisis

Haemolytic crises are acute accelerated drops in haemoglobin level. The red blood cells break down at a faster rate. This is particularly common in patients with coexistent G6PD deficiency **(***Balgir, 2012).* Management is supportive, sometimes with blood transfusions **(***Glassberg, 2011).*

2.3.2 Epidemiology

The highest frequency of sickle cell disease is found in tropical regions, particularly sub-Saharan Africa, tribal regions of India and the Middle-East (*Weatherall and Clegg, 2001)*. Migration of substantial populations from these high prevalence areas to low prevalence countries in Europe has dramatically increased in recent decades and in some European countries sickle-cell disease has now overtaken more familiar genetic conditions such as haemophilia and cystic fibrosis (*Roberts and Montalembert, 2007).* In 2013 it resulted in 176,000 deaths due to SCD up from 113,000 deaths in 1990 (*GBD, 2013).* Sickle-cell disease occurs more commonly among people whose ancestors lived in tropical and sub-tropical sub-Saharan regions where malaria is or was common. Where malaria is common, carrying a single sickle-cell allele (trait) confers a selective advantage—in other words, being a heterozygote is advantageous. Specifically, humans with one of the two alleles of sickle-cell disease show less severe symptoms when infected with malaria (*Wellems et al., 2009).*

- Africa

Three quarters of sickle-cell cases occur in Africa. A recent WHO report estimated that around 2% of newborns in Nigeria were affected by sickle cell anaemia, giving a total of 150,000 affected children born every year in Nigeria alone. The carrier frequency ranges between 10%

and 40% across equatorial Africa, decreasing to 1–2% on the north African coast and <1% in South Africa (*Rees et al.*, 2010).

- United States

The number of people with the disease in the United States is approximately 1 in 5,000, mostly affecting Americans of Sub-Saharan African descent, according to the National Institutes of Health. In the United States, about one out of 500 African-American children and one in every 36,000 Hispanic-American children have sickle-cell anaemia. It is estimated that sickle-cell disease affects 90,000 Americans. Most infants with SCD born in the United States are now identified by routine neonatal screening. Forty-four states along with the District of Columbia, Puerto Rico and the Virgin Islands currently provide universal neonatal screening for SCD (*Pass et al., 2000)*. Sickle cell trait occurs among about 1:13 African-Americans and 1:100 Hispanic-Americans. It is estimated that 2.5 million Americans are heterozygous carriers for the sickle-cell trait (*Aidoo et al., 2002)*.

- United Kingdom

In the United Kingdom (UK) it is thought that between 12,000 and 15,000 people have sickle cell disease with an estimate of 250,000 carriers of the condition in England alone. As the number of carriers is only estimated, all newborn babies in the UK receive a routine blood test to screen for the condition. Due to many adults in high risk groups not knowing if they are carriers, pregnant women and both partners in a couple are offered screening so they can get counselling if they have the sickle cell trait. In addition, blood donors from those in high risk groups are also screened to confirm whether they are carriers and whether their blood filters properly. Donors who are found to be carriers are then informed and their blood, while often used for those of the

same ethnic group, is not used for those with sickle cell disease who require a blood transfusion (*Awasthy et al., 2008).*

2.3.3 Pathophysiology

The loss of red blood cell elasticity is central to the pathophysiology of sickle-cell disease. Normal red blood cells are quite elastic, which allows the cells to deform to pass through capillaries. In sickle-cell disease, low oxygen tension promotes red blood cell sickling and repeated episodes of sickling damage the cell membrane and decreases the cell's elasticity. These cells fail to return to normal shape when normal oxygen tension is restored. As a consequence, these rigid blood cells are unable to deform as they pass through narrow capillaries, leading to vessel occlusion and ischaemia. The actual anaemia of the illness is caused by haemolysis, the destruction of the red cells, because of their shape. Although the bone marrow attempts to compensate by creating new red cells, it does not match the rate of destruction (*Glassberg, 2011)*. Healthy red blood cells typically function for 90–120 days, but sickled cells only last 10–20 days (*Glassberg, 2011).*

2.4 THYROID HORMONES

The thyroid hormones, triiodothyronine (T_3) and its prohormone, thyroxine (T_4), are tyrosine-based hormones produced by the thyroid gland that are primarily responsible for regulation of metabolism. T_3 and T_4 are partially composed of iodine. A deficiency of iodine leads to decreased production of T_3 and T_4, enlarges the thyroid tissue and will cause the disease known as simple goitre. The major form of thyroid hormone in the blood is thyroxine (T_4), which has a longer half-life than T_3. (*Irizarry and Lisandro, 2014).* In humans, the ratio of T_4 to T_3 released into the blood is roughly 20 to 1. T_4 is converted to the active T_3 (three to four times more potent

than T_4) within cells by deiodinases (5'-iodinase). These are further processed by decarboxylation and deiodination to produce iodothyronamine (T_1a) and thyronamine (T_0a). All three isoforms of the deiodinases are selenium-containing enzymes, thus dietary selenium is essential for T_3 production (*Irizarry and Lisandro, 2014).*

2.4.1 Thyroid Hormones Production

Ingested iodine is absorbed through the small intestine and transported in the plasma to the thyroid, where it is concentrated, oxidized, and then incorporated into thyroglobulin (Tg) to form MIT and DIT and later T_4 and T_3. After a variable period of storage in thyroid follicles, Tg is subjected to proteolysis and the released hormones are secreted into the circulation, where specific binding proteins carry them to target tissues. These are the steps involve: (a) iodine availability and absorption; (b) uptake of iodide by the thyroid; (c) oxidation of iodide, which involves the thyroperoxidase (TPO), H_2O_2, and H_2O_2 generation; (d) Tg, whose iodination leads to hormone formation; (e) storage of thyroid hormones in a Tg-bound form; (f) Tg breakdown and hormone release; (g) control of synthesis and secretion by iodine supply and TSH (Françoise *et al*., 2015).

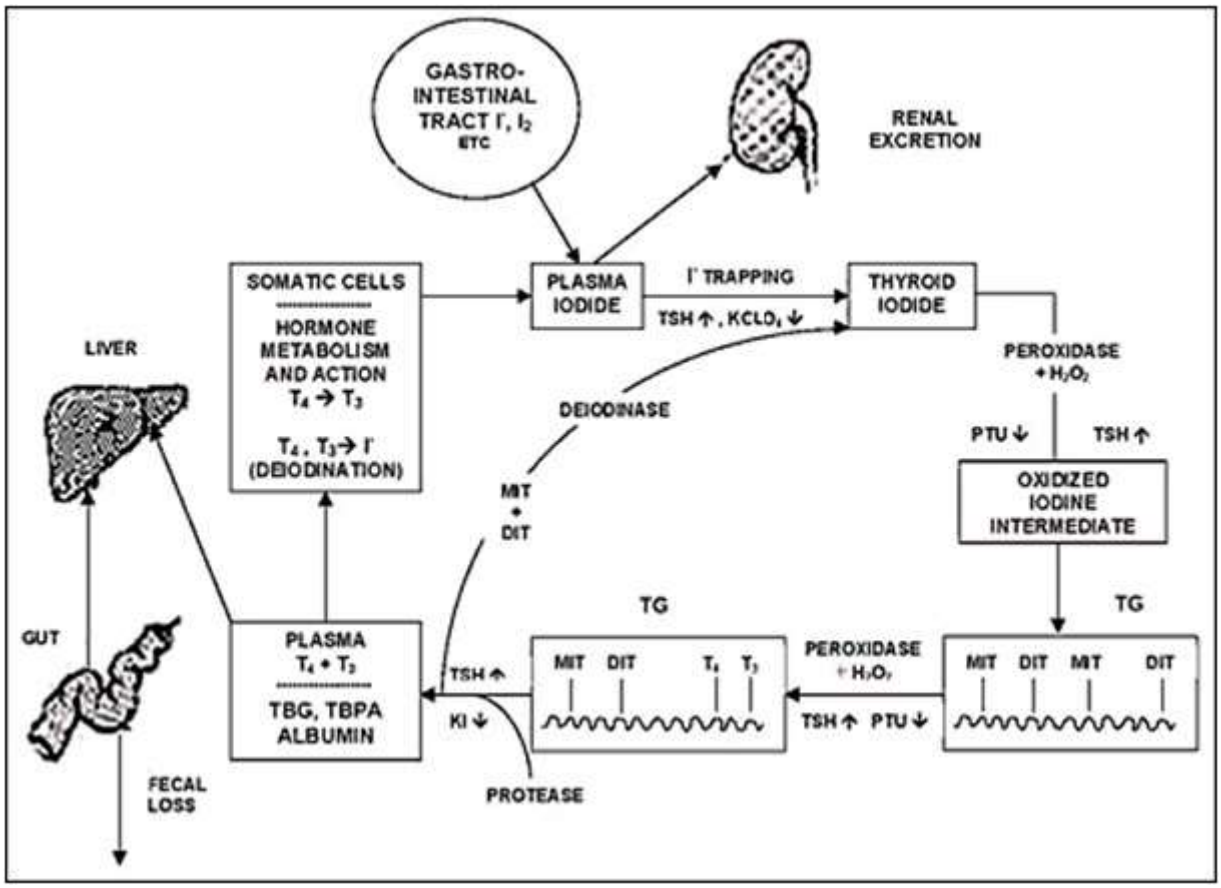

Fig. 2.2.: The iodide cycle.

Ingested iodide is trapped in the thyroid, oxidized, and bound to tyrosine to form iodotyrosines in thyroglobulin (TG); coupling of iodotyrosyl residues forms T_4 and T_3. Hormone secreted by the gland is transported in serum. Some T_4 is deiodinated to T_3. The hormone exerts its metabolic effect on the cell and is ultimately deiodinated; the iodide is reused or excreted in the kidney. A second cycle goes on inside the thyroid gland, with deiodination of iodotyrosines generating iodide, some of which is reused without leaving the thyroid (Françoise *et al*., 2015)

2.4.2 Thyroid Hormones Regulation

The production of thyroxine and triiodothyronine is primarily regulated by thyroid-stimulating hormone (TSH), released by the anterior pituitary. The thyroid, and thyrotropes in the anterior pituitary, form a negative feedback loop: TSH production is suppressed when the free T_4 levels are high. The negative feedback occurs on both the hypothalamus and the pituitary, but it is of particular importance at the level of the pituitary (*Johannes and Dietrich, 2002).* The TSH production itself is modulated by thyrotropin-releasing hormone (TRH), which is produced by the hypothalamus. This is secreted at an increased rate in situations such as cold exposure (to stimulate thermogenesis) which is prominent in case of infants. TSH production is blunted by dopamine and somatostatin (SRIH) which act as local regulators at the level of the pituitary, in response to rising levels of glucocorticoids and sex hormones (estrogen and testosterone), and excessively high blood iodide concentration.

2.4.3 Physiological Effect of Thyroid Hormones

It is likely that all cells in the body are targets for thyroid hormones. While not strictly necessary for life, thyroid hormones have profound effects on many "big time" physiologic processes, such as development, growth and metabolism (Bowen, 2010).

Metabolism: Thyroid hormones stimulate diverse metabolic activities in most tissues, leading to an increase in basal metabolic rate. One consequence of this activity is to increase body heat production, which seems to result, at least in part, from increased oxygen consumption and rates of ATP hydrolysis. By way of analogy, the action of thyroid hormones is akin to blowing on a smouldering fire. A few examples of specific metabolic effects of thyroid hormones include:

- *Lipid metabolism*: Increased thyroid hormone levels stimulate fat mobilization, leading to

increased concentrations of fatty acids in plasma. They also enhance oxidation of fatty acids in many tissues. Finally, plasma concentrations of cholesterol and triglycerides are inversely correlated with thyroid hormone levels - one diagnostic indiction of hypothyroidism is increased blood cholesterol concentration.

- *Carbohydrate metabolism*: Thyroid hormones stimulate almost all aspects of carbohydrate metabolism, including enhancement of insulin-dependent entry of glucose into cells and increased gluconeogenesis and glycogenolysis to generate free glucose.

Growth: Thyroid hormones are clearly necessary for normal growth in children and young animals, as evidenced by the growth-retardation observed in thyroid deficiency. Not surprisingly, the growth-promoting effect of thyroid hormones is intimately intertwined with that of growth hormone, a clear indiction that complex physiologic processes like growth depend upon multiple endocrine controls.

Development: A classical experiment in endocrinology was the demonstration that tadpoles deprived of thyroid hormone failed to undergo metamorphosis into frogs. Of critical importance in mammals is the fact that normal levels of thyroid hormone are essential to the development of the fetal and neonatal brain (Bowen, 2010).

2.4.4 Hyperthyroidism

Hyperthyroidism, also known as overactive thyroid and hypertoxicosis, is the condition that occurs due to excessive production of thyroid hormone by the thyroid gland (*Bahn-Chair et al., 2011)*. Hyperthyroidism may be asymptomatic or present with significant symptoms (*Devereaux et al*., 2014), some of the symptoms of hyperthyroidism include nervousness, irritability, increased perspiration, heart racing, hand tremors, anxiety, difficulty sleeping,

thinning of the skin, fine brittle hair, and muscular weakness—especially in the upper arms and thighs. More frequent bowel movements may occur, and diarrhea is common. Weight loss, sometimes significant, may occur despite a good appetite (though 10% of people with a hyperactive thyroid experience weight gain), vomiting may occur, and, for women, menstrual flow may lighten and menstrual periods may occur less often, or with longer cycles than usual. Thyroid hormone is critical to normal function of cells. In excess, it both overstimulates metabolism and exacerbates the effect of the sympathetic nervous system, causing "speeding up" of various body systems and symptoms resembling an overdose of epinephrine (adrenaline). These include fast heart beat and symptoms of palpitations, nervous system tremor such as of the hands and anxiety symptoms, digestive system hypermotility, unintended weight loss, and (in "lipid panel" blood tests) a lower and sometimes unusually low serum cholesterol (*Bradley, 2012)*. There are several causes of hyperthyroidism. Most often, the entire gland is overproducing thyroid hormone. Less commonly, a single nodule is responsible for the excess hormone secretion, called a "hot" nodule. Thyroiditis (inflammation of the thyroid) can also cause hyperthyroidism (*Andersson et al., 2010)*. Functional thyroid tissue producing an excess of thyroid hormone occurs in a number of clinical conditions. The major causes in humans are:

- Graves' disease. An autoimmune disease (usually, the most common etiology with 50-80% worldwide, although this varies substantially with location- i.e., 47% in Switzerland and 90% in the USA. Thought to be due to varying levels of iodine in the diet (*Andersson et al., 2010).*
- Toxic thyroid adenoma (the most common etiology in Switzerland, 53%, thought to be atypical due to a low level of dietary iodine in this country) (*Andersson et al.,*

2010)

- Toxic multinodular goiter

High blood levels of thyroid hormones (most accurately termed hyperthyroxinemia) can occur for a number of other reasons:

- Inflammation of the thyroid is called thyroiditis. There are several kinds of thyroiditis including Hashimoto's thyroiditis (Hypothyroidism immune-mediated), and subacute thyroiditis (DeQuervain's). These may be *initially* associated with secretion of excess thyroid hormone, but usually progress to gland dysfunction and, thus, to hormone deficiency and hypothyroidism.
- Oral consumption of excess thyroid hormone tablets is possible (surreptitious use of thyroid hormone), as is the rare event of consumption of ground beef contaminated with thyroid tissue, and thus thyroid hormone (termed "hamburger hyperthyroidism").
- Amiodarone, an anti-arrhythmic drug, is structurally similar to thyroxine and may cause either under- or overactivity of the thyroid.
- Postpartum thyroiditis (PPT) occurs in about 7% of women during the year after they give birth. PPT typically has several phases, the first of which is hyperthyroidism. This form of hyperthyroidism usually corrects itself within weeks or months without the need for treatment.
- A struma ovarii is a rare form of monodermal teratoma that contains mostly thyroid tissue, which leads to hyperthyroidism.
- Excess iodine consumption notably from algae such as kelp.

Thyrotoxicosis can also occur after taking too much thyroid hormone in the form of supplements, such as levothyroxine (a phenomenon known as exogenous thyrotoxicosis, alimentary thyrotoxicosis, or occult factitial thyrotoxicosis) (*Biondi and Cooper, 2008)*. Hypersecretion of thyroid stimulating hormone (TSH), which in turn is almost always caused by a pituitary adenoma, accounts for much less than 1 percent of hyperthyroidism cases (*Biondi and Cooper, 2008).*

2.4.5 Hypothyroidism

Hypothyroidism, also called underactive thyroid or low thyroid, is a common disorder of the endocrine system in which the thyroid gland does not produce enough thyroid hormone (Leonard and *Nabeel*, 2013). It can cause a number of symptoms, such as poor ability to tolerate cold, a feeling of tiredness, constipation, depression, and weight gain (Leonard and *Nabeel*, 2013). Occasionally there may be swelling of the front part of the neck due to goiter (Leonard and *Nabeel*, 2013). Untreated hypothyroidism during pregnancy can lead to delays in growth and intellectual development in the baby, which is called cretinism (*Preedy and Victor, 2009)*. Worldwide, too little iodine in the diet is the most common cause of hypothyroidism (*Garber et al., 2012; Chakera et al., 2012)*. In countries with enough iodine in the diet, the most common cause of hypothyroidism is the autoimmune condition Hashimoto's thyroiditis (Leonard and *Nabeel*, 2013). Less common causes include: previous treatment with radioactive iodine, injury to the hypothalamus or the anterior pituitary gland, certain medications, a lack of a functioning thyroid at birth, or previous thyroid surgery (*Persani*, 2012; Leonard and *Nabeel*, 2013). The diagnosis of hypothyroidism, when suspected, can be confirmed with blood tests measuring thyroid-stimulating hormone (TSH) and thyroxine levels (Leonard and *Nabeel*, 2013). Prevention at

the population level has been with the universal salt iodization (*Syed*, 2015). Worldwide about one billion people are estimated to be iodine deficient; however, it is unknown how often this results in hypothyroidism (*Cooper and Braverman, 2012)*. In Western countries, hypothyroidism occurs in 0.3–0.4% of people (*Garber et al., 2012)*. Subclinical hypothyroidism, a milder form of hypothyroidism characterized by normal thyroxine levels and an elevated TSH level, is thought to occur in 4.3–8.5% of people (*Garber et al., 2012)*. Hypothyroidism is more common in women than men (Leonard and *Nabeel*, 2013). People over the age of 60 are more commonly affected (Leonard and *Nabeel*, 2013).

