EFFECT OF AQUEOUS *Dennettia tripetala* BAKER F. LEAF EXTRACT ON HYPERTHYROIDISM IN ALBINO RATS (*Rattus norvegicus* BERKENHOUT)

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FIELD OF STUDY: ANIMAL AND ENVIRONMENTAL PHYSIOLOGY

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A PROJECT SUBMITTED IN PARTIAL FULFILMENT FOR THE AWARD OF MASTERS DEGREE IN ANIMAL AND ENVIRONMENTAL PHYSIOLOGY IN THE DEPARTMENT OF ZOOLOGY AND ENVIRONMENTAL BIOLOGY, FACULTY OF BIOLOGICAL SCIENCES, UNIVERSITY OF NIGERIA, NSUKKA

SUPERVISOR: DR. V. C. EJERE

NOVEMBER, 2017

APPROVAL PAGE

I approve that this project was carried out under my supervision by **Iyikite, Chukwuebuka Johnson,** in the Department of Zoology and Environmental Biology, University of Nigeria, Nsukka, in partial fulfilment of the requirements leading to the award of Masters Degree in Animal and Environmental Physiology.

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DEDICATION

This work is dedicated to the Lord Almighty who made everything possible.

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ABSTRACT

The present study was undertaken to investigate the effect of aqueous *Dennettia tripetala* G. Baker leaf extract on hyperthyroidism in albino rat (*Rattus norvegicus*). A total of 72 adult male albino rats were used for the 28 days study. Hyperthyroidism was induced in the rats of groups B – F, orally, at a dose of 600µg/kg Levothyroxine for 14 days and induction of hyperthyroidism was confirmed by analyzing the serum thyroid hormone level. The serum levels of T_3, T_4, and TSH of the normal animal were 3.33pmol/L, 12.82pmol/L and 2.67mU/L respectively. However, in hyperthyroid rats, the serum levels of T_3 and T_4 value increased to 5.22pmol/L and 26.38pmol/L respectively, while the serum levels of TSH value decreased to 0.14mU/L. Only hyperthyroid rats with equivalent (5.22pmol/L and 26.38pmol/L) or higher serum levels of T_3, T_4, and equivalent (0.14mU/L) or lower serum levels of TSH were used. The extract which was obtained by standard method was screened phytochemically. The rats in the normal control (group A) and hyperthyroid control (group B) were given water and food only, while the rats in the standard control (group C) received 1.35mg/kg of Carbimazole by oral intubation. Furthermore, rats in groups D, E and F were administered graded doses (100mg/kg, 250mg/kg and 500mg/kg) of the extract respectively. The body weights of the rats were determined before the commencement of treatment (week 0) and subsequently during the treatment on a weekly (7 days) interval. Blood samples were also obtained from the orbital sinus of each rat during the treatment on a weekly (7 days) interval for the various analyses. Blood sera from the samples were analysed for thyroid hormone levels (Triiodothyronine, T_3; Thyroxine, T_4; and Thyroid Stimulating Hormone, TSH) and lipid profile (Total cholesterol, TCH; High-Density Lipoprotein Cholesterol, HDL-C; Low-Density Lipoprotein Cholesterol, LDL-C; and Total Triglyceride, TG). In addition, histopathological analysis of the thyroid gland was carried out in the control and treated rats. It was observed that the body weights of the rats did not change significantly in group A (normal control group), while that of the other groups showed a progressive significant ($P < 0.05$) duration dependent increase from weeks 0 to 4. The extract dosed groups and the standard control group showed no significance difference ($P > 0.05$) in T_3 and TSH levels when compared to the normal control, while the T4 levels of the group dosed with 500mg/kg extract was statistically similar to the standard control group but varied significantly ($P < 0.05$) when compared to the normal control group. Also, the total cholesterol and triglyceride levels in the extract dosed groups were significantly higher when compared to the normal and hyperthyroid control groups but significantly lower when compared to the standard control group, while the HDL-C levels in the extract dosed groups and standard control group showed no significant difference ($P > 0.05$) when compared to the normal control group. The histopathological examination of the thyroid gland showed no remarkable histologic differences in normal control, standard control and extract dosed groups. Results, therefore, showed that the aqueous *Dennettia tripetala* leaf extract has the potential to overcome hyperthyroidism in albino rat.

CHAPTER ONE

INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction

Hyperthyroidism encompasses a heterogeneous group of disorders characterised by elevated levels of thyroid hormones in the blood (Peter *et al.,* 2005). Thyroid hormones circulate throughout the body in the bloodstream and act on virtually every tissue and cell in the body. Hyperthyroidism causes many of the body's functions to speed up. It elicits a hyperdynamic circulation with increased cardiac output, heart rate, pulse pressure and blood pressure, as well as decreased vascular peripheral resistance. Contrarily, the hypothyroid state is associated with low cardiac output, heart rate, pulse pressure, blood pressure and elevated vascular peripheral resistance (Dorr *et al.*, 2006). Hyperthyroidism is clinically linked with increased metabolic rate which results in adverse effects on many organs and system activities (Golden *et al*., 2009).

Hyperthyroidism is most commonly caused by the development of Graves' disease, an autoimmune disease in which anomalous antibodies stimulate the thyroid to secrete excessive quantities of thyroid hormones (Siegenthaler, 2007). The disease can progress to the formation of a toxic goiter as a result of thyroid growth in response to lack of negative feedback mechanisms. Treatment of hyperthyroidism has largely been by chemotherapy, radiotherapy, and surgery (Bhaigyabati *et al.*, 2012; Norman, 2015). Whatever method of treatment employed had been dependent on the patient's age, allergies to or side effects of the medications, pregnancy, heart disease and the availability of an experienced thyroid surgeon (Welch, 2008).

Recently, there is an increased demand for using plants in therapy instead of using synthetic drugs with the attendant adverse effects that may be more dangerous than the disease itself. According to Bhaigyabati *et al.* (2012), plant materials have been used as medicine for a wide variety of human ailments due to increase in cost of treatment, side effect of several allopathic drugs and development of resistance to currently used drugs for infectious diseases. More than 50 percent of all modern drugs in use are natural products (Govind, 2011). Drug discovery from medicinal plants has played an important role in the treatment of human diseases and indeed, most new clinical applications of plant secondary metabolites and their derivatives over the last half a century have been made towards combating human ailments (Newman *et al*., 2003).

Four herbs are commonly suggested by Western herbalists, other practitioners of complementary and alternative medicine, and naturopathic medical textbooks for treating hyperthyroidism. Three of the herbs namely: lemon balm (*Melissa officinalis*, Lamiaceae), bugleweed (*Lycopus virginicus*, Lamiaceae), and gromwell (*Lithospermum officinale*, Boraginaceae) appear to have effects on thyroid hormone while the remaining one, motherwort (*Leonurus cardiaca*, Lamiaceae) appears to reduce secondary symptoms of hyperthyroidism (heart palpitations and tachycardia) (Mussig *et al*., 2006; Murray and Bongiorno, 2006; Drum, 2007). Some common plant foods contain substances that can prevent the utilization of iodine, and, subsequently, impact thyroid hormone function. They include, most prominently, members of the family Brassicaceae such as cabbage (*Brassica oleracea*), turnips (*B. rapa*), and rutabagas (*B. napobrassica*); soybeans, peanuts, pine nuts, and millet. These have also been reported to interfere with thyroid iodine uptake (Greer and Astwood, 1948). It is noteworthy that these foods must be consumed raw and in large

quantities to have an antithyroid effect, this may be of clinical significance in some rare cases (Murray and Bongiorno, 2006).

Dennettia tripetala (pepper fruit) is an indigenous fruit tree of the family Annonaceae. It is found in the tropical rainforest region of Nigeria and sometimes in Savana areas (Okwu *et al.,* 2005). It is a medium-sized or small tree which spreads throughout the rainforest and sometimes found in the forest within the Savanna areas. *Dennettia tripetala* fruit serves as a mild stimulant to the consumer (Aiyeloja and Bello, 2006; Ndukwu and Nwadibia, 2006; Oyemitan *et al.,* 2006). The fruits and leaves are used as seasonings which are added to prepared food such as meat, soup, sausage and in some special local dishes and vegetables (Ejechi and Akpomedaye, 2005). *Dennettia tripetala* plant yields a good fuel wood. Its various parts are commonly used as spices and condiment (Oyemitan *et al.*, 2008). The fruit is sold for money especially by rural women. *Dennettia tripetala* is used in traditional medicine (Egharevba and Idah, 2015). The leaves are commonly used by the local herbalists in combination with other medicinal plants to treat various ailments including fever, infantile convulsion, typhoid, cough, worm infestation, vomiting, and stomach upset (Oyemitan, 2006). Similarly, the fruits have been reported to be used for masking mouth odour (Oyemitan, 2006), as well as stimulants (Aiyeloja and Bello, 2006; Ndukwu and Nwadibia, 2006; Oyemitan *et al*., 2006).

1.1.1 Justification for the study

Dennettia tripetala fruit is widely consumed by the inhabitants of West Africa due to its distinctive spicy taste. The leaves are also used in preparing pepper soup delicacies and as condiment in some local dishes for pregnant and postnatal women during which time, the spices and herbs aid in uterine contraction (Achinewhu *et al.,* 1995; Okwu and Morah, 2004;

Adedayo *et al.,* 2010). The highly nutritious fruit is rich in protein, carbohydrates, as well as the antioxidants, vitamins A, C, and E. Elemental analyses showed that *Dennettia tripetala* fruit contains mainly calcium and iron, with traces of magnesium, zinc, manganese, and copper (Egharevba and Idah, 2015).

Medicinally, *Dennettia tripetala* fruit is used for cough treatment and enhancement of appetite (Adedire and Akinkurolere, 2005; Egharevba and Idah, 2015). According to Iseghohi (2015), *Dennettia tripetala* fruit is also used traditionally as a remedy for fever, toothache, diabetes, and nausea. The leaves and roots are commonly used by herbalists in folklore medicine in combination with other medicinal plants to treat various ailment including fever, infantile convulsion, typhoid, worm infestation, vomiting and stomach upset (Ejechi and Akpomedaye, 2005; Oyemitan *et al.,* 2008; Ukeh *et al.,* 2012). *Dennettia tripetala* seeds had been scientifically proven to significantly lower intra ocular pressure in individuals with glaucoma by up to 25% (Timothy and Okere, 2008). Despite the aforementioned medicinal values, there is a dearth of literature on effects of the leaf extract on the endocrine glands and secretions. This informed the present research effort on the effects of the aqueous *D. Tripetala* (G. baker) leaf extract on hyperthyroid rats.

1.1.2 Objectives of the study

The objectives of the study were to:

1. determine the effect of aqueous *D. tripetala* leaf extract on the body weights of hyperthyroid albino rats,
2. evaluate the effect of aqueous *D. tripetala* leaf extract on the thyroid hormone serum levels of hyperthyroid albino rats.

3. determine the effect of aqueous *D. tripetala* leaf extract on the lipid profile of hyperthyroid albino rats.
4. evaluate the effect of aqueous *D. tripetala* leaf extract on the histopathology of thyroid glands of the albino rats.

1.2 Literature Review

1.2.1 *Dennettia tripetala*

Dennettia tripetala (Annonaceae) is a well-known forest fruit and spicy indigenous medicinal plant. It is a plant found mostly in tropical rainforest region and sometimes in the Savannah area (Okwu *et al*, 2005; Okoronkwo *et al.*, 2015). It is widely domesticated in the Southern, Eastern and Western part of Nigeria (Timothy and Okere, 2008). *Dennettia tripetala* is commonly known as pepper fruit in English Language. It is locally called "*Nkarika"* by the Efiks of Carlaba (Ikpi and Nku, 2008), "*Mmimi*" by the Igbos, "*Ata Igbere"* by the Yorubas (Adedayo *et al*., 2010), "*Ako"* by the Edos and "*Opipi"* by the Idomas (Egharevba and Idah, 2015).

Dennettia tripetala tree is medium in size and belongs to the Annonaceae family (Mabberley, 1997). It is a woody plant of at least 3m height with simple leaves and abundant fruits widely consumed in Southern Nigeria (Adedayo *et al*., 2010). The fruit appears red when ripe and green in an unripe form with a pungent spicy taste (see plate 1). The leaf is alternate, oblong cociaceous, apex acuminate with a base cumate margin. It exhibits a symposia growth with a chasmogamous flower. The sepals are green, very broad pubescent and are three in numbers. The petals are fleshy light yellow apocarpous fruit (Brumitt, 1992; Folorunso and Olorude, 2008).