2.4.6 Effects of Thyroid Hormones on Lipid Metabolism

Thyroid hormone plays an important role in the regulation of lipid metabolism. It acts predominantly through its nuclear receptors (thyroid hormone receptor α and β) to regulate the gene expression related to lipid metabolism. Both overt hypothyroidism and hyperthyroidism result in abnormalities of lipid profile (Ting and Xiaochun, 2014). Thyroid Hormone (TH) has multiple effects on the regulation of lipid digestion, absorption, synthesis, and catabolism (Pearce, 2012). Cumulative evidence shows that both overt hypothyroidism and subclinical hypothyroidism can result in hyperlipidemia, leading to increased risk of cardiovascular disease. the elucidation of the molecular mechanism of thyroid hormone action has been done by (Pearce, 2012).

2.4.6.1 Effects of Thyroid Hormones on Cholesterol Metabolism

TH regulates cholesterol synthesis through multiple mechanisms. The liver is the main organ for cholesterol synthesis. 3-Hydroxy-3-Methyl-Glutaryl Coenzyme A Reductase (HMGCR) is the rate-limiting enzyme in cholesterol synthesis, which is regulated by several hormones, such as insulin, glucagon, estrogen, glucocorticoid and thyroid hormone

(Mullur *et al*., 2014). In hypothyroid state, *HMGCR* mRNA levels are reduced and treatment with thyroid hormone restores it to normal level. Thyroid hormone stimulates *HMGCR* transcription and increases its stability (Ness and Chambers, 2000). TH stimulation of *HMGCR* occurs *via* Sterol Regulatory Element Binding Protein-2 (SREBP-2), a cholesterol sensing factor, low density Lipoprotein Cholesterol Receptor (LDL-R) and ATP-Binding Cassette Transporters (ABCA1 and ABCG5/8) (Lopez, 2007). When intracellular cholesterol level is low, thyroid hormone stimulates the transcription of *SREBP2* gene, leads to increasing SREBP-2-mediated *HMGCR* gene transcription (Shin and Osborne, 2003). Thyroid hormone-mediated *LDL-R* and *ABCA1/ABGG5/8* expression plays a major pathway for hepatic cholesterol clearance. TH also reduces cholesterol through enhancing cholesterol clearance pathway. Conversion of cholesterol into bile acids is important for maintaining whole body cholesterol homeostasis. The rate-limiting enzyme in bile acid synthesis is controlled by cholesterol 7-hydroxylase (CYP7A1), which is regulated by thyroid hormone (Hashimoto *et al*., 2006). Recent studies using ChIP-Seq found 3 putative Thyroid Hormone Responsive Elements (TREs) in human *CYP7A1* promoter. These TREs confer T3 transactivation of CYP7A1 in human hepatic cells (HepG2) (Lammel *et al*., 2014). In addition, thyroid hormone increases the activity of the enzymes involved in the metabolism of lipoproteins and reverse cholesterol transport, such as hepatic lipase (HL) (Lithell *et al*., 1981), lipoprotein lipase (LPL) (Kuusi *et al*., 1988), Cholesteryl- Esters Transfer Protein (CETP) (Tan *et al*., 1998) and Lecithin-Cholesterol Acyltransferase (LCAT) (Ridgway and Dolphin, 1985).

2.4.6.2 Effects of Thyroid Hormones on Triglyceride Metabolism

Thyroid hormone plays a role in both lipogenesis and lipolysis. Thyroid hormone regulates

Lipoprotein Lipase (LPL) an essential enzyme that is responsible for removing Triglycerides (TG) from circulating chylomicrons and Very Low Density Lipoproteins (VLDL). LPL catalyzes TG breakdown into non-esterfied fatty acid and transporting to adipose tissue where it is re-esterfied and stored as TG (Kuusi *et al*., 1988). Also, the fatty acids yield from LPL hydrolysis of TG is an energy source for heart. Additionally, TH affects TG levels through angioprotein-like 3 (ANGPTL3), a potent LPL inhibitor. Over expression of ANGPTL3 in mice significantly enhances total cholesterol, non-esterfied fatty acid and TG. Patients carrying ANGPTL3 mutation have increased circulating TG levels (Korstanje *et al*., 2004). Furthermore, in rats, thyroid hormone treatment reduced *ANGPTL3* mRNA expression by 70%. At transcription level, *ANGPTL3* is negatively regulated by thyroid hormone, which is mediated by TRβ but not TRα (Fugier *et al*., 2006). In hyperthyroid condition, TG level showed either no change or decrease, partly, due to T3 down regulate *ANGPTL3* and stimulates *PLP*, leading to hydrolysis TG. Then, T3-mediated LDLR stimulation dramatically increases clearing capacity for LDL (Korstanje *et al*., 2004). Thyroid hormone influences TG homeostasis also involves regulating *APOA5* gene transcription. Apolipoprotein A-V (ApoA5) is associated with HDL, VLDL and chylomicrons. ApoA5 regulates TG level through stimulating LPL-mediated TG hydrolysis and inhibiting hepatic VLDL-TG formation. Patients with single nucleotide polymorphisms or mutation of APOA5 manifest markedly reduced plasma postheparin LPL activity and hypertriglyceridemia (Calandra *et al*., 2006). Thyroid Hormones directly regulates AproA5 gene transcription via TRW in ApoA5 promoter and increases the protein level (Prieur *et al*., 2005). Animal experiment showed that *ApoA5* mRNA significantly reduced in hypothyroid rats. After T3 treatment, *ApoA5mRNA* level returned to normal. Thyroid

hormone regulation of circulating TG level is more complex involving in multi-pathways and positive and negative gene regulations.

2.4.7 Lipid Profiles in Overt Hypothyroidism

Hypothyroidism is a common metabolic disorder in the general population. Indeed, data from the third National Health and Nutrition Examination Survey (NHANES III) showed a 4.6% prevalence of hypothyroidism in the general population, while 9.5% of the Colorado prevalence study participants had elevated levels of TSH (Canaris *et al*., 2000). Serum Total Cholesterol (TC), LDL-C, lipoprotein (a) [Lp(a)], oxi-LDL, ApoB (Pearce, 2012), remnants of VLDL and Chylomicron (CM) levels are increased in overt hypothyroidism (Ito *et al*., 2003), while serum levels of triglyceride, High-Density Lipoprotein Cholesterol (HDL) and VLDL are normal or slightly increased (Mullur *et al*., 2014). All of the lipid abnormalities in overt hypothyroidism are reversible with levothyroxine (L-T4) therapy unless the patient has underlying hyperlipidemia (Kuusi *et al*., 1988). In overt hypothyroidism, thyroid hormone effects LDL receptor expression and cholesterol absorption outweigh the effects of decreased hepatic cholesterol synthesis, leading to high serum levels of LDL, Intermediate Density Lipoprotein Cholesterol (IDC), and total cholesterol levels (Galman *et al*., 2008). Additionally, LPL activity is decreased in hypothyroidism, resulting in higher level of VLDL, TG. Studies showed that Lipoprotein (a) [Lp(a)] levels are increased in patients with overt hypothyroidism and decrease after L-T4 treatment. Lp(a) is a complex of low density lipoprotein in which apolipoprotein (apo) B-100 is linked to apo(a) by a disulfied bridge. Lp(a) promotes foam cell formation and deposition of cholesterol, resulting in increased atherosclerotic and thrombogenicpotential. The mechanism may be related to the decreased clearance of LP (a) mediated by the LDL-

R degradation pathway (Galman *et al*., 2008). The levels of apolipoprotein (B) are higher in both overt and subclinical hypothyroidism and have been shown to decrease after L-T4 treatment (Perez *et al*., 2004). Cholesteryl ester transfer protein (CETP) transfers cholesterol from HDL-cholesterol (C) to LDL-C and VLDL-C. Plasma CETP concentrations are decreased in hypothyroidism and increased in hyperthyroidism, which may lead to the higher serum HDL-C concentrations in hypothyroidism (Dullaart *et al*., 1991). Thyroid hormone analogue (GC-1) can also increase hepatic HDL-C receptor (scavenger receptor B1) (Ito *et al*., 2007), and accelerate the clearance of HDL-C by the liver. HDL-C particles can be subdivided into the smaller HDL2 (primarily incorporating Apo A-I) and larger HDL3 (incorporating Apo A-I and Apo A-II) subfractions. HL can catabolize TG within HDL-C, and regulate the hydrolysis of HDL- 2 to HDL3. HL is decreased in hypothyroidism, leading to the higher HDL2 levels (Tan *et al*., 1998). Apo A-I and Apo A-II are major constituents of HDL-C. Hypothyroidism inhibits the transcription of the Apo A-I gene, but the decreased activity of HL results in slower clearance of Apo A-I, thus leading to increased Apo-AI levels. Human studies show that Apo A-I levels are decreased in hyperthyroidism and increased in hypothyroidism, whereas Apo A-II levels are not influenced by either hyperthyroidism or hypothyroidism (Tan *et al*., 1998). Patients with overt hypothyroidism usually have higher LDL-C, leading to increased oxidized (oxi)-LDL. Oxi-LDLs are taken up by macrophages in the arterial walls to produce foam cells, and as such may be a risk factor for atherosclerosis. The high levels of oxi-LDL are reversible with L-T4 treatment (Duntas *et al*., 2002), both overt hypothyroidism and subclinical hypothyroidism can levels. However, studies show that thyroid status does not affect LDL particle size (Kim *et al*., 2009).

2.4.8 Lipid Profiles in Overt Hyperthyroidism

The incidence of hyperthyroidism was lower (2.2%) (Canaris *et al*., 2000), compare with hypothyroidism in the general population. Similarly, a decreased prevalence of hyperthyroidism is evident in hyperlipidemic patients, since only 3 out of the 248 patients in a study conducted by Tsimihodimos *et al*., (1999), had thyrotoxicosis. Despite the increased activity of the HMG-CoA reductase, levels of TC, LDL-C, ApoB and Lp(a) tend to decrease in patients with clinical or subclinical hyperthyroidism. This is due to increased LDL receptor gene expression resulting in enhanced LDL receptor-mediated catabolism of LDL particles (Kung *et al*., 1995). However, no difference in LDL subfraction distribution was been observed between subclinical or overt hyperthyroid versus euthyroid subjects (Kim *et al*., 2009). In overt hyperthyroidism, serum levels of total cholesterol, LDL-C, and HDL-C (mainly HDL2) are decreased, while triglyceride levels are slightly elevated, normal or reduced (Tan *et al*., 1998), and oxi-LDL levels are increased (Azizi *et al*., 2003), whether the decreased HDL-C levels and increased oxi-LDL levels lead to atherosclerosis in hyperthyroidism is not known. Reason for this mild hypertriglyceridemia is unclear. Lipolysis is augmented in hyperthyroidism with elevation of free fatty acids in plasma, but hepatic lipogenesis is also augmented due to increased free fatty acid flux from adipose tissue to the liver (Duntas and Brenta, 2012). After treatment for hyperthyroidism, the hypertriglyceridemia caused by overt hyperthyroidism can be reversed.

2.5 EFFECT OF MEDICINAL PLANTS ON THYROID GLAND

Oduola *et al*., (2016) reported the effect of *Vitellaria paradoxa* stem bark extract on T_3, T_4, and TSH. Results showed that T_3, T_4 and TSH values of animals that received higher doses were significantly decreased ($p<0.05$) than the control groups. It was suggested that

Vitellaria paradoxa stem bark can be used in the management of hyperthyroidism condition (Oduola *et al*., 2016).

The effect of aqueous extract of *Murraya koenigii* leaves on some hormonal (T_3, T_4 and TSH) parameters in rat was reported by Sushmita and Manoranjan, (2015). The results showed that thyronine and thyroxine were increased, while thyroid stimulating hormone was decreased significantly ($P < 0.05$) at high doses. The study suggests that the extracts can act as a stimulant to thyroid functions activity, this suggests that *M. koenigii* leaf extracts could be used in hypothyroidism condition to normalize hormone levels (Sushmita and Manoranjan, 2015).

Shahnaz *et al*., (2012) reported the effect of *Physalis alkekengi* plant extract belonging to *Solanaceae* family on the concentration of the pituitary-thyroid axis hormones. The results showed significant increases in plasma concentrations of thyroxine (T_4) and triiodothyronine (T_3) in the maximum dose group ($p<0.05$) with no significant changes in plasma concentrations of thyroid-stimulating hormone (TSH). Increases in T_3 and T_4 levels with no changes in TSH concentration indicate hyperthyroidism euthyroidism in which the levels of thyroid hormones increase while the amount of TSH remains constant. It was suggested that *Physalis alkekengi* can be use in the management of hypothyroidism condition (Shahnaz *et al*., 2012)

Sharif *et al*., (2012) reported the effects of *Nigella sativa* L. ethanolic extract on thyroid function. The results showed a significant increase in the T_3 and T_4, serum concentration and TSH in the normal rats compared to control groups. It was suggested that *Nigella sativa* L can be use in the management of hyporthyroidism condition (Sharif *et al*., 2012).

Kar *et al*., (2002) reported the effect of *Bacopa monnieri* in the regulation of thyroid

hormone concentrations in male mice. The results showed a significant increase in T_3 concentration, T_4 concentration and TSH compared to control groups. It was suggested that *Bacopa monnieri* can be use in the regulation of hyporthyroidism. The effect of *Aegle marmelos* in the alteration of thyroid hormones concentration was also reported, the results showed a significant decrease in T_3, T_4 and TSH concentration compared to control groups by A. *marmelos* which was about 62% indicating its possible use in the regulation of hyperthyroidism. Kar *et al*., (2002) reported the effect of *Aloe vera* in the alteration of thyroid hormones concentration. The results showed a significant decrease in T_3, T_4 and TSH concentration compared to control groups. It was suggested that Aloe vera can be use in the regulation of hyperthyroidism (Kar *et al*., 2002).

2.5.1 Effect of Medicinal Plant on Lipid Metabolism

Aja *et al*., (2015) reported the effect of ethanol extract of *Moringa oleifera* leaves on lipid profiles in rats model. The results showed that there were significant ($P<0.05$) decrease in the total cholesterol level at high dose compared to control groups. A significant ($P<0.05$) increase was also observed in HDL-C level at high dose. While a significant ($p<0.05$) increase was observed in LDL-C level in rats when *Moringa oleifera* ethanol leaf extract was administered. The result also showed a significant ($p<0.05$) increase in triglyceride level. It was suggested that *Moringa oleifera* leaves may be useful in the management of cardiovascular disease (Aja *et al*., 2015).

Nasser *et al*., (2015) reported the effect of *Peganum harmala L.* on lipid metabolism. P. *harmala* decreased plasma cholesterol and decreased plasma triglycerides at high dose compared to control groups. *Peganum harmala* supplementation decreased very low density lipoprotein cholesterol and increased high density lipoprotein cholesterol

significantly (P<0.05). P. *harmala* at the low dose was similarly effective on lipid metabolism but differences were not significantly. It is concluded that methanolic extract of P. *harmala* could be effectively used to optimize serum lipid profile (Nasser *et al*., 2015).

The effects of methanolic extract of *Peganum harmala* L. on serum lipid profile of rat model was reported by Ali *et al*., (2014) total cholesterol (TC) and low density lipoprotein cholesterol (LDL-C) showed gradual significant decreased (P<0.05) by methanolic extract of *P. harmala* compared to control groups and there was a gradual increased of high density lipoprotein cholesterol (HDL-C) at high dose compared to the control groups. It is concluded that methanolic extract of *P. harmala* could be effectively used to optimize serum lipid profile (Ali *et al*., 2014).

Wazida *et al*., (2013) reported the effect of aqueous leaf extract of *Moringa oleifera* for its ameliorative effect in the regulation of lipid metabolism in rat model. The extract significantly reduced (p<0.05) total cholesterol concentration (TC) and low density lipoproteins cholesterol (LDL) concentration in the serum while it had no significant effect on serum High density lipoprotein (HDL) cholesterol concentration at all doses administered when compared with controls. The results suggested that the extract may have beneficial effect on serum cholesterol concentration (Wazida *et al*., 2013).

Anti-hyperlipidemic activity of methanol leaf extract of *Persea americana* was reported by Kolawole *et al.,* (2012). The changes observed in the plasma levels of total cholesterol (TC), triglycerides (TG), low density lipoprotein (LDL) and high density lipoprotein (HDL) of hyperlipidemic control rats were reversed by *Persea americana* in a dose-dependent manner. At low dose *Persea americana* significantly (p<0.05) reduced TC, TG and LDL plasma levels and increased HDL plasma level. At a higher dose *Persea americana* reduced

TC, TG and LDL levels while HDL was increased. The anti-hyperlipidemic effect of *Persea americana* was comparable to that of the standard drug, cholestyramine. The results showed that *Persea americana* could be a source of good alternative remedy for hyperlipidemia. (Kolawole *et al*., 2012).

Sodipo *et al*., (2012) reported the effect of the aqueous fruit extract of *Solanum macrocarpum* on the total lipid profile on chronic titron-induced hyperlipidemic rats. The increase in HDL-C was dose-dependent and statistically significant ($p<0.05$). There was no change ($p>0.05$) with increase in extract dose for both total cholesterol and triglycerides, while the decrease in LDL-C was significant ($p<0.05$). The VLDL-C reduced with increase in extract dose. The decrease in VLDL-C was only significant ($p<0.05$) at high dose. The results showed that the plant may be capable of reducing circulating lipids in chronic triton-induced hyperlipidemic rats probably by reducing absorption of lipids, thus, reducing hyperlipidemia. At the same time, the aqueous fruit extract probably has the potential to reduce the risk of development of heart diseases since VLDL-C has been shown to be beneficial and indicative of a lower risk of coronary heart diseases. Also, a reduction in percent atherosclerosis is desirable as this implies that atherosclerosis is reduced (Sodipo *et al*., 2012).