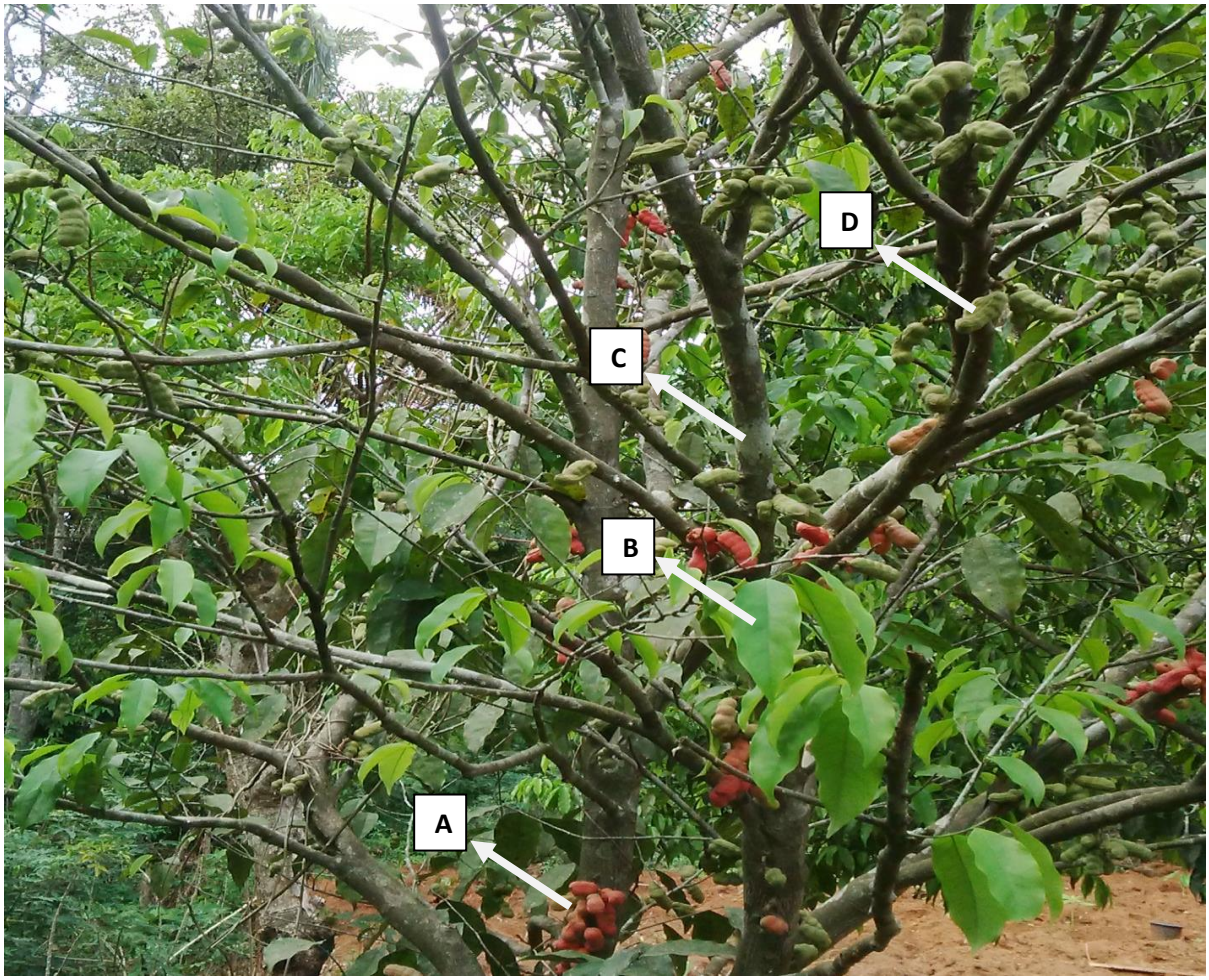

Plate 1: *Dennettia tripetala* plant showing A (ripped fruit), B (the leaves), C (the stem) and D (unripped fruit)

The plant possesses phytochemicals that have been shown to elicit antimicrobial, insecticidal, analgesic, and anti-inflammatory properties. The leaf and stem bark oils had been demonstrated to possess antimicrobial activity against *Staphylococcus aureus* (Osisiogu, 1975). The essential oil from the fruit has been reported to inhibit the growth of tomato-rot fungi (Ejechi *et al*., 1999). The fruit extract had been shown to possess strong antifungal activities against *Saccharomyces cerevisiae*, *Candida tropicalis*, *Cryptococcus* sp., *Geotrichum* sp., *Rhizopus stolonifer*, *Aspergillus* and *Fusarium* sp. (Ejechi *et al.,* 1999). It can also serve as an insecticide against 3rd instars larvae of *Aedes aegypti* mosquito and bioinsecticides for the control of rice weevil, *Sitophilus zeamais*. The ethanolic extract has been used traditionally in Nigeria to combat the growth of *Ostrinia nubiliries* that affects significantly corn, cotton as well as other vegetable crops (Ewete *et al*., 1996). The essential oil of *D. tripetala* has been reported to inhibit the growth of tomato-rot fungi (Ejechi *et al.,* 1999). Onyechi *et al*. (2013) have made use of the fruits in the formulation of herbal tablets by direct compression. Oyemitan *et al*. (2008) reported the antinociceptive and anti-inflammatory activities of the essential oil of the fruit in mice. In addition, D. *tripetala* finds application in food preservation and seasoning.

1.2.2 Classification of *Dennettia tripetala*

Dennettia tripetala is classified as follows: Kingdom: Plantae; Phylum: Agnoliophyta; Class: Magnolidae; Order: Magnoliales; Family: Annonaceae; Genus: *Dennettia*; Species: *Dennettia tripetala*.

1.2.3 Uses of *Dennettia tripetala*

1.2.3.1 Nutritional values

The parts of the plant used include the leaves, fruits, seeds, roots and stem bark (Ejechi, *et al.*, 1999). The mature fruits constitute the main edible portions (Ikpi and Nku, 2008). The fruits are mainly chewed raw in different forms (fresh green, fresh ripened brown, black dry fruits, and dry seeds). Researchers have shown that the fruit of *Dennettia tripetala* when dry mainly consists of carbohydrates (Okwu and Morah, 2004; Ihemeje *et al.*, 2013). *Dennettia tripetala* also contains protein, fiber, ash, lipids, moisture, trace elements, minerals, and water-soluble vitamins (Iseghohi, 2015). Okwu *et al* (2005) also reported that *D. tripetala* fruits contain important nutritive substances such as vitamins, minerals, and fibre.

The fruits as well as other parts of the plant possess strong pepperish and pungent taste. The peppery fruits of *D. tripetala* are applied to the food meant for pregnant women and are important in the diets of postpartum women, during which time it is claimed that spices and herbs aid uterine contraction (Okwu and Morah, 2004; Achinewhu *et al*., 1995). They are popularly used as spices and condiments in flavouring food (Oyemitan *et al.*, 2008), and as a seasoning which is added to prepared food such as meat, sausages, soups, and vegetable (Lebouef and Caver, 1972). The leaves are commonly used in pepper soup delicacies, and as a condiment in some special local dishes (Ejechi and Akpomedaye, 2005). The young leaves have a spicy taste and are sometimes used to prepare pepper soup.

1.2.3.2 Medicinal values

Some communities in parts of Southern Nigeria also utilized the leaves and roots, in addition to the fruits for medicinal purpose (Iwu, 1989). The leaves and seeds have been found to be useful in the treatment of cough, fever, toothache, diabetes and are also used to

enhance appetite, clear throats, check excess saliva, relieve coated tongues and stop nausea (Okoronkwo *et al.,* 2015). The leaves and roots are commonly used by local herbalists in folklore medicine in combination with other medicinal plants to treat various ailments including fever, infantile convulsion, typhoid, worm infestation, vomiting and stomach upset (Ejechi and Akpomedaye, 2005; Oyemitan *et al.,* 2008; Ukeh *et al.,* 2012). The leaves and fruits are used with other herbs for the treatment of cough, infantile convulsion, vomiting, worm infestation and typhoid (Ejechi and Akpomedaye, 2005; Ukeh *et al*., 2012). *Dennettia tripetala* is used as masticators, which when chewed produces a unique peppery effect (Keay, 1989). The peppery spicy taste of mature *D. tripetala* fruits usually serves as a mild stimulant to the consumer. The plant has also been shown to possess chemotherapeutic, antihyperglycemic, and antioxidant properties (Iseghohi, 2015).

1.2.3.3 Socio-economic values

The plant usually produces fruit between the months of March and May. For this reason, local traders preserve the seeds of pepper fruit by drying it under the sun in order to ensure continuous availability until the next harvest (Iseghohi, 2015). The fruits are sometimes taken with kola nuts, garden egg and palm wine, especially in the Southern part of Nigeria where it serves also for cultural entertainment of guests, particularly during coronation, new yam festivals, weddings and marriage festivals (Enwere, 1998; Keay, 1989). The wood is used as fuel. Also, the plant possesses phytochemicals that have been shown to elicit antimicrobial, insecticidal, analgesic, and anti-inflammatory properties (Iseghohi, 2015).

1.2.4 Biochemical composition of *Dennettia tripetala*

The essential oil of *Dennettia tripetala* seeds obtained using the simultaneous distillation extraction technique has been analysed using gas chromatography-mass spectrometry technique (Iseghohi, 2015). According to Elekwa *et al*. (2011), twenty-five compounds were identified in the n-hexane seed extract, including linoleic acid, ethyl ester, caryophyllene, 3-carene, phenyl ethyl alcohol, and cubebene. Phytochemical screening of *D tripetala* ethanolic seed extract revealed the presence of tannins, alkaloids, steroids, flavonoids, cardiac glycosides, saponins, and terpenoids (Elekwa *et al*., 2011). These constituents provide a scientific basis for the use of *Dennettia tripetala* in traditional medicine. Saponins, tannins, and flavonoids, for instance, are effective against diabetes (Iseghohi, 2015). *Dennettia tripetala* also contains carbohydrates, protein, fiber, ash, lipids, moisture and traces of elements which include minerals, and water-soluble vitamins (Ihemeje *et al.*, 2013). According to Ihemeje *et al*. (2013), the phytochemicals and water-soluble vitamin contents tend to increase significantly with ripening.

The ethanolic seed extract also possesses antimicrobial and anti-inflammatory properties (Reihemann *et al.*, 1999; Sparg *et al.,* 2004). Cardiac glycosides can be used in the treatment of asthma (Trease and Evans, 1989). Alpha-linoleic acid has been shown to reduce the risk of cardiovascular disease (William, 2000) as well as prostate cancer in men (Koralek *et al.*, 2006). The presence of these bioactive constituents in *Dennettia tripetala*, no doubts potentiate the plant for the treatment of these diseases.

1.2.5 Anti-microbial properties of *Dennettia tripetala*

The essential oil and phenolic acid extracts of *Dennettia tripetala* seeds have been reported to inhibit the growth of food-borne microorganisms such as *Staphylococcus aureus*,

Salmonella sp., *Escherichia coli*, and a host of others (Ejechi and Akpomedaye, 2005). This potentiality, therefore, makes pepper fruit a prime agent of preservation of food substances such as meat which is prone to rapid decomposition in places without constant electricity. More recently, the leaves of *Dennettia tripetala* were found to be effective in inhibiting the growth of the cocoyam rot-causing fungus, *Sclerotium rolfsii* in both in-vitro and in-vivo situation (Nwachukwu and Osuji, 2008).

1.2.6 Analgesic and anti-inflammatory effects of *Dennettia tripetala*

The essential oil of *Dennettia tripetala* fruits has been found to possess analgesic effects as great as that induced by the powerful opioid, morphine as well as aspirin and indomethacin (Iseghohi, 2015). This oil also relieved inflammation in rodents with edema to levels comparable with that of dexamethasone (Oyemitan *et al.*, 2008). The mechanism by which *Dennettia tripetala* exhibits its analgesic effects was inferred by the fact that Naloxone, which inhibits the analgesic effect of morphine, was also able to inhibit that of *Dennettia tripetala*. This result backs up the use of *Dennettia tripetala* in pain and fever in folk medicine (Iseghohi, 2015). In addition, the seeds of *Dennettia tripetala* have been found to be effective in reducing intraocular pressure in normotensive emmetropic humans (Timothy and Okere, 2008). This suggests that *Dennettia tripetala* could be put to use in the possible prevention and management of glaucoma (Iseghohi, 2015).

1.2.7 Anti-hyperglycemic effect of *Dennettia tripetala*

Recent researches have provided evidence and a preliminary mechanism for the antihyperglycemic effect of ethyl acetate extract of *Dennettia tripetala*. Anaga and Asuzu (2010) showed that *Dennettia tripetala* fruit extract can reduce the plasma glucose level in drug-induced hyperglycemic rats to levels comparable with that of normal rats. This effect

was found to be more pronounced than that of Tolbutamide. Using 3T3-L1 adipocytes and brefeldin, these same researchers investigated the possible mechanism for this observed phenomenon in *Dennettia tripetala*. It was found that *Dennettia tripetala* exerts this effect partly by recruiting glucose uptake proteins from the interior of the cell to the plasma membrane (Anaga and Asuzu, 2011).

1.2.8 Anti-oxidant effect of *Dennettia tripetala*

In living organisms, reactive oxygen species (ROS) are generated as a part of metabolism. These ROS are usually hindered from causing oxidative damage to cellular constituents by antioxidants present in the organism. Some of these antioxidants are produced in the body in the form of antioxidant enzymes, while others have to be consumed from plants in the form of antioxidant nutrients (Oboh and Akindahunsi, 2004). Preliminary phytochemical analysis has revealed the presence of antioxidants such as flavonoids and ascorbic acid in *Dennettia tripetala* (Ihemeje *et al.*, 2013).