The possible hypolipemic effect of *Persea americana* on hypercholestrolemic rats was reported by Asaolu *et al*., (2010) however, treatment with various doses of the methanolic extract of the seeds of *Persea americana* caused a significant reduction in the levels of TC, TG, LDLC and VLDLC while the levels of HDLC increased significantly compared to control groups. These effects were dose dependent as marked changes were observed at the highest dose of the methanolic extract of *Persea americana* seeds. It was concluded that

Persea americana seeds showed hypolipemic effect and may serve as possible alternative treatment for hyperlipemia and hypertension **(**Asaolu *et al*., 2010).

Ben *et al*., (2006) reported the effect of an aqueous extract prepared from the leaves of *Viscum album* (Mistletoe) on lipid profile in male wistar rats. Results showed significant increases ($P<0.01$) in the level of total cholesterol (TC) and high density lipoproteins (HDL) compared to the control groups. The LDL levels did not show any significant change from the control values. From the results, it was suggested that the crude aqueous extract from mistletoe leaf may be relatively safe for therapeutic use as it neither predisposes to cardiovascular risk (Ben *et al*., 2006).

2.6 LIPID METABOLISM

2.6.1 Cholesterol Metabolism

Cholesterol is a precursor for steroid hormones and bile acids, and also a crucial component of lipid membranes. Daily intake of cholesterol (0.5g) constitutes around 25% of total body cholesterol turnover, the rest is endogenously synthesized primarily by the liver and the intestine (Dietschy, 1984). The rate-limiting enzyme in cholesterol synthesis is 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGCoAR) that catalyzes the formation of mevalonate from 3-hydroxy-3-methylglutaryl coenzyme A (Siperstein and Fagan, 1964; Siperstein and Fagan, 1966). Cholesterol synthesis has a diurnal rhythm that peaks around midnight and reaches its nadir at noon in both humans and rodents (Edwards *et al*., 1972). Transcription of HMGCoAR is regulated by a feedback mechanism; when intracellular sterol levels are depleted, the sterol regulatory element binding protein 2 (SREBP2) is activated and translocated to the cell nucleus where it initiates transcription of HMGCoAR. When sterol levels are restored, SREBP2 is inactivated by insulin induced gene (INSIG)

(McPherson and Gauthier, 2004). HMGCoAR is also regulated by hormones e.g. estrogen (Angelin, 1992).[9], glucagon, insulin (Ness *et al*., 1994), and thyroid hormone (Simonet and Ness, 1988).

2.6.2 Lipoprotein Metabolism

Due to its amphipathic structure, cholesterol is not soluble in blood, and has to be transported within lipoproteins. These are aggregates of lipids and proteins (apolipoproteins) and represent a continuous array of particles of varying size, composition, and density. They can be separated into chylomicrons, very low density lipoproteins (VLDLs), low density lipoproteins (LDLs), high density lipoproteins (HDLs), and lipoprotein(a) [Lp(a)]. Despite differences in size, composition, and density, lipoproteins share a common structure. The polar ends of apolipoproteins, cholesterol, and phospholipids face the blood while their nonpolar ends point inwards to the lipoprotein core, where nonpolar lipids such as cholesteryl esters and triglycerides reside (Edwards *et al*., 1972).

2.6.2.1 Chylomicrons

Chylomicrons are formed in the enterocytes postprandially. They are the largest class of lipoproteins and approximately 90% of the lipid content is triglycerides, 5% cholesterol, and 5% phospholipids. After assembly, chylomicrons enter the lymphatic system and reach the circulation via the thoracic duct. Chylomicrons contain mostly apoB48 but may in addition contain apoAI, apoAII, apoAIV, apoCII and apoE. In peripheral tissues, apoCII and apoE activate lipoprotein lipase (LPL) that hydrolyses chylomicron triglycerides, resulting in free fatty acids (FFAs) to be used as energy supply in muscle and adipose tissue or to be stored. After hydrolysis, excess surface lipids and some of the apolipoproteins are

transferred from the chylomicron remnant to HDLs, from which it acquires cholesterol esters. Still located on the chylomicron remnant, apoE interacts with hepatic LDL receptors (LDLRs) and LDLR-related proteins (LRPs) that clear the remnant particle from the circulation. The half-life of chylomicrons is approximately less than 15 min and chylomicrons are thus not normally present in blood samples from fasted subjects (Jones and Kubow, 1999).

2.6.2.2 Very Low Density Lipoprotein

VLDL are formed in the liver. The lipid content of VLDL is approximately 70% triglycerides, 15% cholesterol, and 15% phospholipids. In humans, VLDL contain apoB100 while in rodents, VLDL may contain either apoB48 or apoB100. Although apoB is continuously synthesized, it is degraded unless sufficient lipids associate with it, and the secretion of VLDL is thus dependent on the hepatic lipid level. Secreted VLDLs acquire additional apolipoproteins such as apoCI, apoCII, apoCIII, and apoE. LPL activated by apoCII and apoE hydrolyzes the triglycerides within VLDLs, and FFAs are released to be taken up by peripheral cells. The half-life of VLDLs is approximately 2 hours. The VLDL remnant, also referred to as intermediate density lipoprotein (IDL), can either be cleared from the circulation by interaction of the apoB100 and apoE with LDLRs, or be further hydrolyzed by hepatic lipase (Dietschy *et al*., 1993).

2.6.2.3 Low Density Lipoprotein

LDLs are formed in the circulation by hydrolysis of triglycerides within intermediate density lipoprotein. In humans, LDLs transport the major part, more than 70%, of the circulating cholesterol, while in rodents, HDL is the main cholesterol transporting particle.

LDLs contain a single apoB100 that interacts with the LDLR resulting in the internalization of the LDL particle. Most LDLRs are located in the liver and 70-80% of the LDL-cholesterol is removed from the circulation by hepatic LDLRs. There is also a LDLR-independent pathway for uptake of LDLs which appears to benonsaturable and therefore strictly dependent on the LDL concentration. The half-life of LDLs is 2-3 days. Increased LDL-cholesterol levels is an independent risk factor for atherosclerosis (Jones and Kubow, 1999).

2.6.2.4 High Density Lipoprotein

HDLs are formed in the circulation after hepatic or intestinal secretion of free or partly lipidated

apoAI, which is the major apolipoprotein of HDLs. ApoAI acts on peripheral cells in order for cholesterol to be transferred from these to the HDLs. This is the first step in a process called reverse cholesterol transport in which cholesterol is moved from peripheral tissues, unable to dispose excess cholesterol, to the liver where it can be eliminated from the body through biliary secretion. The cholesterol taken up by HDLs is esterified by the action of lecithin:cholesterol acyltransferase (LCAT) and moves to the core of HDL. ApoAI and apoAIV are thought to be activators of LCAT. The cholesteryl esters are either taken up by the hepatic scavenger receptor class B type I (SRBI), or transferred to apoB-containing lipoproteins in exchange for triglycerides by the action of cholesteryl ester transfer protein (CETP). HDLs can also be taken up by LDLRs and possibly by a separate apoE receptor present on hepatocytes. The half-life of HDLs is approximately 2-3 days. Other than apoAI, HDLs also contain apoAII, the second most abundant apo of HDL but of unclear physiological function, and apoAIV, apoCII, apoCIII and apoE. ApoCII is important in the

activation of LPL, while apoCIII may inhibit LPL action. HDL is thought to protect against atherosclerosis (Jones and Kubow, 1999).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 PLANT COLLECTION AND IDENTIFICATION

The leaves of *Gliricidia sepium* plants were collected from Osogbo metropolis of Osun State, Nigeria. The plant was identified and authenticated by G.A. Ademoriyo at the Herbarium unit of Botany Department, Obafemi Awolowo University, Ile Ife, by

comparing with established Herbarium specimen with voucher number IFE/17460 which was deposited at the Herbarium.

3.2 PREPARATION AND EXTRACTION

Fresh leaf of G. *sepium* was collected and air-dried at room temperature over a period of 6 weeks. It was ground manually using mortar and pestle. One kilogram (1kg) of the ground material was soaked in five litre (5L) of absolute methanol for 72 hours on a mixer to ensure maximum extraction by percolation method using maceration technique under room temperature. This was followed by periodic stirring. The resulting crude extract was filtered using whattman number one filter paper and then the filtrate was concentrated in an oven at 48°C to dryness. The dried crude extract was stored in a refrigerator at low temperature (4°C) in sterile plastic bottles, at the Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University Sokoto, until required for use.

3.3 EXPERIMENTAL ANIMALS

Wistar albino rats weighing between 150g to 170g were purchased from the animal house, of the Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University, Sokoto. They were maintained as described by Aniagu *et al*., (2005) in clean metabolic cage, placed in a well ventilated room with a temperature of 26°C to 28°C, photoperiods of 12 hours light and 12 hours darkness.

The animal were maintained on a pellet feeds (vital), obtained from Grand cereals oil mills limited, Jos, Nigeria, and were supplied with drinking water *ad libitum*. Cleaning of the animal cages was carried out daily. All the experimental protocols were in compliance with the Institutional Animal Ethics Committee guidelines as well as internationally accepted practices for use and care of laboratory animals as contained in US guidelines (National

Institute of Health, 1992), and also in accordance with the recommendation of the International Association for the study of pain (IASP) (Zimmerman, 1983).

3.4 RESEARCH DESIGNS

3.4.1 Acute Toxicity Study

Acute toxicity study was performed in accordance with the procedure outlined by the Organisation for Economic Co-operation Development guidelines 425 (OECD, 2010). Eighteen (18) wistar rats of both sexes were used for this study. The rats were randomly divided into 3 groups comprising of 6 animals each, 3 males and 3 females in separate cages, with the first group as the control group. A single dose of 2000 and 5000mg/kg of the extract was administered to the rats in group 2 and 3 respectively by intra gastric gavage using oral cannula. After the administration of the extract, each animal was observed for signs of toxicities and mortality within the first 4 hours, 24 hours, 48 hours, 72 hours and up to 14 days.

3.4.2 Sub-Chronic Toxicity Study

Sub-chronic toxicity study was carried out in accordance with OECD 425 (2010) guidelines. Fifty (50) rats of both sexes, were divided into five (5) groups of ten (10) rats each, five (5) males and five (5) females in separate cages. Group 1 served as control group and received normal saline. Graded doses of the extract 250, 500, 750 and 1000mg/kg body weight respectively, were administered orally to the rats in group 2, 3, 4 and 5 daily for 28 days. All the rats had free access to food and water throughout the duration of the experiment and were observed daily for general symptoms of toxicity and mortality (Aniagu *et al*., 2005). After 28 days period, the animals were fasted overnight, and

anaesthetised using chloroform. Blood samples were collected from the animals through cardiac puncture, into clean lithium heparinised bottle. The rats were sacrificed through lumbar dislocation and their thyroid glands were removed and put in 10% formal saline as fixative. The blood samples collected were centrifuge at 5000 rpm for 10 minutes. The plasma of each sample were separated and kept frozen at -4°C until required for analysis (Aniagu *et al*., 2005).

3.4.3 Histopathological Examination

The thyroid glands carefully removed were transferred into specimen bottles containing 10% formalin for proper fixation. The samples were taken to department of histopathology UDUTH where they were grossed and processed using automatic tissue processor. This was followed by embedding the grossed tissues in molten paraffin wax. Section of 5-6µm in thickness were cut and made onto slides. These tissue sections were stained using Haematoxylene and eosin method for photo-microscopic examination of general tissue structures as reported by Orchard and Nation (2012).

3.5 LABORATORY ANALYSIS

3.5.1 Thyroid Stimulating Hormone (TSH)

TSH was determined using ELISA method for the quantitative determination in serum by microplate as described by Hopton and Harrap (1986).

3.5.1.1 Principle

Immunoenzymometric assay: the essential reagent for immunoenzymometric assay include high affinity and specificity antibodies (enzyme conjugated and immobilized), with different and distinct epitope recognition, in excess, and native antigen the immobilization

takes place during the assay at the surface of a microplate well through the interaction of streptavidin coated on the well and exogenously added biotinylated monoclonal anti TSH antibody. Upon mixing monoclonal biotinylated antibody, the enzymes labeled antibody and a serum containing the native antigen, reaction results between the native antigen and the antibodies, without competition or steric hindrance, to form a soluble sandwich complex.

3.5.1.2 Procedure

Fifty microlitre (50µl) of the appropriate reference control was pipetted into the assigned wells. Fifty microlitre (50µl) of the specimen was pipetted into the assigned well. One hundred microlitre (100µl) of the TSH enzymes reagent was pipetted to each well. The microplate was swirl gently for 20-30 seconds, it was mixed and covered. The microplate was incubated for 60 minute at room temperature. The content of microplate was decanted, the plate was washed five (5) times with a wash buffer using automatic manual plate washer, tapped and blotted dried with absorbent paper. One hundred microlitre (100µl) of working substrate solution was added to all wells. The microplate was incubated at room temperature for 15 minutes. Fifty microlitre (50µl) of stop solution was added to each well and It was mixed gentle for 15-20 seconds. The absorbance of each well were read at 450nm (using a reference wavelength of 620-630nm to minimize well imperfections).

3.5.1.3 Calculation

A dose response curve was used to ascertain the concentration of thyroid stimulating hormone in unknown specimens.

3.5.2 Triiodothyronine (T_3)

T_3 was determine using ELISA method for the quantitative determination in serum by

microplate as described by Hopton and Harrap (1986).

3.5.2.1 Principle

Immunoenzymometric assay: the essential reagent for immunoenzymometric assay include high affinity and specificity antibodies (enzyme conjugated and immobilized), with different and distinct epitope recognition, in excess, and native antigen the immobilization takes place during the assay at the surface of a microplate well through the interaction of streptavidin coated on the well and exogenously added biotinylated monoclonal anti triiodothyronine antibody. Upon mixing monoclonal biotinylated antibody, the enzymes labeled antibody and a serum containing the native antigen, reaction results between the native antigen and the antibodies, without competition or steric hindrance, to form a soluble sandwich complex.

3.5.2.2 Procedure

Fifty microlitre (50µl) of the appropriate reference control was pipetted into the assign well. Fifty microlitre (50µl) of the specimen was pipetted into the assigned well. One hundred microlitre (100µl) of the T3 enzymes reagent was pipetted to each well. The microplate was swirl gently for 20-30 seconds to. It was mixed and covered. The microplate was incubated for 60 minute at room temperature. The content of microplate was decanted, the plate was washed five (5) times with a wash buffer using automatic manual plate washer, tapped and blotted dried with absorbent paper. One hundred microlitre (100µl) of working substrate solution was added to all wells. The microplate was incubated at room temperature for 15 minutes. Fifty microlitre (50µl) of stop solution was added to each well and it was mixed gentle for 15-20 seconds. The absorbance of each well was read at 450nm (using a reference wavelength of 620-630nm to minimize well imperfections).

3.5.2.3 Calculation

A dose response curve was used to ascertain the concentration of triiodothyronine (T3) in unknown specimens.

3.5.3 Thyroxine (T_4)

T_4 was determine using ELISA method for the quantitative determination in serum by microplate as describe by Hopton and Harrap (1986).

3.5.3.1 Principle

Immunoenzymometric assay: the essential reagent for immunoenzymometric assay include high affinity and specificity antibodies (enzyme conjugated and immobilized), with different and distinct epitope recognition, in excess, and native antigen the immobilization takes place during the assay at the surface of a microplate well through the interaction of streptavidin coated on the well and exogenously added biotinylated monoclonal anti thyroxine antibody. Upon mixing monoclonal biotinylated antibody, the enzymes labeled antibody and a serum containing the native antigen, reaction results between the native antigen and the antibodies, without competition or steric hindrance, to form a soluble sandwich complex.

2.5.3.2 Procedure

Twenty five microlitre (25µl) of the appropriate reference control was pipetted into the assign well. Twenty five microlitre (25µl) of the specimen will be pipetted into the assigned well. One hundred microlitre (100µl) of the T3 enzymes reagent was pipetted to each well. The microplate was swirl gently for 20-30 seconds it was mixed and covered. The microplate was incubated for 60 minute at room temperature. The content of microplate was decanted, the plate was washed five (5) times with a wash buffer using

automatic manual plate washer, tapped and blotted dried with absorbent paper. One hundred microlitre (100µl) of working substrate solution was added to all wells. The microplate was incubated at room temperature for 15 minutes. Fifty microlitre (50µl) of stop solution was added to each well and it was mixed gentle for 15-20 seconds. The absorbance of each well was read at 450nm (using a reference wavelength of 620-630nm to minimize well imperfections).

3.5.3.3 Calculation

A dose response curve was used to ascertain the concentration of thyroxine (T4) in unknown specimens.

3.5.4 Total Cholesterol (TC)

Enzymatic colorimetric method was used for the estimation of total cholesterol as described by Trinder (1969).

3.5.4.1 Principle:

Cholesterol ester + H_20 $\xrightarrow{\text{cholesterol esterase}}$ cholesterol + fatty acid

Cholesterol + O_2 $\xrightarrow{\text{cholesterol Oxidase}}$ 4-cholesten-3-one + H_2O_2

$2H_2O_2$ + phenol + aminoantipyrin $\xrightarrow{\text{peroxidase}}$ Red quinine + $4H_2O$

The absorbance of red colour is proportional to the concentration of cholesterol in the sample.

3.5.4.2 Procedure

One mililitre (1ml) of cholesterol reagent was added into test, standard and blank tubes. Ten microlitre (10µl) of serum was added into test and ten microlitre (10µl) of standard was

added into standard tube. The tubes were mixed and incubated at 37^0 C for 5 minutes. The absorbance was read against the blank within 60 minutes at 505nm.