Adedayo *et al.* (2010) found that the phenol content of *Dennettia tripetala* fruit increases with ripening, while the ascorbic acid and flavonoid content do not change. According to them, the aqueous extract of unripe *Dennettia tripetala* fruit possesses greater antioxidant ability compared to ripe fruit. This was typified by higher reducing power, greater ability to scavenge 2, 2′-azinobis (3-ethylbenzothiaziline-6-sulfonate) (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), and hydroxide (OH), as well as higher Fe reducing and chelating potential. They, therefore, concluded that as *Dennettia tripetala* fruit undergoes ripening, its total phenol content increases, but its antioxidant potentials decrease. Aderogba *et al.* (2011) isolated two flavonoid glycosides from the ethyl acetate fraction of a 20% aqueous methanol

extract of *Dennettia tripetala* leaves which were found to instantly bleach the purple colour of DPPH, indicating free radical scavenging potential

Okolie *et al.* (2014) discovered that the ethanolic extract of *Dennettia tripetala* roots exhibits the ability to reduce ferric ion in a concentration-dependent manner. The extract also possesses a H_2O_2-scavenging ability similar to that of ascorbic acid. Furthermore, the extract inhibits lipid peroxidation in frozen animal samples to an extent comparable with that of vitamins C and E (Okolie *et al.*, 2014). They, therefore, concluded that *Dennettia tripetala* may be useful in the preservation of frozen meat. Other in-vitro antioxidant studies have been done using the methanol extract of *Dennettia tripetala* leaves and results show that *Dennettia tripetala* possesses strong antioxidant potentials (Odoh *et al.*, 2014).

1.2.9 Effect of *Dennettia tripetala* on cancer

Dennettia tripetala extract has also been reported to inhibit the growth of prostate cancer cells. Jagla (2013) tested the efficacy of ethanolic extract of *Dennettia tripetala* seeds on prostate cancer cell lines PC3 and LNCaP. The extract of *Dennettia tripetala* fruit was found to possess growth-inhibitory and cytotoxic effects on the prostate cancer cell lines in-vitro (Jagla, 2013).

1.2.10 Acute toxicity of *Dennettia tripetala*

Earlier reports showed that the median lethal dose (LD_{50}) values of the ethanol extract of *Dennettia tripetala* seeds were estimated to be above 5000mg/kg. Eighteen (18) albino mice (both sexes) were used, and at all the doses of ethanol extract of *D. tripetala* seed administered orally to the mice for acute toxicity study, there were neither clinical signs nor mortality after 24 hours post-treatment observation (Anosike *et al.*, 2016). According to Oyemitan (2006), the estimated LD_{50} values of the essential oil following oral (per os) and

intraperitoneal (i.p.) routes in rats were 1,265 mg/kg (p.o.) and 775 mg/kg (i.p.), while the values in mice were 2,150 mg/kg (p.o.) and 470 mg/kg (i.p.) respectively. The reported mechanism(s) of the behavioural effects (novelty-induced and exploratory behaviours) of the essential oils of the plant has been linked to opioidergic and GABA-ergic pathways (Oyemitan, 2006).

1.2.11 Thyroid gland

The thyroid gland is one of the largest endocrine glands in the body and consists of two connected lobes. It is a butterfly-shaped organ located just below Adam's apple in the neck (Videla *et al.*, 2007). The thyroid gland is specialized for production, storage and release of the thyroid hormones thyroxine (T_4) and triiodothyronine (T_3). They are major regulators of basal metabolic rate and energy expenditure in homeothermic animals, and they are required for normal cell growth and development. Apart from general metabolic disturbance, an impairment of thyroid hormone production causes serious intellectual and behavioural abnormalities that may affect the patient's daily functioning and result in additional stress and depression (Longo *et al*., 2012). Therefore, the research on the thyroid gland has not only significant medical but also social implications.

1.2.11.1 Histology of thyroid gland

At the microscopic level, there are three primary features of the thyroid gland namely: follicles, follicular cells and parafollicular cells (Fawcett and Jensh, 2002). The thyroid gland is composed of spherical follicles that selectively absorb iodine (as iodide ions, I^-) from the blood for production of thyroid hormones, and also for storage of iodine in thyroglobulin (Fawcett and Jensh, 2002). Twenty-five percent of the body's iodide ions are in the thyroid gland (Brady, 2014). Inside the follicles, in a region called the follicular lumen, colloid serves

as a reservoir of materials for thyroid hormone production and, to a lesser extent, acts as a reservoir for the hormones themselves (Fawcett and Jensh, 2002). A colloid is rich in a protein called thyroglobulin. The follicles are surrounded by a single layer of follicular cells, which secrete T_3 and T_4. When the gland is not secreting T_3 and T_4 (inactive), the epithelial cells range from low columnar to cuboidal cells. When active, the epithelial cells become tall columnar cells (Fawcett and Jensh, 2002). Scattered among follicular cells and in spaces between the spherical follicles are another type of thyroid cell, parafollicular cells (also called C cells), which secrete calcitonin (Fawcett and Jensh, 2002).

1.2.11.2 Physiology of thyroid

The primary function of the thyroid is the production of the hormones T_3, T_4, and calcitonin. The surface-layer cells (epithelium) of the thyroid's follicles perform the processes of synthesis and release of thyroid hormones (Welch, 2008). Once released into the blood plasma, T_4 and T_3 bind reversibly to plasma proteins. Most circulating thyroid hormones are protein bound, yet only the free (unbound) fraction is available to tissues, whose cells actively take up the hormone molecules (Farwell and Braverman, 2005). Thyroid hormones bind to a specific receptor located in the nucleus of most cells. Activation of this receptor affects many cellular functions, primarily cell growth and metabolism by direct influence on gene transcription and subsequent protein synthesis, or by direct effects on the cell or on mitochondria through stimulation of cell growth and respiration (Welch, 2008).

Up to 80% of the T_4 is converted to T_3 by organs such as the liver, kidney, and spleen. T_3 is several times more powerful than T_4, which is largely a prohormone, perhaps four or even ten times more active (Nussey and Whitehead, 2001). The thyroid also contains another type of cell: parafollicular cells, known as "C cells," which synthesize and release the

hormone calcitonin (Welch, 2008). Calcitonin lowers plasma calcium levels (Schteingart, 2003).

1.2.11.3 Thyroid hormones production and action

The thyroid gland is specialized for production, storage and release of the thyroid hormones, thyroxine (T_4) and triiodothyronine (T_3). Thyroxine (T_4) is synthesised by the follicular cells from the tyrosine residues of the protein called thyroglobulin (Tg) (Boron and Boulpaep, 2003). It has 123 tyrosine residues, but only 4-6 are active (Ekholm and Bjorkman, 1997). Iodine is captured with the "iodine trap" by the hydrogen peroxide generated by the enzyme, thyroid peroxidase (TPO) (Ekholm and Bjorkman, 1997). This is linked to the 3' and 5' sites of the benzene ring of the tyrosine residues on Tg sequentially on tyrosine residue forming monoiodotyrosine (MIT) and then diiodotyrosine (DIT) by iodination. Two diiodotyrosine can couple to form T_4 hormone attached to thyroglobulin releasing one alanine. Upon stimulation by the thyroid-stimulating hormone (TSH), the follicular cells reabsorb Tg and cleave the iodinated tyrosines from Tg in lysosomes, forming free T_4, DIT, MIT, T_3 and traces of reverse triiodothyronine (RT_3). Deiodinase releases the sequestered iodine from MIT and DIT. Deiodinase enzymes convert T_4 to T_3 and RT_3, which is a major source of both RT_3 (95%) and T_3 (87%) in peripheral tissues (Bianco *et al.*, 2002).