3.5.4.3 Calculation

$$\frac{absorbance\ of\ Test}{absorbance\ of\ standard}\ \text{x}\ conc\ of\ standard\ (200mg/dl)$$

3.5.5 High Density Lipoprotein-Cholesterol (HDL-C)

Enzymatic colorimetric method was used for the estimation of HDL-C as described by Trinder (1969).

3.5.5.1 Principle:

The chylomicrons, very low density lipoprotein (VLDL) and low density lipoprotein (LDL) were precipitated by phosphotungstic acid and magnesium ions. After centrifugation HDL remained in the supernatant and was measure by enzymatic method as in cholesterol above.

3.5.5.2 Procedure

Precipitation:

Two hundred microlitre (200µl) of sample was pipetted into a test tube. Five hundred (200µl) of HDL reagent was added. It was mixed and Incubated at room temperature for 10 minutes mixed and centrifuge at 4000rpm for 10 minutes. Clear supernatant was separated and HDL-C concentration was determined using cholesterol reagent.

HDL-C determination

One thousand microlitres (1000µl) of cholesterol reagent was pipetted into labeled test, standard and blank test tubes. Fifty microlitre (50µl) of HDL-C supernatant was pipetted into test and fifty (50µl) of standard into standard tube. They were mixed and incubated at 37^0 C for 5 minutes. The absorbance of test and standard were read against the blank.

3.5.5.3 Calculation

$$\frac{absorbance\ of\ Test}{absorbance\ of\ standard}\ \text{x}\ N$$

N=standard concentration (50mg/dl)

3.5.6 Low Density Lipoprotein-Cholesterol (LDL-C)

LDL-C was calculated by Friedwald formulae (Friedwald, *et al*., 1974)

LDL-C = TC— (HDL-C + TG/5)

3.5.7 Very Low Density Lipoprotein-Cholesterol (VLDL-C)

VLDL-C was calculated by Friedwald formulae (Friedwald, *et al*., 1974)

VLDL-C = TG/5

3.5.8 Triglycerides (TG)

Enzymatic colorimetric method was used for the estimation of triglycerides as described by Trinder (1969**).**

3.5.8.1 Principle:

Triglycerides + H_2O $\xrightarrow{\text{lipoprotein lipase}}$ Glycerol + fatty acid

Glycerol + ATP $\xrightarrow{\text{glycerol kinase}}$ Glycerol-3-phosphate + ADP

Glycerol-3-phosphate + O_2 $\xrightarrow{\text{glycerol-3-phosphate}}$ Dihydroxy acetone phosphate + H_2O_2

H_2O_2 + 4-aminoantipyrine + P-chlorophenol $\xrightarrow{\text{peroxidase}}$ Red quinoimine.

The absorbance is proportional to the concentration of TG in the sample.

2.5.8.2 Procedure

One mililitre (1ml) of TG reagent was pipetted into test, standard and blank tube. Ten microlitre (10µl) of serum, standard and distilled water was pipetted into test, standard and blank respectively. It was mixed and incubated at 37^0 C for 5 minutes. The absorbance was

read against blank within 60 minutes at 505nm.

3.5.8.3 Calculation

$$\frac{absorbance\ of\ Test}{absorbance\ of\ standard}$$ x *conc of standard (200mg/dl)*

3.6 DATA ANALYSIS

The data obtained from this study were analysed using statistical package for Social Science (SPSS) for windows, version 21.0 (SPSS Inc., Chicago, IL, USA). The data were presented as the mean ± standard deviation (S.D) of the concentration. Analysis of variance (ANOVA) at 95% confidence interval was used to evaluate the significance of the difference between the mean values of the measured parameters in the respective test and control groups. A mean difference was considered significant when $p<0.05$

CHAPTER FOUR

4.0 RESULTS

4.1 ACUTE TOXICITY

The results obtained are presented in tables 4.1 to 4.3 and Figures 4.1 to 4.3.

Table 4.1: shows the result of oral acute toxicity in the Wistar rats. The result of oral acute toxicity showed that no death was recorded in the rats after 24 hours and up to 14 days post oral treatment.

Table 4.2: shows the results of the effect of oral administration of *Gliricidia Sepium* aqueous leaf extract on thyroid hormones (T_3, T_4, TSH) in wistar rats. T_3 was significantly higher ($p< 0.05$) in the test groups when compared with controls, while T_4 and TSH were significantly lower ($p< 0.05$) than that of control group.

Table 4.3: shows the results of the effects of oral administration of *Gliricidia Sepium* aqueous leaf extract on lipid profiles (TC, TG, HDL, LDL, and VLDL) in wistar rats. There were no statistically significant differences ($p>0.05$) in lipid profiles in the test group when compared with control groups.

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Table 4.1: Acute oral toxicity study of *Gliricidia Sepium* aqueous leaf extract in wistar rats

Groups	Dosa (mg/kg)	Observation period	Behavioural changes	Mortality
Control	D/H_2O	Up to 72 hours	None	None
Group 2	2000	Up to 72 hours	None	None
Day 1: 1st rat	2000	Up to 72 hours	None	None
Day 2: 2nd rat	2000	Up to 72 hours	None	None
Day 3: 3rd rat	2000	Up to 72 hours	None	None
Group 3	5000	Up to 72 hours	None	None
Day 1: 1st rat	5000	Up to 72 hours	None	None
Day 2: 2nd rat	5000	Up to 72 hours	None	None
Day 3: 3rd rat	5000	Up to 72 hours	None	None

Table 4.2: Thyroid hormones levels in wistar rats exposed to *Gliricidia Sepium* aqueous leaf extract in acute oral toxicity study

	Dose(mg/kg)				
Parameters	Control	2000	5000	F	P-value
	n=6	n=6	n=6		
T_3 (ng/dl)	$0.070^a \pm 0.094$	$0.174^b \pm 0.103$	$0.370^b \pm 0.187$	8.71	0.0028
T_4 (µg/dl)	$2.438^a \pm 0.913$	$1.104^b \pm 1.112$	$1.840^a \pm 0.456$	3.80	0.0447
TSH (IU/ml)	$0.663^a \pm 0.642$	$0.069^b \pm 0.102$	$0.030^b \pm 0.051$	4.71	0.024

(P<0.05) indicates significant differences, (ns) indicates not significant

Post hoc test	**(T_3) P-value**	**(T_4) P-value**	**(TSH) P-value**
Grp 1 vs 2	0.001	0.001	0.001
Grp 1 vs 3	0.001	ns	0.001
Grp 2 vs 3	ns	0.001	ns

(P<0.05) indicates significant differences, (ns) indicates not significant

Table 4.3: Lipid profiles levels in wistar rats exposed to *Gliricidia Sepium* aqueous leaf extract in acute oral toxicity study

	Dose (mg/kg)				
Parameter	Control	2000	5000	F	P-value
	n=6	n=6	n=6		
TC (mmol/l)	58.5 ± 8.78	56.67 ± 13 92	59.40 ± 26.39	0.04	0.9600
TG (mmol/l)	64.375 ± 27.313	45.333 ± 7.916	46.80 ± 12.438	2.04	0.162
HDL (mmol/l)	26.25 ± 5.922	28.17 ± 3.250	21.6 ± 7.470	1.89	0.184
LDL (mmol/l)	23.0 ± 13.784	19.83 ± 13.014	30.34 ± 30.409	0.43	0.660
VLDL (mmol/l)	13.0 ± 5.425	9.0 ± 1.414	9.40 ± 2.408	2.27	0.135

(P<0.05)

Post hoc test (TC) P-value	**(TG) P-value**	**(HDL) P-value**	**(LDL) P-value**	**(VLDL) P-value**
Grp 1 vs 2	ns	ns	ns	ns	ns
Grp 1 vs 3	ns	ns	ns	ns	ns
Grp 2 vs 3	ns	ns	ns	ns	ns

($P<0.05$) indicates significant differences, (ns) indicates not significant

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Plate 4.1: Photomicrograph of thyroid gland tissue in control rats showing normal thyroid follicles (black arrow) and follicular epithelial cells (red arrow) (H&E X 100).

Plate 4.2: Photomicrograph of thyroid gland tissue in group 2 (2000mg/kg) rats showing normal thyroid follicles (black arrow) and follicular epithelial cells (red arrow) (H&E X 100).

Plate 4.3: Photomicrograph of thyroid gland tissue in group 3 (5000mg/kg) rats showing normal thyroid follicles (black arrow) and follicular epithelial cells (red arrow) (H&E X 100).

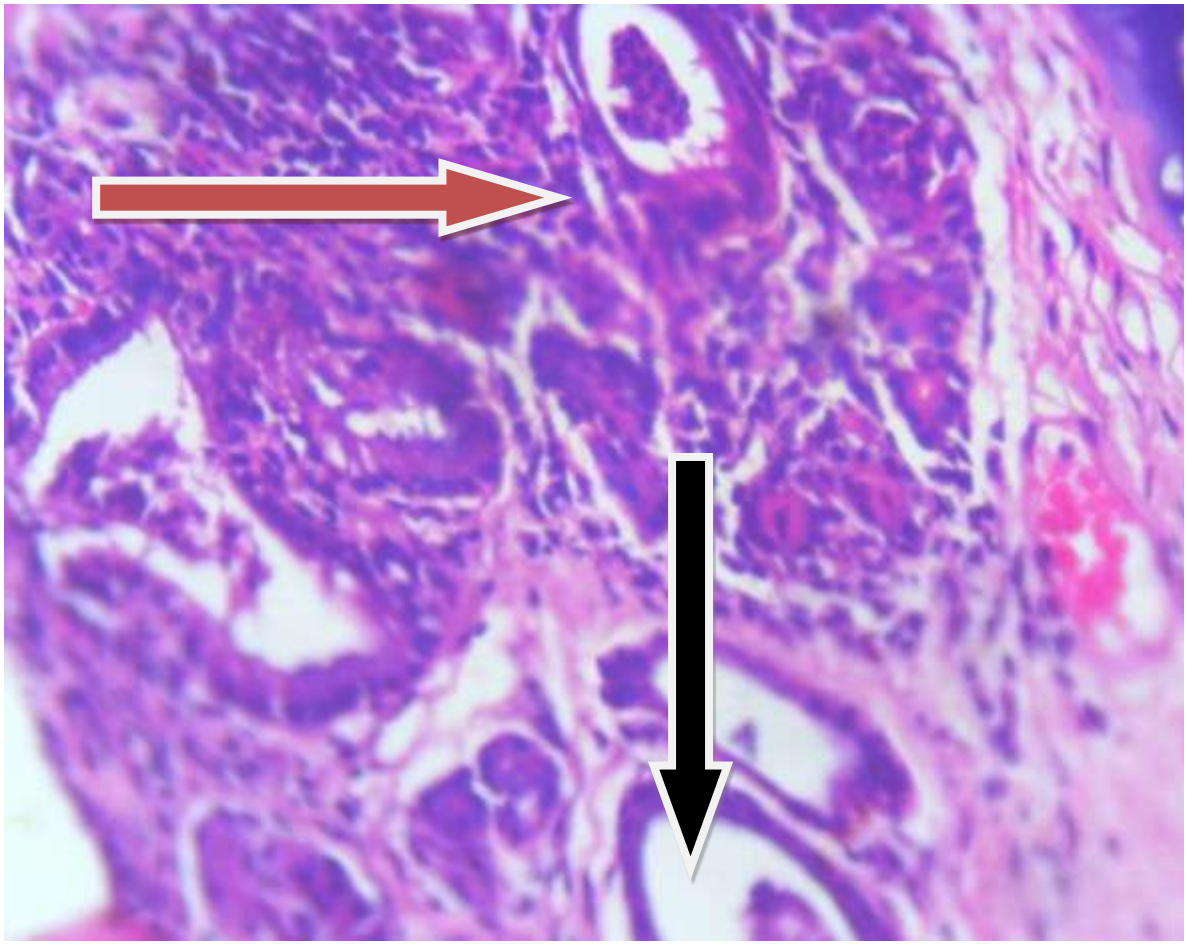

Plate 4.1: Photomicrograph of thyroid gland tissue in control rats showing normal thyroid follicles (black arrow) and follicular epithelial cells (red arrow). (H & E X 100).

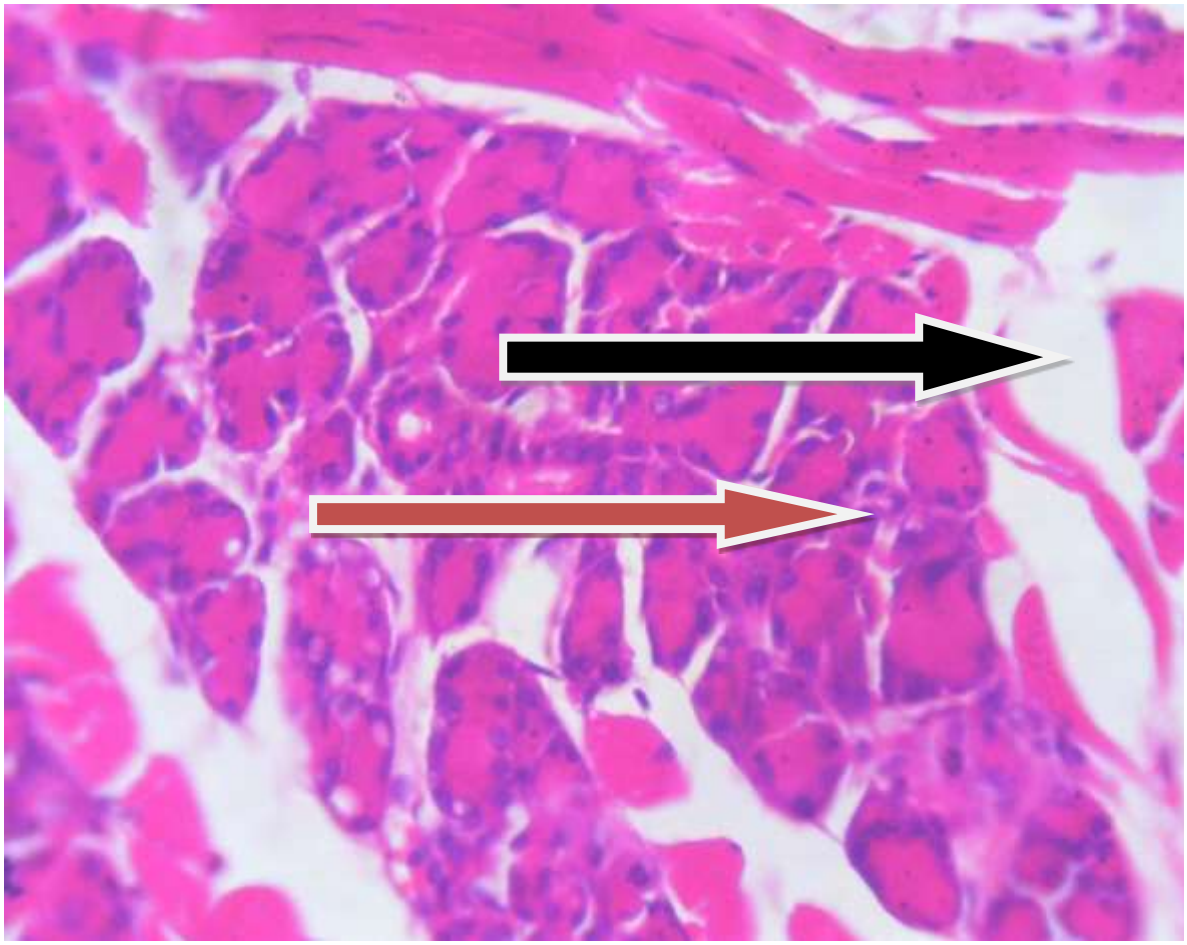

Plate 4.2: Photomicrograph of thyroid gland tissue in group 2 (2000mg/kg) rats showing normal thyroid follicles (black arrow) and follicular epithelial cells (red arrow). (H & E X 100).

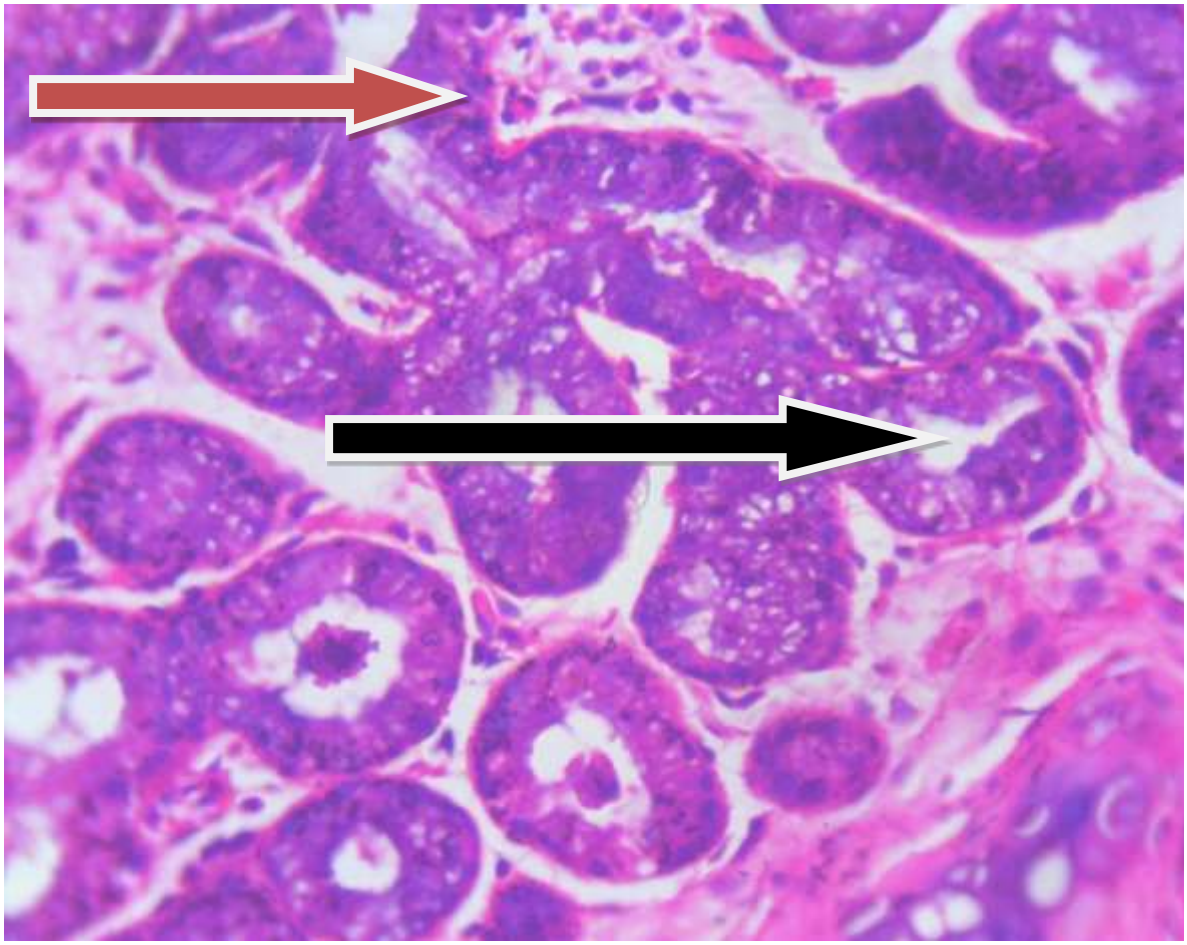

Plate 4.3: Photomicrograph of thyroid gland tissue in group 3 (5000mg/kg) rats showing normal thyroid follicles (black arrow) and follicular epithelial cells (red arrow). (H & E X100).

4.2 SUB-CHRONIC TOXICITY

The results obtained are presented in tables 4.4 and 4.5 and Figures 4.4 to 4.8

Table 4.4 shows the results of the effects of sub-chronic ingestion of *Gliricidia Sepium* aqueous leaf extract on thyroid hormone (T_3, T_4, TSH) in wistar rats. T_3 and TSH in the test group were not statistically significant differences ($p>0.05$) when compared with control group, while T_4 was significantly higher ($p< 0.05$) at lower dose (250mg) and significantly lower at higher dose (500mg, 750mg and 1000mg) in the test group when compared with the control groups.

Table 4.5 shows the results of the effects of sub-chronic ingestion of *Gliricidia Sepium* aqueous leaf extract on lipid profiles (TC, TG, HDL, LDL, and VLDL) in wistar rats. HDL was significantly lower ($p< 0.05$) in the test group when compared with the control group, while TC, TG, LDL and VLDL values from the test group were not statistically significant differences ($p>0.05$) when compared with the control groups.

Table 4.4 Thyroid hormones levels in wistar rats exposed to *Gliricidia Sepium* aqueous leaf extract in sub-chronic toxicity study

	Dose (mg/kg)					
Parameters	Control	250	500	750	1000	F	P-value
		n=10	n=10	n=10	n=10		
T_3	0.070±0.094	0.168±0.104	0.094±0.108	0.052±0.022	0.067±0.058	1.71	0.175
T_4	2.438^{a}±0.913	2.680^{a}±1.209	1.085^{b}±0.615	1.071^{b}±0.439	1.229^{b}±0.682	6.48	0.0007
TSH	1.038±1.140	0.031±0.046	0.096±0.006	0.186±0.114	4.560±11.659	0.89	0.480

Difference superscript alphabet along rows indicates significant difference ($P<0.05$)

Post hoc test	(T_3) P-value	(T_4) P-value	(TSH) P-value
Grp 1 vs 2	ns	ns	ns
Grp 1 vs 3	ns	0.001	ns
Grp 1 vs 4	ns	0.001	ns
Grp 1 vs 5	ns	0.001	ns
Grp 2 vs 3	ns	0.001	ns
Grp 2 vs 4	ns	0.001	ns
Grp 2 vs 5	ns	0.001	ns
Grp 3 vs 4	ns	ns	ns
Grp 3 vs 5	ns	ns	ns
Grp 4 vs 5	ns	0.001	ns

(P<0.05) indicates significant differences, (ns) indicates not significant

Table 4.5: Lipid profiles levels in wistar rats exposed to *Gliricidia Sepium* aqueous leaf extract in sub-chronic toxicity study

	Dose (mg/kg)						
Parameters	Control	250	500	750	1000	F	P-

value						
	n=10	n=10	n=10	n=10	n=10	
TC	58.5±8.783	56.8±22.309	44.714±10.657	54.571±17.444	50.143±14.531	1.00
0.4240						
TG	25.00±4.44	15.20±4.38	22.00±3.79	24.86±8.55	24.29±6.40	0.60
0.663						
HDL	25.00^{a}±4.44	15.20^{b}±4.38	22.00^{ab}±3.79	24.86^{a}±8.55	24.29^{a}±6.40	2.77
0.045						
LDL	23.0±13.78	25.75±20.90	9.57±6.48	14.86±12.21	12.43±9.68	1.85
0.147						
VLDL	13.00±5.42	15.75±4.11	14.71±2.84	14.86±3.85	12.43±5.71	0.55
0.7009						

Difference superscript alphabet along rows indicates significant difference (P<0.05)

Post hoc test	**(TC) P-value**	**(TG) P-value**	**(HDL) P-value**	**(LDL) P-value**	**(VLDL) P-value**
Grp 1 vs 2	ns	ns	0.001	ns	

ns					
Grp 1 vs 3	ns	ns	ns	ns	
ns					
Grp 1 vs 4	ns	ns	ns	ns	
ns					
Grp 1 vs 5	ns	ns	ns	ns	
ns					
Grp 2 vs 3	ns	ns	ns	ns	
ns					
Grp 2 vs 4	ns	ns	ns	ns	
ns					
Grp 2 vs 5	ns	ns	ns	ns	
ns					
Grp 3 vs 4	ns	ns	ns	ns	
ns					
Grp 3 vs 5	ns	ns	ns	ns	
ns					
Grp 4 vs 5	ns	ns	ns	ns	
ns					

(P<0.05) indicates significant differences, (ns) indicates not significant

Plate 4.4: Photomicrograph of thyroid gland tissue in control rats showing normal thyroid follicles (black arrow) and follicular epithelial cells (red arrow) (H&E X 100).

Plate 4.5: Photomicrograph of thyroid gland tissue in group 2 (250mg/kg) rats showing normal thyroid follicles (back arrow) and follicular epithelial cells (red arrow) (H&E X 100).

Plate 4.6: Photomicrograph of thyroid gland tissue in group 3 (500mg/kg) rats showing normal thyroid follicles (black arrow) and follicular epithelial cells (red arrow) (H&E X 100).

Plate 4.7: Photomicrograph of thyroid gland tissue in group 4 (750mg/kg) rats showing normal thyroid follicles (black arrow) and follicular epithelial cells (red arrow) (H&E X 100).

Plate 4.8: Photomicrograph of thyroid gland tissue in group 5 (1000mg/kg) rats showing normal thyroid follicles (black arrow) and follicular epithelial cells (red arrow) (H&E X 100).

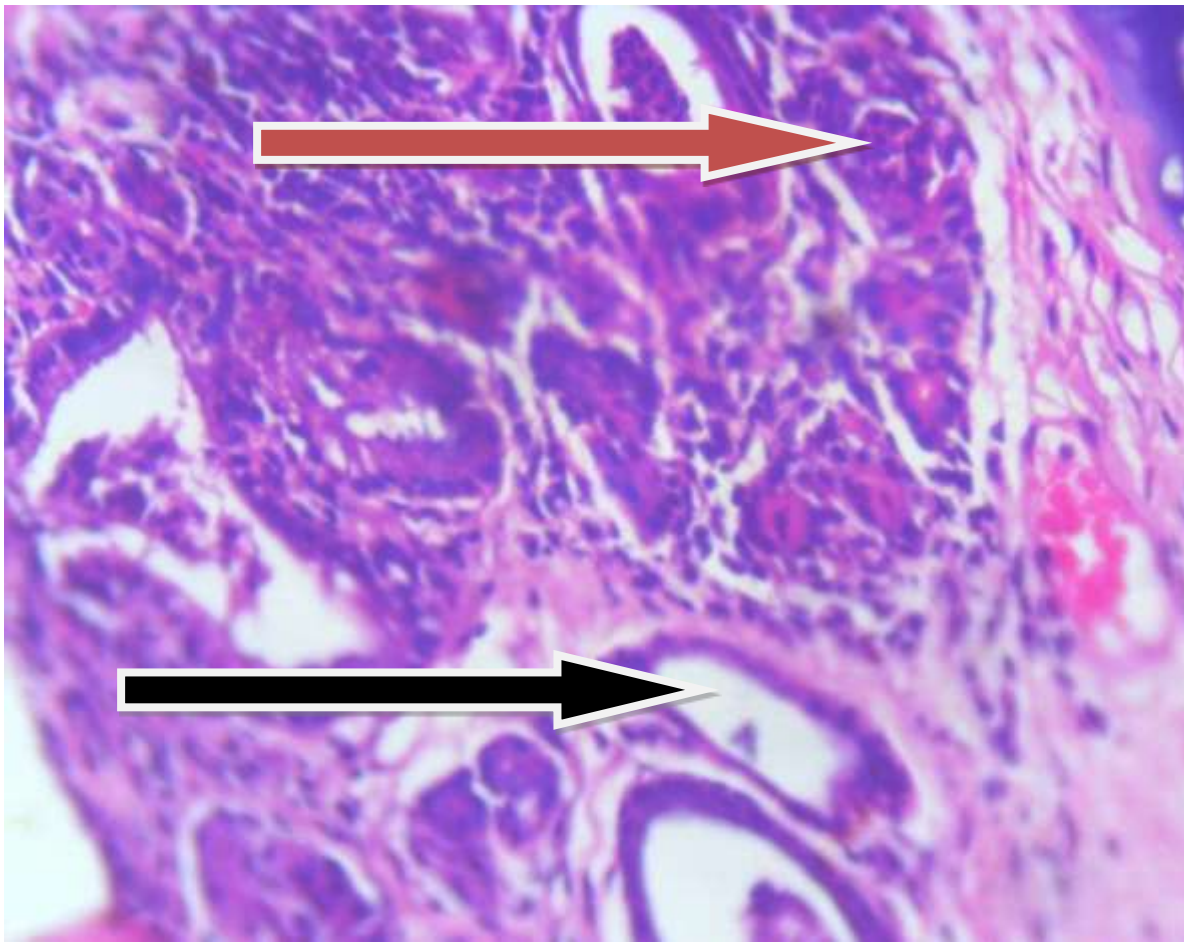

Plate 4.4: Photomicrograph of thyroid gland tissue in control rats showing normal thyroid follicles (black arrow) and follicular epithelial cells (red arrow). (H & E X 100).

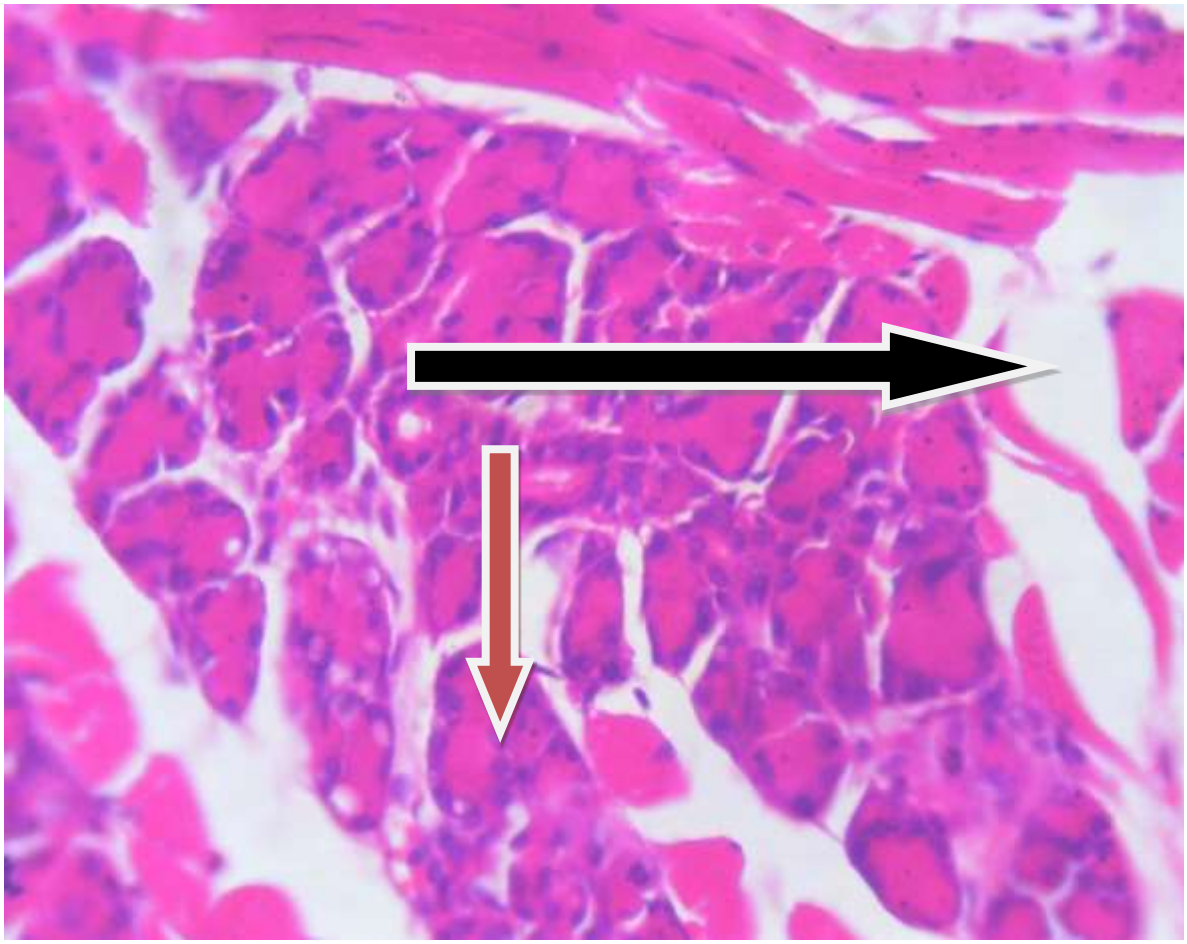

Plate 4.5: Photomicrograph of thyroid gland tissue in group 2 (250mg/kg) rats showing normal thyroid follicles (black arrow) and follicular epithelial cells (red arrow). (H & E X 100).

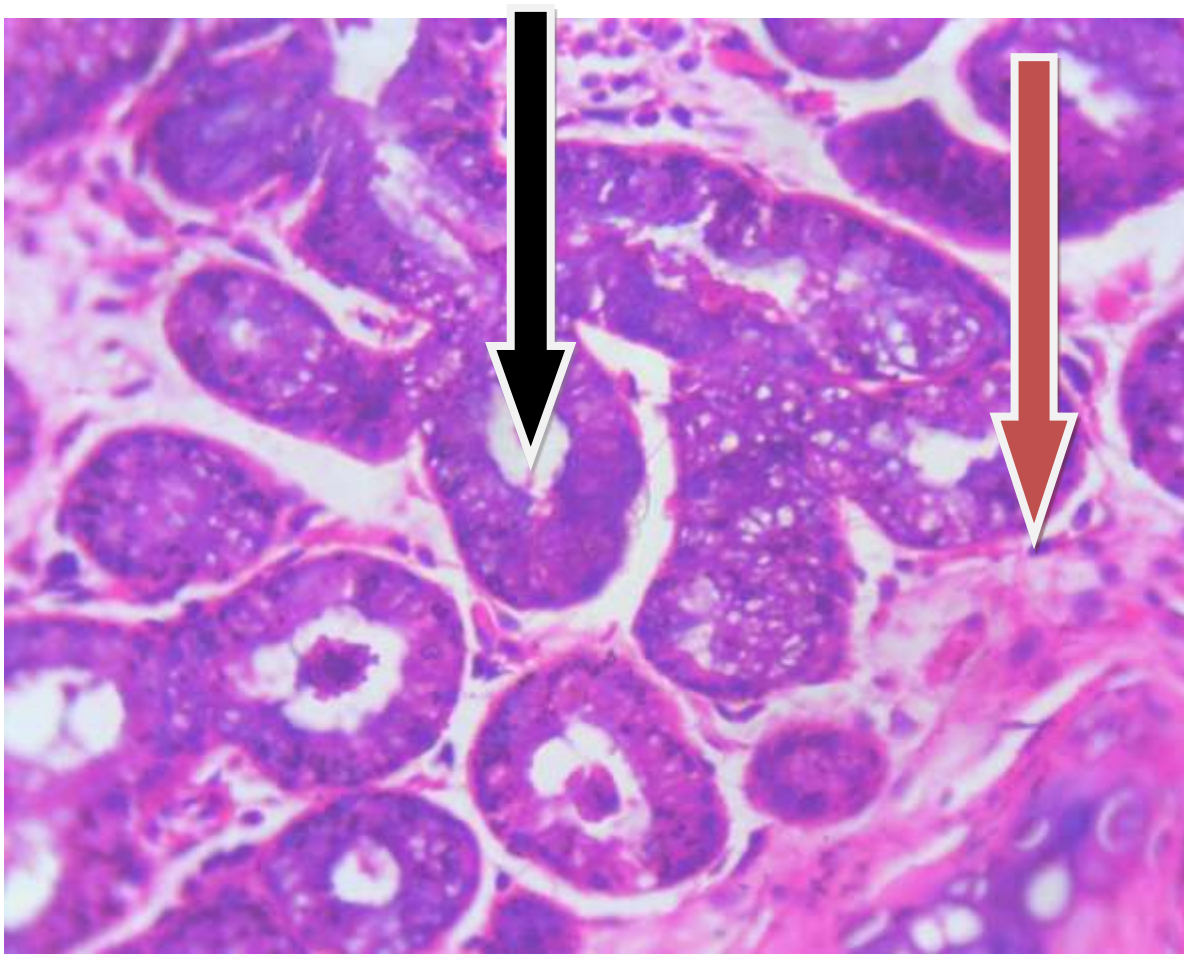

Plate 4.6: Photomicrograph of thyroid gland tissue in group 3 (500mg/kg) rats showing normal thyroid follicles (black arrow) and follicular epithelial cells (red arrow). (H & E X 100).