1.2.11.4 T_3 and T_4 regulation

The production of thyroxine and triiodothyronine is primarily regulated by thyroid-stimulating hormone (TSH), released by the anterior pituitary. To ensure stable levels of thyroid hormones, the hypothalamus monitors circulating thyroid hormone levels and responds to low levels by releasing thyrotropin-releasing hormone (TRH). This TRH then stimulates the pituitary to release thyroid stimulating hormone (TSH) (Segerson, *et al*., 1987;

Dyess, *et al*., 1988). When thyroid hormone levels increase, production of TSH decreases, which in turn slows the release of new hormone from the thyroid gland. The negative feedback occurs on both the hypothalamus and the pituitary, but it is of particular importance at the level of the pituitary (Johannes, 2002). Cold temperatures can also increase TRH levels. This is thought to be an intrinsic mechanism that helps keep us warm in cold weather (Arancibia *et al*., 1996). Elevated levels of cortisol, as seen during stress and in conditions such as Cushing's syndrome, lower TRH, TSH and thyroid hormone levels as well (Tsigos and Chrousos, 2002; Roelfsema *et al*., 2009). 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 (Johannes, 2002).

The parafollicular cells of the thyroid gland produce calcitonin in response to hypercalcemia. This hormone regulates calcium levels in the blood (Johannes, 2002). Calcitonin stimulates the movement of calcium into bone, in opposition to the effects of parathyroid hormone (PTH). However, it does appear that calcitonin seems far less essential than PTH. This is more so as calcium metabolism remains clinically normal after removal of the thyroid gland (thyroidectomy), but not the parathyroids (Johannes, 2002).

1.2.11.5 Functions of thyroid hormones

The thyroid hormones act on nearly every cell in the body. They act to increase the basal metabolic rate, affect protein synthesis, help regulate long bone growth (synergy with growth hormone) and neural maturation, and increase the body's sensitivity to catecholamines (such as adrenaline) by permissiveness. The thyroid hormones are essential for proper development and differentiation of all cells of the human body. These hormones also regulate

protein, fat, and carbohydrate metabolism, affecting how human cells use energetic compounds. They also stimulate vitamin metabolism. Numerous physiological and pathological stimuli influence thyroid hormone synthesis.

Thyroid hormone leads to heat generation in humans. However, the thyronamines function through some unknown mechanism to inhibit neuronal activity; this plays an important role in the hibernation cycles of mammals and the moulting behaviour of birds. One effect of administering the thyronamines is a severe drop in body temperature.

1.2.12 Hyperthyroidism

The thyroid gland is prone to several distinct diseases, some of which are extremely common. One of these diseases is hyperthyroidism. The term hyperthyroidism refers to any condition in which there is too much thyroid hormone produced in the body (American Thyroid Association, 2014). In other words, the thyroid gland is overactive. Hyperthyroidism is sometimes called thyrotoxicosis, which refers to high thyroid hormone levels in the bloodstream, irrespective of their source. Hyperthyroidism causes many of the body's functions to speed up. It shows a hyperdynamic circulation with increased cardiac output, heart rate, pulse pressure and blood pressure and decreased vascular peripheral resistance whereas the hypothyroid state is associated with the low cardiac output, heart rate, pulse pressure and blood pressure and elevated vascular peripheral resistance (Dorr, *et al.*, 2006). Hyperthyroidism is clinically linked with an increased metabolic rate which results in adverse effects on many organs and system activities (Golden *et al*., 2009). Chemotherapy, radiotherapy, and surgery are only three existing modes of treatment in modern medicine (Bhaigyabati *et al.*, 2012; Norman, 2015; Peterson, 2015).

1.2.12.1 Causes of hyperthyroidism

There are various causes of hyperthyroidism. Graves' disease, also known as toxic diffuse goiter, is the most common cause of hyperthyroidism. This type of hyperthyroidism tends to run in families and it occurs more often in young women (American Thyroid Association, 2014). According to Reid and Wheeler (2005) and American Thyroid Association (2014), in more than 70% of cases, hyperthyroidism is caused by an autoimmune disorder called Graves' disease. Normally, the immune system protects the body from infection by identifying and destroying bacteria, viruses, and other potentially harmful foreign substances. But in autoimmune diseases, the immune system attacks the body's own cells and organs. With Graves' disease, the immune system makes an antibody called thyroid stimulating immunoglobulin (TSI) that attaches to thyroid cells. TSI mimics the action of TSH and stimulates the thyroid to make too much thyroid hormone. Little is known about the causes of this disease in specific individuals (American Thyroid Association, 2014).

Other causes of hyperthyroidism include: thyroid nodules, thyroiditis, or inflammation of the thyroid, consuming too much iodine and overmedicating with synthetic thyroid hormone, which is used to treat an underactive thyroid. Thyroid nodules, also called adenomas, are lumps in the thyroid. Thyroid nodules are common and usually noncancerous. They may become overactive and produce too much hormone. A single overactive nodule is called a toxic adenoma. Multiple overactive nodules are called toxic multinodular goiter. Often found in older adults, toxic multinodular goiter can produce a large amount of excess thyroid hormone (Gharib *et al*., 2010). Also, people may temporarily have symptoms of hyperthyroidism if they have a condition called thyroiditis. This condition is caused by a problem with the immune system or a viral infection that causes the gland to leak stored thyroid hormone (NIDDK, 2012). The same symptoms can also be caused by taking too

much thyroid hormone in tablet form. These last two forms of excess thyroid hormone are only called thyrotoxicosis since the thyroid is not overactive (NIDDK, 2012).

1.2.12.2 Symptoms of hyperthyroidism

Thyroid hormone plays a significant role in the pace of metabolism in the body (American Thyroid Association, 2014). If there is too much thyroid hormone, every function of the body tends to speed up. The symptoms of hyperthyroidism are nervousness, irritability, increased perspiration, heart racing, hand tremors, anxiety, difficulty in sleeping, thinning of the skin, goiter, fine brittle hair and weakness in the muscles - especially in the upper arms and thighs (American Thyroid Association, 2014). Since hyperthyroidism increases metabolism, many individuals initially have a lot of energy. However, as the hyperthyroidism continues, the body tends to break down, so being tired is very common (American Thyroid Association, 2014). Also, there may be more frequent bowel movements, but diarrhea is uncommon. The person may lose weight despite a good appetite and, for women, menstrual flow may lighten and menstrual periods may occur less often (American Thyroid Association, 2014).