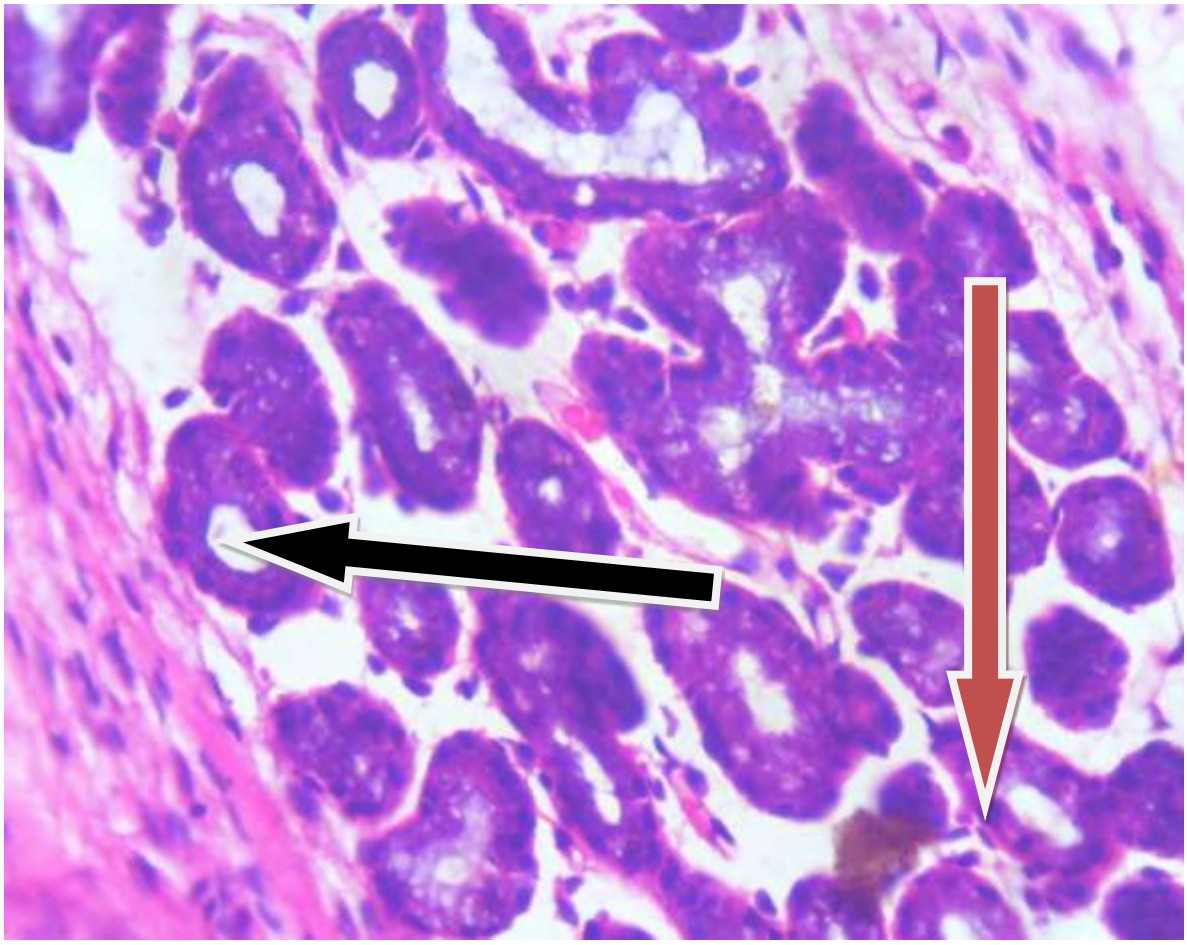

Plate 4.7: Photomicrograph of thyroid gland tissue in group 4 (750mg/kg) rats showing normal thyroid follicles (black arrow) and follicular epithelial cells (red arrow). (H & E X 100).

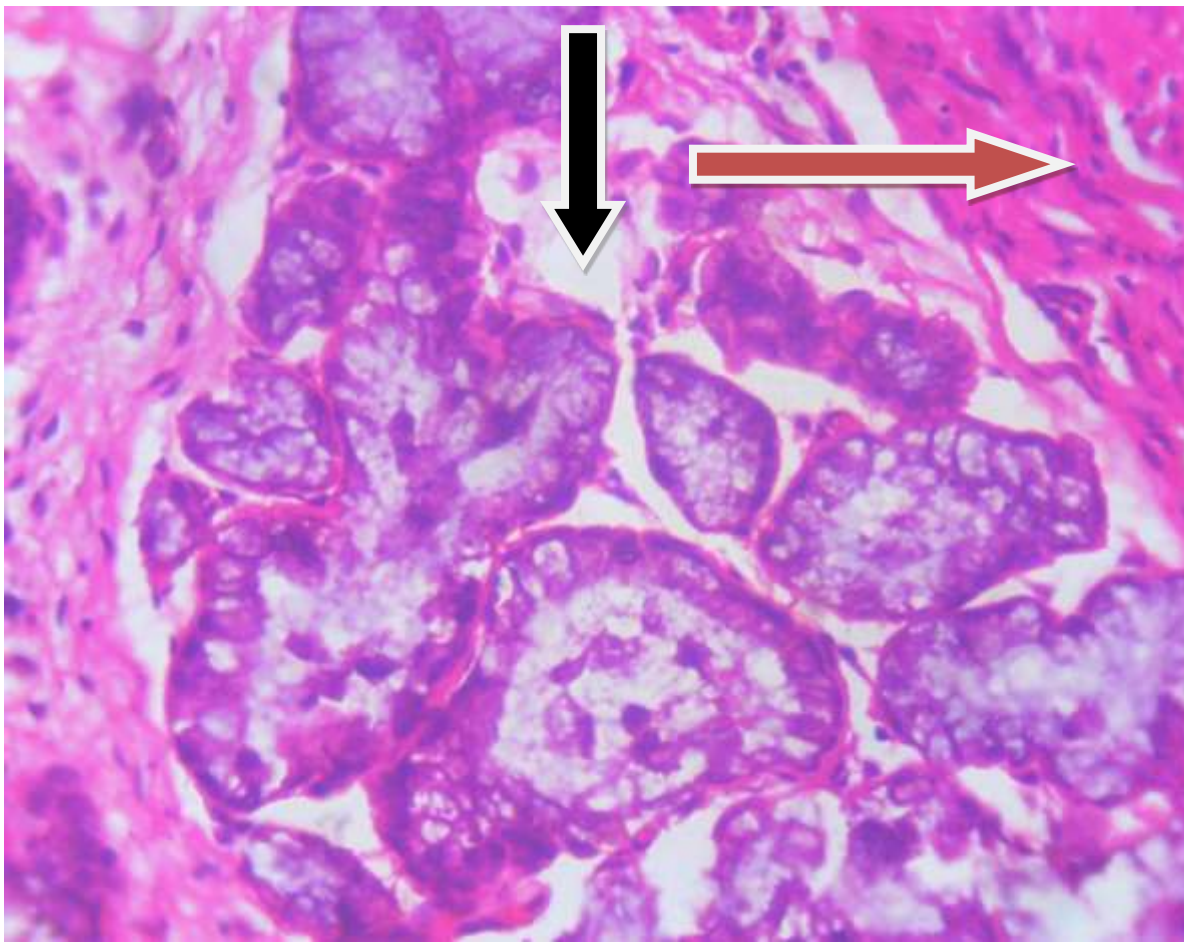

Plate 4.8: Photomicrograph of thyroid gland tissue in group 5 (1000mg/kg) rats showing normal thyroid follicles (black arrow) and follicular epithelial cells (red arrow). (H & E X 100).

CHAPTER FIVE

5.0 DISCUSSION

Medicinal plants have played a key role in the world health care with about 80% of Africans depending on phytomedicine which has shown a wide range of uses in the treatment of diseases, especially priority diseases of Africa such as sickle cell disease, malaria, diabetes and hypertension (Ekeanyanwu, 2011). Herbal products, due to their natural origin, used to be considered as safe for human consumption but however, the potential risks involved with the use of such plants have been reported (Jordan *et al*., 2010). Oduola *et al*., (2016) documented antisckling properties in *Gliricidia sepium* leaf extract.

From the present research, in the oral acute toxicity study it was found that single doses of 2000mg/kg and 5000mg/kg body weight of aqueous leaf extract of *Gliricidia sepium* to Wistar albino rats at 2000 mg/kg and 5000 mg/kg body weight did not cause any behavioral changes or mortality. Therefore, the median lethal dose (LD_{50}) was higher than 5000 mg/kg. Presently, the chemical labeling and classification of acute systemic toxicity based on oral LD_{50} values recommended by the Organization for Economic Co-operation and Development (OECD, Paris, France) (Walum, 1998) are as follows: very toxic, $\leq$ 5mg/kg; toxic, $>$ 5 $\leq$ 50mg/kg; harmful, $>$ 50 $\leq$ 500mg/kg, and no label $>$ 500 $\leq$ 2000mg/kg. According to Globally Harmonized System of Classification and Labeling of Chemicals (GHS) and Office of Pesticide Program (OPP) draft (2004), toxic chemicals are classified and labeled as follow: Category 1 and 2: LD_{50} $>$ 5mg/kg $\leq$ 50mg/kg; fatal if swallowed, Category 3: LD_{50} $>$50mg/kg $\leq$ 300mg/kg; toxic if swallowed, Category 4: LD_{50} $>$ 300mg/kg $\leq$ 2000mg/kg; harmful if swallowed, Category 5: LD_{50} $>$ 2000mg/kg $\leq$ 5000mg/kg; may be harmful if swallowed, LD_{50} $>$ 5000mg/kg; not classified. This means

that the extract is relatively safe, justifying the users claim that the extract is not harmful for human consumption.

Although in oral acute toxicity, T_3 was significantly higher ($p<0.05$) in the test groups when compared with the control group while T_4 and TSH were significantly lower ($p<0.05$) in the test groups when compared with the control group, the significant differences may not be dose response related. Also in sub-chronic toxicity there were no statistically significant differences ($p>0.05$) in T_3 and TSH in the test groups when compared with the control group, while T_4 was significantly higher ($p< 0.05$) at lower dose (250mg) and significantly lower at higher dose (500mg, 750mg and 1000mg) in the test groups when compared with the control groups, the significant differences may not be dose - response related. The differences may not be dose - response related because the results did not follow a definite pattern. Even if it were dose-response related it could not be ascertained what was responsible for the differences because the extract administered was a crude one, until the crude extract fractionated, purified and the bioactive compound(s) characterized and the experiment repeated using the pure compound.

In the oral acute toxicity study, TG, TC, VLDL, LDL-C and HDL-C in the test groups were not statistically significant differences ($p>0.05$) when compared with the control group. In sub-chronic toxicity study, HDL-C was significantly lower ($p< 0.05$) in the test groups when compared with the control group, while TG, TC, VLDL and LDL-C in the test groups were not statistically significant differences ($p>0.05$) when compared with the control group, the significant differences may not be dose response related because they did not follow a definite pattern.

The thyroid gland tissue of the experimental rats in both acute and sub-chronic toxicity

studies revealed normochromic normocytic cells tha tissue section of the thyroid gland from the control and experimental rats stain H&E were essentially normal.

CHAPTER SIX

6.0 CONCLUSIONS AND REOMMENDATIONS

6.1 CONCLUSIONS

1. In conclusion, from the present study it has been shown that the LD_{50} of *Gliricidia sepium* aqueous leaf extract is higher than 5000mg/kg in wistar rats, an indication that it is relatively safe for human consumption.

2. It has also been shown that ingestion of *Gliricidia sepium* aqueous leaf extract has no adverse effect on thyroid hormones and lipid profiles in wistar rats. Hence it may not induce thyroid and lipid disorders in the users.

6.2 RECOMMENDATIONS

1. It is recommended that toxicity studies of G. *sepium* on liver, kidney, cardiac, and bone marrow functions be carried out in wistar rats.

2. If it does not have any adverse effect on these organs, it is recommended that clinical trial should be carried out.

REFERENCES

Abulude, F.O., and Adebote, V.T. (2009). Antibacterial Investigation of Crude Extracts of The root bark of *Gliricidia Sepium*. *Continental Journal of Microbiology*, **3**: 23 – 26.

Ahmed, R. and Sani, A. (2013). Antimycotic activity and toxicological effects of stem bark extract of vitellaria paradoxa in wistar rats. *Science. International*, **25**(**1**): 1013-5316

*Aidoo, M., Terlouw, D.J., Kolczak, M.S., McElroy, P.D., Ter Kuile, F.O., Kariuki, S., Nahlen, B.L., Lal, A.A., and Udhayakumar, V. (2002). Protective effects of the sickle cell gene against malaria morbidity and mortality". Lancet, **359 (9314)**: 1311–1312.*

Aja, P.M., Ibiam, U.A., Nwali, B.U., Orji, O.U., Edwin, N., and Afiukwa, C.A. (2015). Evaluation of Effect of Ethanol Extracts of Moringa oleifera and Cajanus cajan Leaves on Lipid Profiles in Alloxan Induced Diabetic Albino Rats. *Global Journal of Biotechnology* & *Biochemistry*, **10**(**2**): 71-76

Akharaiyi, F.C., Boboye, B., and Adetuyi F.C. (2012). Antibacterial, Phytochemical and Antioxidant Activities of the Leaf Extracts of *Gliricidia sepium* and Spathodea campanulata. *World Applied Sciences Journal*, **16**(**4**): 523-530.

Ali, M.E., Hoda, F., Majid, M.F., Mohammad, M., Mehraneh, D., and Mehdi, A.F. (2014). Effect of Peganum harmala L. on Lipid parameters in hypercholesterolemia-induced male Wistar Rat; *Academia Journal of Medicinal Plants* **2**(**5**): 074-078.

*Andersson, M., and Zimmermann, M.B. (2010). Influence of Iodine Deficiency and Excess on Thyroid Function Tests Endocrine Updates, **28**: 45–69.*

Angelin, B., (1992). Hepatic cholesterol metabolism in estrogen-treated men. Gastroenterolog, **103**(**5**): 1657-1663.

Aniagu, S.O., Nwinyi, F.C., Akumka, D.D., Ajoku, G.A., and Dzarma, D. (2005). Toxicity studies in rats fed nature cure bitters, *African Journal of Biotechnology*, **4:** 72-78

*Anie, K.A., and Green, J. (2012). Psychological therapies for sickle cell disease and pain Cochrane Database of Systematic Reviews, **2***

Asaolu, M.F., Asaolu, S.S., Oyeyemi, A.O., and Aluko, B.T. (2010). Hypolipemic effects of methanolic extract of persea americana seeds in hypercholestrolemic rats; *Journal of Medicine and Medical Sciences*, **1**(**4**): 126-128

Asvold, B.O., Vatten, L.J., Nilsen, T.I., Bjoro, T. (2007). The association between TSH within the reference range and serum lipid concentrations in a population-based study. The HUNT Study. European Journal of Endocrinol, **156**:181–186.

*Awasthy, N., Aggarwal, K.C., Goyal, P.C., Prasad, M.S., Saluja, S., and Sharma, M. (2008). Sickle cell disease: Experience of a tertiary care center in a nonendemic area". Annals of Tropical Medicine and Public Health, **1** (**1**): 1–4*

Azizi, F., Raiszadeh, F., Solati, M., Etemadi, A., Rahmani, M., and Arabi, M. (2003). Serum paraoxonase 1 activity is decreased in thyroid dysfunction. *Journal of Endocrinology Investigation*, **26**: 703-709.

Bahn-Chair, R.S., Burch, H.B., Cooper, D.S., Garber, J.R., Greenlee, M.C., Klein, I., Laurberg, P., McDougall, I.R., Montori, V.M., Rivkees, S.A., Ross, D.S., Sosa, J.A., and Stan, M.N. (2011). Hyperthyroidism and other causes of thyrotoxicosis: management

*guidelines of the American Thyroid Association and American Association of Clinical Endocrinologists Thyroid, **21 (6)**: 593–646*

*Balgir, R.S. (2012). Community expansion and gene geography of sickle cell trait and G6PD deficiency, and natural selection against malaria: experience from tribal land of India". Cardiovascular & Hematological Agents in Medicinal Chemistry, **10(1)**: 3–13.*

Barnes, P.M., Powell-Griner, E., McFann, K., and Nahin, R.L. (2004). Complementary and Alternative Medicine Use among Adults: United States, 2002. *Advance data from vital and health statistics; no 343; National Center for Health Statistics, p. 20*

Beena, J and Joji R.L. (2010). Evaluation Of Antibacterial Activity of The Leaf And Flower Essential Oils of *Gliricidia sepium* From South India. *International Journal of Applied Pharmaceutics*, **2** (**3**): 177-179.

Ben, E.E., Eno1, A.E., Ofem, O.E., Aidem, U., and Itam, E.H. (2006). Increased plasma total cholesterol and high density lipoprotein levels produced by the crude extract from the leaves of viscum album (mistletoe). *Nigerian Journal of Physiological Sciences*, **21** (**1**-**2**): 55-60

Bennison, J.J. and Paterson, R.T. (1993). Use of Trees by Livestock 3: Gliricidia.. *Chatham*, UK

Natural Resources Institute

*Biondi, B.1., and Cooper, D.S. (2008). The clinical significance of subclinical thyroid dysfunction. Endocrine Reviews, **29 (1)**: 76–131.*

Bowen, R. (2010). Mechanism of Action and Physiologic Effects of Thyroid Hormones *Endocrine Index*, p.1

Bradley, P.A. (2012). Depression and Psychosis in Neurological Practice. neurology in clinical practice. (6th ed.). Philadelphia, pp. 102–103.