1.2.12.3 Diagnosis of hyperthyroidism

Hyperthyroidism is usually detected by an enlarged thyroid gland and a rapid pulse. Other indicators include moist, smooth skin and a tremor of the fingertips. The diagnosis of hyperthyroidism is however usually confirmed by laboratory tests that measure the amount of thyroid hormones - thyroxine (T_4) and triiodothyronine (T_3) - and thyroid-stimulating hormone (TSH) in the blood (American Thyroid Association, 2014). A high level of thyroid hormone in the blood coupled with a low level of TSH is common with an overactive thyroid gland. If blood tests show that the thyroid is overactive, the doctor may want to obtain a

picture of the thyroid (a thyroid scan). The scan will find out if the entire thyroid gland is overactive or whether there is a toxic nodular goiter or thyroiditis (thyroid inflammation). A test that measures the ability of the gland to collect iodine (a thyroid uptake) may be done at the same time (American Thyroid Association, 2014).

1.2.12.4 Treatment of hyperthyroidism

Hyperthyroidism can be treated with medications, radiotherapy, or thyroid surgery (Bhaigyabati *et al.*, 2012; Norman, 2015 and Peterson, 2015). The aim of treatment is to bring the thyroid hormone levels to a normal state, thus preventing long-term complications, and to relieve uncomfortable symptoms. No single treatment works for everyone. Treatment depends on the cause of hyperthyroidism and how severe it is. When choosing a treatment, health care providers consider a patient's age, possible allergies to or side effects of the medications. They also consider such conditions as pregnancy, heart disease, and the availability of an experienced thyroid surgeon (NIDDK, 2012).

Drugs known as antithyroid agents - methimazole (Tapazole®), carbimazole or in rare instances propylthiouracil (PTU) - may be prescribed if the doctor chooses to treat the hyperthyroidism by blocking the thyroid gland's ability to make new thyroid hormone. Antithyroid drugs cause allergic reactions in about 5% of patients who take them. Common minor reactions are red skin rashes, hives, and occasionally fever and joint pains (American Thyroid Association, 2014). A rarer (occurring in 1 of 500 patients), but more serious side effect is a decrease in the number of white blood cells. Radioactive iodine has been used to treat patients for hyperthyroidism for over 60 years and has been shown to be generally safe. The radioactive iodine used in this treatment is administered by mouth, usually in a small

capsule that is taken just once. This destroys the thyroid cells that make thyroid hormone (American Thyroid Association, 2014) but does not affect other body tissues (NIDDK, 2012).

Hyperthyroidism can be permanently cured by surgical removal of most of the thyroid gland. This procedure is best performed by a surgeon who has much experience in thyroid surgery. After the thyroid gland is removed, the patient will likely become hypothyroid. However, the thyroid hormone levels can be restored to normal by treatment once a day with thyroid hormone supplements (American Thyroid Association, 2014). Beta Blockers are also useful in the treatment of hyperthyroidism. They block the action of thyroid hormone on the body. They usually make the patient feel better within hours to days, even though they do not change the high levels of thyroid hormone in the blood (American Thyroid Association, 2014).

CHAPTER TWO

MATERIALS AND METHODS

2.1 Procurement of *Dennettia tripetala*

Fresh leaves of *Dennettia tripetala* were obtained from Ukehe in Igbo-Etiti local government area, Enugu state. Identification was authenticated at the Taxonomy unit, Department of Plant Science and Biotechnology, University of Nigeria, Nsukka.

2.2 Procurement of Experimental Drugs

Levothyroxine was purchased from Elofex Pharmaceutical shop, Nsukka, Enugu State while Carbimazole was purchased from Virgil Pharmaceutical shop, Nsukka, Enugu State.

2.3 Procurement and Management of Experimental Animals

A total of 72 adult male albino rats (*Rattus novergicus*) were used for the study. The animals were purchased from the Genetics and Animal Breeding Unit, Department of Zoology and Environmental Biology, University of Nigeria, Nsukka. The rats were fed with standard rodent diet until the initiation of treatment. All animals received human care, as outlined in the guide for the care and use of laboratory animals. All the animals were maintained under the standard laboratory condition at temperature, 26 ± 2^0C, and humidity.

2.4 Preparation of Extracts

Extracts were prepared using the modified method of Case (2005). The fresh leaves were washed in running tap water and thereafter rinsed 3 times in distilled water. The leaves were air dried and ground into coarse powder. 5 kg of pulverized leaf material was used to prepare an infusion in 10 litres of warm (45ºC) distilled water. The infusion was left

overnight under refrigeration (4ºC) to prevent any possible contamination. After 24 hours, the extract was kept in a rotary shaker at 100 rpm for 1 hour and filtered with Whatman No.1 filter paper and subsequently subjected to lyophilization at –47.5ºC in the Department of Pharmaceutical Chemistry, Nnamdi Azikiwe University, Awka, Anambra State. The frozen extract was then freeze-dried to a powder, weighed and preserved at 4ºC for future use.

2.5 Phytochemical Analysis

The alkaloid, flavonoids, tannins, saponins, and total phenol contents of aqueous *D. tripetala* leaf extract were determined as detailed below:

2.5.1 Determination of alkaloids

The alkaloid content was determined using the method described by Harborne (1973). 5 g of the extract was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added and covered. The solution was allowed to stand for 4 hours. Thereafter, the solution was filtered with Whatman filter paper (number 1) and the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop-wise to the filtrate until the precipitation was complete. The whole solution was allowed to settle and the precipitate was filtered off with a weighed filter paper and, the filtrate in the filter paper was dried in the oven at 60^0C and reweighed to determine the weight of the filter paper and alkaloid

$$\text{Alkaloid } (\%) = \frac{(\text{weight of filter paper} + \text{alkaloid}) - (\text{weight of filter paper})}{\text{weight of sample used}} \times 100$$

2.5.2 Determination of total phenol content

The method described by Obadoni and Ochuko (2001) was used for phenol determination. 2 g of the sample was weighed into a flask and was defatted with petroleum ether. The residue was allowed to stand for few minutes to air dry. Thereafter, the residue

was boiled with 50 ml of diethyl ether for 15 minutes to extract the phenol. A total volume of 5 ml of the aliquots of the boiled sample was transferred into a test tube, and 2 ml of ammonia solution followed by 5 ml of amyl alcohol were added and allowed to stand for 30 minutes for colour development. The blank and the standard were then prepared. Then, the absorbance of the sample and the standard was read against the blank using spectrophotometer.

$$\text{Phenol (mg/100g)} = \frac{\text{A sample}}{\text{A standard}} \times \text{Concentration of the standard}$$

2.5.3 Determination of total flavonoid content

The flavonoid content was determined by using the method described by Bohm and Kocipai-Abyazan (1994). 10 g of the plant sample was extracted repeatedly with 100 ml of 80% aqueous methanol (left to stand for 45minutes) at room temperature. The whole solution was filtered through Whatman filter paper No 42 (125 mm). The filtrate was later transferred in a crucible and evaporated to dryness over a water bath and weighed to a constant weight.