Calandra, S., Priore, O.C., Tarugi, P., Bertolini, S. (2006). APOA5 and triglyceride metabolism, lesson from human APOA5 deficiency. *Current Opinion Lipidol*, **17**: 122-127.

Canaris, G.J., Manowitz, N.R., Mayor, G., Ridgway, E.C. (2000). The Colorado thyroid disease prevalence study. Arch Intern Med;**160**: 526–534.

Castelli, L. (1984). Epidemiology of Coronary Heart Disease. *American Journal of Medicine*; **76**, 4-12.

Chadhokar, P.A. (2010). *Gliricidia* maculate, Apronising legume forage plant. *World Animal Review*, **44**: 36-43.

*Chakera, A.J., Pearce, S.H., and Vaidya, B. (2012). Treatment for primary hypothyroidism current approaches and future possibilities. Drug Design Development and Therapy (Review), **6**: 1–11.*

Cloudio, O.D. (2007). The role of medicinal plants in the provision of health care. *Journal of Medicinal Plants Research*, **1**(**3**): 50-59

Cook, B.G., Pengelly, B.C., Brown, S.D., Donnelly, J.L., Eagles, D.A., Franco, M.A., Hanson J

Mullen, B.F., Partridge, I.J., Peters, M., and Schultze-Kraft, R. (2005). Tropical forages

CSIRO, DPI&F(Qld), CIAT and ILRI, Brisbane, Australia

Cooper, D.S., and Braverman, L.E. (2012). Werner & Ingbar's the thyroid a fundamental and clinical text (10th ed.). Philadelphia: Wolters Kluwer/Lippincott Williams & Wilkins Health. p. 552

Crunkhorn, S. and Patti, M.E. (2008). Links between thyroid hormone action, oxidative metabolism and diabetes risk Thyroid, **18**: 227–237.

Csurhes, S. and Edwards, G. (1998). Potential environmental weeds in australia; candidate species for preventative control. Queens land department of natural resources, 164-168.

*Devereaux, D., and Tewelde, S.Z. (2014). Hyperthyroidism and thyrotoxicosis. Emergency Medicine Clinical North America, **32 (2)**: 277–92.*

Dietschy, J.M., (1984). Regulation of cholesterol metabolism in man and in other species. *Klinische Wochenschrift*, **62**(**8**):338-345.

Dietschy, J.M., Turley, S.D., and Spady, D.K. (1993). Role of liver in the maintenance of cholesterol and low density lipoprotein homeostasis in different animal species, including humans. *Journal of lipid research*, **34**(**10**): 1637-59.

Dullaart, R.P., Hoogenberg, K., Groener, J.E., Dikkeschei, L.D., Erkelens, D.W., and Doorenbos, H. (1991). The activity of cholesteryl ester transfer protein is decreased in hypothyroidism: a possible contribution to alterations in high-density lipoproteins. *European Journal of Clinical Investigation*, **20**: 581-587.

Duntas, L.H., and Brenta, G. (2012). The effect of thyroid disorders on lipid levels and metabolism. *Medicine Clinical North Am*, **96**: 269-281.

Duntas, L.H., Mantzou, E., and Koutras, D.A. (2002). Circulating levels of oxidized low-density lipoprotein in overt and mild hypothyroidism. *Thyroid*, **12**: 1003-1007.

DRAFT. (2004). Chemical hazard classification and labeling: comparison of OPP requirement

and the GSH; http://www.epa.gov/sites/production/files/2015-09/decuments/ghscriteria

-summary.pdf

Ecocrop database. (2009). Animal feed resources information system. Ecocrop database. FAO

http://ecocrop.fao.org/ecocrop/srv/en/home

Edwards, P.A., Muroya, H., and Gould, R.G. (1972). In vivo demonstration of the circadian thythm of cholesterol biosynthesis in the liver and intestine of the rat. *Journal of lipid research*, **13**(**3**): 396-401.

Ekeanyanwu, C.R., (2011). Traditional medicine in Nigeria: current status and the future, *Research Journal of Pharmacology*, **5**(**6**): 90-94

*Elvin-Lewis, M. (2001). Should we be concerned about herbal remedies. Journal of Ethnopharmacology, **75 (2–3)**: 141–164.*

*Ernst, E. (2007). Herbal medicines: balancing benefits and risks. Novartis Found. Symp. Novartis Foundation Symposia, **282**: 154–167.*

Ernst, E. (2010). Harmless Herbs A Review of the Recent Literature" *The American Journal of Medicine, **104 (2)**: 170–178.*

Evans, M., Roberts, A., Davies, S., and Rees, A. (2004). Medical Lipid Regulating Therapy: Current Evidence, Ongoing Trials and Future Developments. *Drugs*, **64**, 1181-1196.

Fabricant, D.S. and Farnsworth, N.R. (2001). The value of plants used in traditional medicine for drug discovery. *Environ. Health Perspect, **1(1)**: 69–75.*

FAO. (2009). Grassland Index. A searchable catalogue of grass and forage legumes. FAO feedipedia.org/node/552. Last update on May 11, 2015. 14:34

Françoise, M., Corinne, D., Jacques, D.M.D., and Bernard, R. (2915). Chapter 2 Thyroid Hormone Synthesis And Secretion *Endotext*; 1

Friedwald, W.T., Levy, D., and Fredricson, D.S. (1974). Estimation of the concentration of low- Density Lipoprotein Cholesterol in plasma without the use of preparative ultracentrifugation ; *Clinical Chemistry*, **18**(**6**): 499-502.

Fugier, C., Tousaint, J.J., Prieur, X., Plateroti, M., Samarut, J., and Delerive, P. (2006). The lipoprotein lipase inhibitor ANGPTL3 is negatively regulated by thyroid hormone. *Journal Biological Chemimistry*, **281**: 11553-11559.

Galman, C., Bonde, Y., Matasconi, M., Angelin, B., and Rudling, M. (2008). Dramatically increased intestinal absorption of cholesterol following hypophysectomy is normalized by thyroid hormone. *Gastroenterology*, **134**: 1127-1136.

*Garber, J.R., Cobin, R.H., Gharib, H., Hennessey, J.V., Klein, I., Mechanick, J.I., Pessah-Pollack, R., and Singer, P.A. (2012). Clinical Practice Guidelines for Hypothyroidism in Adults Thyroid, **22 (12)**: 1200–1235.*

Gary, H.G. *(2015). What Are the Signs and Symptoms of Sickle Cell Disease?* http://www.nhlbi.nih.gov. *Updated December 7, 2015, retrieve August 2, 2016*

Gary, H.G. *(2015). What Causes Sickle Cell Disease?* http://www.nhlbi.nih.gov. *Updated December 7, 2015, retrieve August 2, 2016*

Gary, H.G. *(2015). What Is Sickle Cell Disease?* http://www.nhlbi.nih.gov. *Updated December 7, 2015, retrieve August 2, 2016*

GBD, (2013). Mortality and Causes, Collaborators "Global, regional, and national age-sex specific all-cause and cause-specific mortality for 240 causes of death, a systematic analysis for the Global Burden of Disease Study". *Lancet, **385**: 117–171*

GBDS (2013). *Prevalence, and years lived with disability for 301 acute and chronic diseases and injuries in 188 countries Global Burden of Disease Study, Collaborators"Global, regional, and national incidence, Lancet, **386 (9995)**: 743–800.*

Gbile, Z.O., and Adesina, S.K. (1986). Nigerian flora and its pharmaceutical potentials. *Journal of Enthopharmacology*; 19:1-6.

*Glassberg, J. (2011). Evidence-based management of sickle cell disease in the emergency department. Emergency Medicine Practice, **13 (8)**: 1–20*

Gotto, A.M.J. (1998). Triglyceride the Forgotten Risk Factor. *Circulation,* **97**, 1027-1028.

Gutsaeva, D.R., Montero-Huerta, P., Parkerson, J.B., Yerigenahally, S.D., Ikuta, T., Head, C.A. (2014): Molecular mechanisms underlying synergistic adhesion of sickle red blood cells by hypoxia and low nitric oxide bioavailability. *Blood*, **123**(**12**): 1917-1926

Harries, A.D., and Cullinan, T. (1994). The danger of traditional eye medicine, *Lancet*; **344**: 1588-1589.

Hashimoto, K., Cohen, R.N., Yamada, M., Markan, K.R., Monden, T., Satoh, T. (2006). Cross- talk between thyroid hormone receptor and liver X receptor regulatory pathways is revealed in a thyroid hormone resistance mouse model. *Journal of Biological Chemistry*, **281**: 295-302.

Hokanson, J.E., and Austen, M.A. (1996). Plasma Triglyceride Level Is a Risk Factor for Cardiovascular Disease Independent of High Density Lipoprotein Cholesterol Levels: A Meta-Analysis of Population Based Prospective Studies. *Journal of Cardiovascular Risk*, **3**: 213.

Hopton, M.R. and Harrap, J.J. (1986). Immunoradiometric assay of thyrotropin as a first line

thyroid function test in the routine laboratory. *Clinical Chemistry*; **32**: 691.

Hsieh, C.J. and Wang, P.W. (2008). Serum concentrations of adiponectin in patients with hyperthyroidism before and after control of thyroid function. Endocrine Journal, **55**: 489– 494.

Hughes, C.E. (1987). Biological considerations in designing a seed collection strategy for *Gliricidia sepium*. *Commonwealth Forestry Review*, **66**; 31-48.

ILDIS. (2002). International Legume Database and Information Service. University of

Southampton, UK. p. 108

Irizarry, H., and Lisandro, E. (2014). Thyroid Hormone Toxicity. *Medscape. WedMD LLC*. Retrieved 2 May 2014

Ito, M., Arishima, T., Kudo, T., Nishihara, E., Ohye, H., and Kubota, S. (2007). Effect of levo- thyroxine replacement on non-high-density lipoprotein cholesterol in hypothyroid patients. *Journal of Clinical Endocrinology Metab*, **92**: 608-611.

Ito, M., Takamatsu, J., Matsuo, T., Kameoka, K., Kubota, S., and Fukata, S., (2003). Serum concentrations of remnant-like particles in hypothyroid patients before and after thyroxine replacement. *Clinical Endocrinology*, **58**: 621-626.

Iyamu, E.W., Turner, E.A., Asakura, T., and Niprisan, B. (2003). improves the survival rates of transgenic sickle cell mice under severe hypoxic conditions. *British Journal of Haematology*, **122**: 1001-1008.

*Jacquart, D. (2008). Islamic Pharmacology in the Middle Ages: Theories and Substances". European Review, **16 (2)**: 219–227.*

James, A. and Duke, J.A. (2000). Returning to our Medicinal Roots. *Journal of mother earth news, 26–33.*

Johannes, W. and Dietrich, A. (2002). Der Hypophysen-Schilddrüsen-Regelkreis. *Berlin, Germany: Logos-Verlag Berlin.* ISBN 978-3-89722-850-4.

Jones, P., and Kubow, S. (1999). Lipids, sterols, and their metabolites. 9th ed. Modern nutrition in health and disease; p. 67-94.

Jordan, S.A., Cunningham, D.G., and Marles, R..J. (2010). Assessment of herbal medicinal products: Challenges, and opportunities to increase the knowledge base for safety assessment. *Toxicology and Applied Pharmacology*, **243(2)**: 198-216.

*Kala, C. and Prakash S. (2007). Revitalizing Indian systems of herbal medicine by the National Medicinal Plants Board through institutional networking and capacity building. Current Science, **93 (6)**: 797–806.*

Kaliyamoorthy, K., Shanmugam, D., Kuppusamy, E., and Larvicidal, O.P. (2012). Activities of Gliricidia sepium [Jacq.] [Leguminosae] against the malarial vector Anopheles stephensi Liston [Culicidae: Diptera] Phytochemistry: Asian Pacific *Journal of Tropical Medicine*, **5**(**8**): 598–604.

Kar, A., Panda, S. and Bharti, S. (2002). Relative efficacy of three medicinal plant extracts in the alteration of thyroid hormone concentrations in male mice, *Journal of Ethnopharmacology*, **8**: 281-285

*Kester, M.H., Martinez, D.E., Mena, R., Obregon, M.J., Marinkovic, D., Howatson, A., Visser, T.J., Hume, R., Morreale, D.E., and Escobar, G. (2004). Iodothyronine levels in the human developing brain: major regulatory roles of iodothyronine deiodinases in different areas. Journal of Clinical Endocrinology Metab, **89 (7)**: 3117–3128.*

*Khatib, R., Rabah, R., Sarnaik, S.A. (2009). The spleen in the sickling disorders: an update. Pediatric Radiology, **39 (1)**: 17–22.*

Kim, C.S., Kang, J.G., Lee, S.J., Ihm, S.H., Yoo, H.J., and Nam, J.S (2009). Relationship of low- density lipoprotein (LDL) particle size to thyroid function status in Koreans. *Journal of Clinical Endocrinology*, **71**: 130-136.

Kim, D.W., Jung, S.L., and Baek, J.H. (2013). The prevalence and features of thyroid pyramidal

lobe, accessory thyroid, and ectopic thyroid as assessed by computed tomography: a multicenter study. *Thyroid*, **23**(**1**): 84-91

Knudsen, N., Laurberg, P., and Rasmussen, L.B. (2005). Small differences in thyroid function may be important for body mass index and the occurrence of obesity in the population. Journal of Clinical Endocrinol Metab, **90**: 4019–4024.

Kola, P.K., Vadite, S.N., Bhuvan C.V., Lavanya, R., Narendra, K.K., Bhagyasree, V., Soumya, B., and Lakshmi, S. (2014). Evaluation of In vitro and In vivo Anti-Inflammatory Activity of Aqueous Extract of Gliricidia sepium Flowers in Rats *International Journal of Pharmacognosy and Phytochemical*, **6**(**3**): 477-481.

Kolawole, O.T., Kolawole, S.O., Ayankunle, A.A., and Olanira, I.O. (2012). Methanol Leaf Extract of Persea Americana Protects Rats against Cholesterol-Induced Hyperlipidemia, *British Journal of Medicine* & *Medical Research*, **3**(**10**): 2225-7217

Korstanje, R., Eriksson, P., Samnegård, A., Olsson, P.G., Forsman-Semb, K., and Sen, S. (2004). Locating Ath8, a locus for murine atherosclerosis susceptibility and testing several of its candidate genes in mice and humans. *Atherosclerosis*, **177**: 443-450.

Kuusi, T., Taskinen, M.R., and Nikkila, E.A. (1988). Lipoproteins, lipolytic enzymes, and hormonal status in hypothyroid women at different levels of substitution. *Journal of Clinical Endocrinol Metab*, **66**: 51-56.

Kung, A.W., Pang, R.W., Lauder, I., Lam, K.S., and Janus, E.D. (1995). Changes in serum lipoprotein(a) and lipids during treatment of hyperthyroidism. Clinical Chemistry, **41**: 226–231.

*Lai, P.K. and Roy, J. (2004). Antimicrobial and chemopreventive properties of herbs and spices. Current Medical Chemistry, **11(11)**: 1451–60.*

Lammel, L.J.A., Angajala, A., Engler, D.A., Webb, P., and Ayers, S.D. (2014). Thyroid hormone induction of human cholesterol 7 alpha-hydroxylase (Cyp7a1) in vitro. *Molecular Cell Endocrinology*, **388**: 32-40.

*Lee, M.R. (2006). Solanaceae III henbane, hags and Hawley Harvey Crippen". Journal R Coll Physicians Edinb **36 (4)**: 366–373.*

Leonard, W. and Nabeel, B. (2013). Hypothyroidism" National Institute of Diabetes and Digestive and Kidney Diseases.

*Lin, S. Yang, S.S., Chau, T. and Halperin, M.L. (2003). An unusual cause of hypokalemic paralysis: chronic licorice ingestion. American Journal of Medical Sciences, **325(3)**: 153–156.*

Lithell, H., Boberg, J., Hellsing, K., Ljunghall, S., Lundgvist, G., and Vessby, B. (1981). Serum lipoprotein and apolipoprotein concentrations and tissue lipoprotein-lipase activity in overt and subclinical hypothyroidism: the effect of substitution therapy. *Europian Journal Clinical Investigation*, **11**: 3-10.

Lopez, D., Abisambra, S.J.F., Bedi, M., and Ness, G.C. (2007). Activation of the hepatic LDL receptor promoter by thyroid hormone. *Biochim Biophys Acta*, **1771**: 1216-1225.

*Lynch, C.R., Folkers, M.E., and Hutson, W.R. (2006). Fulminant hepatic failure associated with the use of black cohosh: a case report. Liver Transplant, **12(6)**: 989–992.*

Makani, J., Cox, S.E., Soka, D., Komba, A.N., amd Oruo, J. (2011). Mortality in sickle cell anemia in Africa: A prospective cohort study in Tanzania. plos one, **6**(**2**): 1-6.

Manwani, D., and Frenette, P.S. (2013). Vaso-occlusion in sickle cell disease: Pathophysiology and novel targeted therapies. *Blood* , **122**(**24**): 3892-3898.

McPherson, R. and Gauthier, A. (2004). Molecular regulation of SREBP function: the Insig-SCAP connection and isoform-specific modulation of lipid synthesis. *Biochemistry and cell biology*, **82**(**1**): 201-211.

Mpiana, P.T., Misakabu, F.S., Tshibangu, D.S.T., Ngbolua, K.N., and Mwanangombo, D.T. (2014). Antisickling activity and membrane stabilizing effect of anthocyanins extracts from Adansonia digitata L. Barks on Sickle Blood Cells. *International Blood Research & Reviews*, **2**(**5**): 198-212

*Müller, J.L. (1998). Love potions and the ointment of witches: historical aspects of the nightshade alkaloids. Journal Toxicology Clinical Toxicology, **36(6)**: 617–627.*

Mullur, R., Liu, Y.Y., and Brent, G.A. (2014). Thyroid hormone regulation of metabolism. *Physiology Review*, **94**: 355-382.

Nasser, K., Ali, M.E., Yaser, S., Hoda, F., and Mehdi, A.F. (2015). Effect of Peganum harmala L. on lipid metabolism andchanges HMGcoA reductase in Hypercholesterolemiainduced male wistar Rat; *The European Proceedings of Social & Behavioural Sciences*, 1330-2357

*National Cholesterol Education Program . (2002). Expert Panel On Detection, "Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) final report". Circulation, **106 (25)**: 3143–3421.*

National Institute of Health. (1992). Institutional Animal Care and Use Committee Guidbook, NIH Publication no. 92-3415. Washington, D.C. U.S. Government Printing Office.

Nazli, R., Akhter, M., Ambreen, S., Solangi, A.H., and Sultana, N., (2008). Insecticidal, Nematicidal and antibacterial activities of *Gliricidia sepium, Pakisthan Journal of. Botony*, **40** (**6**): 2626-2629.

Nazli, R., Sohail, T., Nawab, B., and Yaqeen, Z. (2011). Antimicrobial property of Gliricidia sepium plant extract.*Pakisthan Journal of. agric*. *Resources*, **24**: 1-4.

*Nekvindová, J. and Anzenbacher, P. (2007). Interactions of food and dietary supplements with drug metabolising cytochrome P450 enzymes". Ceska Slov Farm, **56(4)**: 165–173.*

Ness, G.C., and Chambers, C.M. (2000). Feedback and hormonal regulation of hepatic 3-hydroxy-3-methylglutaryl coenzyme A reductase: the concept of cholesterol buffering capacity. *Proc Soc Exp Biological Medicine*, **224**: 8-19.

Ness, G.C., Zhao, Z., and Wiggins, L. (1994). Insulin and glucagon modulate hepatic 3-hydroxy-3-methylglutaryl-coenzyme A reductase activity by affecting immunoreactive protein levels. *The Journal of biological chemistry*, **269**(**46**): 29168-29172.

Nostro, A., Germano, M.P., D'Angelo, V., Marino, A., and Cannatelli, M.A. (2000). Extraction methods and bioautography for evaluation of medicinal plant antimicrobial activity, *Letters in Applied Microbiology*, **30**: 379-384.

Nwaoguikpe, R.N., Ujowundu, C.O., and Okwu, G.N. (2013). The antisickling potentials of four curcubits (T. occidentalis, C. maxima; C. sativus and C. lonatus). *School Journal of App. Medical. Sciences,* **1**(**3**): 191-198.

Nyoka, B.I., Simons, A.J., and Akinnifesi, F.K. (2012). Genotype-environment interaction in *Gliricidia sepium*: phenotypic stability of provenances for leaf biomass yield. *Agriculture, Ecosystems & Environment*; **157**:87-93.

Oduola, T., Dallatu1, M.K., Muhammed, A.O., Ndakotsu, M.A., Adebisi, I.M., and Hassan, S.W. (2016). *Gliricidia sepium* Aqueous Leaf Extract Possesses Antisickling Property *International Blood Research & Reviews*, **5**(**3**): 1-6

Oduola, T., Adeniyi, F.A.A., Ogunyemi, E.O., Bello, I.S., and Idowu, T.O. (2006). Antisickling agent in an extract of unripe pawpaw (*Carica papaya*): Is it real? *African Journal of Biotechnology,* **5**(**20**): 1947-1949.

Oduola, T., Abdulahi, A., Ngaski, Hussein, M., Abdurrasheed, O., Muhammed, Moses O., Akiibinu, and Ayooye S.A. (2016). Thyrotoxic Evaluation and Lipid Peroxidation in Wistar Albino Rats Exposed to *Vitellaria paradoxa* Stem Bark, *British Journal of Pharmaceutical Research*, **10**(**4**): 1-7

OECD (2000). Guidance Document on Acute Oral Toxicity . Environmental Health and Safety Monograph Series on Testing and Assessment No 24.

O'Hara, M., Keifer, D., Farrel, K., and Kemper, K. (2010). A review of 12 commonly used medicinal herbs. *Archives of family medicine*, **7:**523-536

Ohlsen, S., and Rogers, D. (2004). Reducing Hyperlipideamia and CHD. *The Pharmaceutical*

Journal, **273**: 116-118.

Okoli, R.I., Aigbe, O., Ohaju-Obodo, J.O., and Mensah, J.K. (2007). Medicinal Herbs Used for Managing Some Common Ailments among Esan People of Edo State, *Nigeria. Pakistan Journal of Nutrition*, **6**(**5**): 490-496

*Olujohungbe, A., and Burnett, A.L. (2013). How I manage priapism due to sickle cell disease. British Journal of Haematology, **160(6**): 754–65.*

Orchan, G. and Nation, B.(2012). Oxford histopathology, fundamental biomedical science, New York; **55**. 73-74

Pankaj, T. and Anand, K. (2000). Role of Moringa oleifera ln the regulation of thyroid hormones status. *Pharmacological Research*, **41**(**3**): 391-323.

*Pass, K.A., Lane, P.A., Fernhoff, P.M., Hinton, C.F., Panny, S.R., Parks, J.S., Pelias, M.Z., Rhead, W.J., Ross, S.I., Wethers, D.L., and Elsas, L.J. (2000). US newborn screening system guidelines II: follow-up of children, diagnosis, management, and evaluation. Journal Pediatric, **137(37**): 1–46.*

*Paul, R.N., Castro, O.L., Aggarwal, A., and Oneal, P.A. (2011). Acute chest syndrome: sickle cell disease. Europian. Journal of Haematology, **87(3**): 191–207.*

Pauline, N., Cabral, B.N.P., Anatole, P.C., Jocelyne, A.M.V., Bruno, M., and Jeanne, N.Y. (2013). The in vitro antisickling and antioxidant effects of aqueous extracts Zanthoxyllum heitzii on sickle cell disorder. BMC *Complementary and Alternative Medicine*, **13**: 162.

Pearce, EN. (2012). Update in lipid alterations in subclinical hypothyroidism. *Journal Clinical Endocrinology Metabolism*, **97**: 326-333.

Pearson, H.A. (1977). Sickle cell anemia and severe infections due to encapsulated bacteria" *Journal of Infectious Diseases, **136**:25–30.*

Perez, A., Cubero, J.M., Sucunza, N., Ortega, E., Arcelús, R., and Rodriguez- Espinosa, J. (2004). Emerging cardiovascular risk factors in subclinical hypothyroidism: lack of change after restoration of euthyroidism. *Metabolism*, **53**: 1512-1515.

*Persani, L. (2012). Clinical review: Central hypothyroidism: pathogenic, diagnostic, and therapeutic challenges. The Journal of Clinical endocrinology and Metabolism (Review), **97 (9)**: 3068–78.*

Pertchik, B. and Pertchik, H. (1951). Flowering Trees of the Caribbean. Rhinehart & Co., New York; 125 pp.

Philomena, G. (2011). Concerns regarding the safety and toxicity of medicinal plants – An overview. *Journal Applied Pharmaceutical Sciences*, **01**(**6**): 40-44.

*Pinn, G. (2001). Adverse effects associated with herbal medicine. Aust Fam Physician; **30 (11)**: 1070–1075.*

Preedy, A., and Vator, M,. (2009). Comprehensive Handbook of Iodine Nutritional, Biochemical, Pathological and Therapeutic Aspects. Burlington: Elsevier; p. 616.

Prieur, X., Huby, T., Coste, H., Schaap, F.G., Chapman, M.J., and Rodríguez, J.C. (2005). Thyroid hormone regulates the hypotriglyceridemic gene APOA5. *Journal Biological Chemistry*, **280**: 27533-27543.

Rani, B.D. (2007). Ecological Basis of Agroforestry. *CRC Press; p.44*

Rastrelli, L., Berger, I., Kubelka, W., Caceres, A., Tommasi, D.N., and Simone, D.F. (1999). New 12a-Hydroxyrotenoids from Gliricidia sepium bark. *Journal Nat prod*, **62** (**1**): 188- 190

*Rees, D.C., Williams, T.N., and Gladwin, M.T. (2010). Sickle-cell disease. Lancet; **376 (9757)**: 2018–2031.*

Rico-Gray, V., Chemas, A. and Mandujano, S. (1991). Uses of tropical deciduous forest species by the Yucatan Maya. Agroforestry Systems, **14**: 149-161.

Ridgway, N.D., and Dolphin, P.J. (1985). Serum activity and hepatic secretion of lecithin:cholesterol acyltransferase in experimental hypothyroidism and hypercholesterolemia. *Journal Lipid Res*, **26**: 1300-1313.

Roberts, I., Montalembert, M.D. (2007). Sickle cell disease as a paradigm of immigration hematology: new challenges for hematologists in Europe *Haematologica; **92(7)**: 865– 871.*

Sankar N.S. (2013). Phytochemical profiles and antioxidant activities of the leaf extracts of *gliricidia sepium*; *International Journal of Innovations in Biological Sciences*, **3**(**3**): 87- 91

Santamarina, F.S., Gonzalez, N.H., Freeman, L., Wagner, E., and Nong, Z. (2004). Hepatic lipase lipoprotein metabolism, and atherogenesis. Arterioscler Thromb Vasc Biol, **24**: 1750 1754.

Serjeant, G.R. (2010). One hundred years of sickle cell disease. *British journal of haematology, **151(5)**: 425–429.*

Shahnaz, S. Saeed, C., Bijan, A., Mohammad, M., Attari, A.Z., and Majid, R. (2012). The Effect of Alcoholic Extract of *Physalis alkekengi* on Serum Concentration of Thyroid Hormones in Rats *Zahedan Journal of Research In Medical Sciences*, **14**(**5**): 7-11

Sharif, S.H., Elmahdi, B.M., Mohammed, A.M., and Mohammed, A.H. (2012). The effects of *Nigellasativa* L. ethanolic extract on thyroid function in normal and alloxan-induced diabetic rats. *Thyroid Resources Practice,* **9**(**2**): 48-52

Shin, D.J., and Osborne, T.F. (2003). Thyroid hormone regulation and cholesterol metabolism are connected through Sterol Regulatory Element- Binding Protein-2 (SREBP-2). *Journal Biological Chemistry,* **278**: 34114-34118.

*Sidhu, D. and Naugler, C. (2012). Fasting Time and Lipid Levels in a Community-Based Population. Archives of Internal Medicine, **172(22)**: 1–4.*

Simonet, W.S. and Ness, G.C. (1988). Transcriptional and posttranscriptional regulation of rat hepatic 3-hydroxy-3-methylglutaryl-coenzyme A reductase by thyroid hormones. *The Journal of biological chemistry,* **263**(**25**): 12448-12453.

Simons, A.J. (1996). Ecology and reproductive biology. Gliricidia sepium. Genetic Resources

for Farmers. Tropical Forestry Paper 33. Oxford, UK: Oxford Forestry Institute, 19-31

Siperstein, M.D. and Fagan, V.M. (1964). Studies on the feed-back regulation of cholesterol synthesis. *Advances in enzyme regulation,* **2**:249-64.

Siperstein, M.D. and Fagan, V.M. (1966). Feedback control of mevalonate synthesis by dietary cholesterol. *The Journal of biological chemistry,* **241**(**3**): 602-609.

*Slavov, S.N., Kashima, S., Pinto, A.C., and Covas, D.T. (2011). Human parvovirus B19: general considerations and impact on patients with sickle-cell disease and thalassemia and on blood transfusions". FEMS Immunology and Medical Microbiology, **62(3)**: 247–262.*

Sodipo, O., Adebola, A., Inna, F., and Sandabe, U. K. (2012). Total Lipid Profile, Faecal Cholesterol, very Low Density Lipoprotein Cholesterol (VLDL-C), Atherogenic Index (A.I) and Percent Atherosclerosis with Aqueous Fruit Extract of Solanum macrocarpum in Chronic Triton-Induced Hyperlipidemic Albino Rats; *Journal of Biological Sciences*, **4**(**2**): 206-214.

Spolarich, A.E. and Andrews, L. (2007). An examination of the bleeding complications associated with herbal supplements, antiplatelet and anticoagulant medications. *Journal of Dental Hygen, **81(3)**: 67.*

Stephen, N. and Saffron, W. (2001). The thyroid gland in Endocrinology: Published by BIOS

Stewart, J.L., Allison, G.E., and Simons, A.J. (1996). *Gliricidia sepium Genetic resources for farmers*. Oxford Forestry Institute, University of Oxford, UK.

Stewart, J.L., Dunsdon, A.J., Hellin, J.J., and Hughes, C.E. (1992). Wood Biomass Estimation of Central American Dry Zone Species. Tropical Forestry Paper 26, Oxford Forestry Institute, pp 83

Stuttle, J.M. (2015). Gliricidia sepium (Jacq.) *Food and Agriculture Organization of the United Nations.* Retrieved 29 November 2015

Sushmita, C. and Manoranjan, P.S. (2015). Effect of aqueous extract of Murraya koenigii on haematological, hormonal and lipid profile of Albino rats, *Journal of Coastal Life Medicine*, **3**:5-63

Swain and Tony. (1968). Plants in the Development of Modern Medicine. Harvard University Press.

*Syed, S. (2015). Iodine and the "near" eradication of cretinism.". Pediatrics, **135(4)**: 594–6.*

*Talalay, P. and Talalay, P. (2001). The importance of using scientific principles in the development of medicinal agents from plants. Academic Medicine, **76(3)**: 238–47.*

Tan, K.C., Shiu, S.W., and Kung, A.W. (1998). Effect of thyroid dysfunction on high-density lipoprotein subfraction metabolism: roles of hepatic lipase and cholesteryl ester transfer protein. *Journal Clinical Endocrinology Metabolism,* **83**: 2921-2924.

*Tapsell, L.C., Hemphill, I., and Cobiac, L. (2006). Health benefits of herbs and spices: the past, the present, the future". Medical Journal of Aust, **185(4)**: 4–24.*

Tiez, (1994). Textbook of clinical chemistry, 2nd ed. Philadelphia: W.B saunders.

Ting, J., and Xiaochun, T. (2014). Role of Thyroid Hormone in Metabolic Homeostasis. *Journal of Endocrinology, Diabetes & Obesity*, 2333-6692

Trinder, P. (1969). Annals of biochemistry, 6:24. In, Cheesbrough, M. (1992). Medical laboratory manual for tropical countries,ELBS, Cambridge. Vol.**1** (2nd ed.): 527-545.

Tshilanda, D.D., Mpiana, .PT., Onyamboko, D.N.V., Mbala, B.M., and Ngbolua, K.N. (2014). Antisickling activity of butyl stearate isolated from Ocimum basilicum (Lamiaceae). *Asian Pac Journal Tropical Biomed*, **4**(**5**): 393-398.

Tsimihodimos, V., Bairaktari, E., Tzallas, C., Miltiadus, G., Liberopoulos, E., and Elisaf, M. (1999). The incidence of thyroid function abnormalities in patients attending an outpatient lipid clinic. Thyroid, **9**:365–368.

Vickers, A.J. (2007). Which botanicals or other unconventional anticancer agents hould we take to clinical trial?*" Journal of Soc Integr Oncology, **5(3)**: 125–129.*

WAC (2005). Agroforestree database. World Agroforestry Centre: Nairobi, Kenya. http://www.worldagroforestry.org/Sites/TreeDBS/AFT/AFT.htm

Walum, E. (1998). Acute oral toxicity. Environmental Health Perspective, ***106****: 497-503*

Wazida, T., Arunka, R.K., and Sinha, M.P. (2013). Effect of Moringa Oleifera leaf extract on regulation of hypothyroidism and lipid profiles. An international Quarterly, Journal of Life Science, ***8(2****): 665-669*

Weatherall, D.J., and Clegg, J.B. (2001). Inherited haemoglobin disorders: an increasing global health problem". *Bull. World Health Organization,* ***79(8****): 704–712.*

Wellems, T.E., Hayton, K., and Fairhurst, R.M. (2009). The impact of malaria parasitism: from corpuscles to communities". *Journal Clinical Investigation,* ***119(9****): 2496–2505.*

Wong, W.Y., Powars, D.R., Chan, L., Hiti, A., Johnson, C., and Overturf, G. (1992). Polysaccharide encapsulated bacterial infection in sickle cell anaemia: a thirty year epidemiologic experience". American Journal of Hematology, ***39(3****): 176–82.*

World Health Organisation. (2006). Management of birth defects and haemoglobin disorders: Report of a joint WHO-March of Dimes Meeting. Geneva, Switzerland 17-19 May

Zimmermann, M. (1983). Ethical guidelines for investigation of experimental pain in conscious animal. *Pain*, **16:** 109-110

APPENDICES

APPENDIX I

LIST OF EQUIPMENTS:

1. Cotton wool (Penox super absorbent cotton BP, Kano)
2. Syringe (5ml syringe, Innoson Syringe set)
3. Test Tubes (Pyrex England)
4. Deep freezer (Model HS-546, Italy)
5. Micropipette (Scipette, Model CO- 35397, United Kindom)
6. Micro-pipette tips (Thermo-electron)
7. Centrifuge machine (Centrifuge, universal Model 320, Hettich, Germany)
8. Water bath (Digital, Model DK-420, Hettich, Germany)
9. Spectrophotometer (UV/VIS, Beckman Coulter, Model DU-520, Thailand)
10. Semi-auto analyser (Microlab 300 Vital scientific)
11. Hot air oven (Uniscope)
12. Sensitive weighing balance (Santex)

APPENDIX II

REAGENTS AND CHEMICALS

All the reagents used for the study were of analytical grade, all the reagent kits were purchased from Nums Diagnostics. Abuja, Nigeria.