Calculation:

Weight of empty beaker = W_1

Weight of empty beaker + Sample after drying = W_2

$$\text{Weight of residue} = \frac{W2 - W1}{\text{weight of sample used}} \times \frac{100}{1}$$

2.5.4 Determination of saponin content

The method used was that of Obadoni and Ochuko (2001). 20 g of the sample powder was put into a conical flask and 100 ml of 20% ethanol was added into the flask. The mixture was heated over a hot water bath set at 55°C for 4 hours with continuous stirring. The mixture was then filtered and the residue re-extracted with another 200 ml of 20% ethanol

and was concentrated in water bath to 40 ml at about 90°C. The concentrate was transferred into a 250 ml separating funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated and thereafter, 60 ml of n-butanol was added. The combined n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. Thereafter, the extract was added into a weighed crucible, evaporated in oven at 60^0C and the weght of the saponin was quantified in percentage as follows

$$\text{Saponins (\%)} = \frac{(\text{weight of crucible} + \text{saponin}) - (\text{weight of empty crucible})}{\text{weight of sample used}} \times 100$$

2.5.5 Determination of tannin content

The tannin content was ascertained after Van-Burden and Robinson (1981) methods**.** 5g of the finely ground sample was weighed and transferred into 250 ml conical flask and 50 ml of distilled water added and shook vigorously for an hour in a mechanical shaker. The resulting solution was filtered into a 50 ml volumetric flask and made up to the mark. 5ml of the filtrate was pipetted out into a test tube. 0.1g of tannic acid was dissolved in 100ml of water to form a tannic acid solution. 5ml of the tannic acid solution was pipetted out into another 50ml volumetric flask. A blank sample was also prepared using 5ml of distilled water. The three samples were incubated for 1.5 hours at 20 – 30^0C and the samples were then filled with distilled water up to a mark of 50 ml of the volumetric flask. The absorbance of the three samples was measured at 760 nm using spectronic 21D. The values generated were used to calculate the tannin content as follows:

Calculation

Let absorbance of 50ml of the extract = X

Let absorbance of tannic solution = Y

Let absorbance of blank = Z

$$\text{Tannin (mg/100g)} = \frac{X - Z}{Y - Z}$$

$$= \frac{\text{extract} - \text{blank}}{\text{standard} - \text{blank}}$$

2.6 Lethal Dose (LD_{50}) Determination

The lethal dose (LD_{50}) of the extract was determined according to the method of Lorke (1983). A total of 18 mice were used with the following assumptions made:

a. Substances more toxic than 1mg/kg are highly toxic that it is not important to calculate the LD_{50} exactly.
b. LD_{50} value greater than 5000mg/kg is of no practical interest.
c. An approximate figure for the LD_{50} is usually adequate to estimate the risk of acute intoxication.

The experiment involved first, a preliminary test in which three groups of mice (n = 3) were orally administered 10, 100, and 1000mg/kg of aqueous leaf extract of *Dennettia tripetala*. The animals were constantly observed for first 2 hours, intermittently for the next 4 hours and then overnight. No death of the animals per group was recorded after 24 hours. From the result obtained above, another three higher doses of 1600, 2900, and 5000mg/kg were administered orally to three groups of mice (n = 3).

2.7 Experimental Design

A total of seventy two (72) adult male albino rats were used for the study. The rats were allowed to acclimatize under a standard photoperiodic condition in a clean rat cage in the Postgraduate Research Laboratory of Department of Zoology and Environmental Biology, University of Nigeria, Nsukka. The rats were divided into six (6) groups (A – F)

with twelve rats in each cage as shown below. Each group was further replicated three times comprising of four rats each.

Group A: Control (no treatment).

Group B: Hyperthyroid rats without treatment (negative control).

Group C: Hyperthyroid rats treated with the standard drug (1.35mg/kg Carbimazole) (positive control).

Group D, Group E and Group F: Hyperthyroid rats administered different dosages (100mg/kg, 250mg/kg and 500mg/kg) of the plant extract. Measurement of all the parameters was taken at day zero before the commencement of treatment and thereafter on 7days interval. All the rats were allowed free access to water and feed in accordance with standard experimental procedures.

Table 1: Design that was used for the Experiment

Groups	Treatment (mg/kg)	Week 1	Week 2	Week 3	Week 4	Total
A	Normal Control	3	3	3	3	**12**
B	Hyperthyroid Control	3	3	3	3	**12**
C	Standard Control	3	3	3	3	**12**
D	100mg/kg	3	3	3	3	**12**
E	250mg/kg	3	3	3	3	**12**
F	500mg/kg	3	3	3	3	**12**
Total Number of Animals		**18**	**18**	**18**	**18**	**72**

2.8 Induction of Hyperthyroidism in Rat

Hyperthyroidism was induced in the rats of groups B – F, orally, at a dose of 600µg/kg Levothyroxine for 14 days and induction of hyperthyroidism was confirmed by analyzing the serum thyroid hormone levels. The serum levels of the thyroid hormones in hyperthyroid rats have been found to be increased and the TSH decreased. The serum levels of T_3, T_4, and TSH of the normal animal were 3.33pmol/L, 12.82pmol/L and 2.67mU/L respectively. However, in Hyperthyroid rats, the serum levels of T_3 and T_4 increased to 5.22pmol/L and 26.38pmol/L respectively, while the serum levels of TSH decreased to 0.14mU/L (Rodiques *et al.*, 2009; Bhaigyabati *et al.*, 2012; Donzelli *et al.*, 2016). Only hyperthyroid rats with equivalent (5.22pmol/L and 26.38pmol/L) or higher serum levels of T_3, T_4, and equivalent (0.14mU/L) or lower serum levels of TSH were regarded as hyperthyroid and subsequently used.

2.9 Treatment of the Rats with Standard Drug (Carbimazole)

Hyperthyroid rats of group C were treated with the standard drug, 1.35mg/kg Carbimazole orally for twenty eight (28) days.

2.10 Treatment of the Rats with Aqueous Leaf Extract of *D. tripetala*

The Hyperthyroid rats of groups D, E and F were treated with three different dosages of 100mg/kg, 250mg/kg and 500mg/kg of the aqueous *D. tripetala* leaf extract respectively orally for twenty eight (28) days. At seven (7) days interval, one animal was taken from all replicates of each group for weight measurement and blood collection. The Serum from experimental rats was analyzed for thyroid hormone levels as well as the lipid profile. At the end of the experiment, the thyroid glands of the rats were dissected out and fixed in formal buffer solution for histopathological study.

2.11 Collection of Blood Samples

Blood samples were collected from the intraorbital sinus (Parasuraman *et al*., 2010) using a capillary tube. The collected serum was separated by centrifugation at 3000rpm for 15min in a normal table centrifuge and used for the analysis. Sera from the experimental rats were analysed for thyroid hormone levels (ELISA method) and lipid profile (Kit method) before and during the experiment. Serum samples were stored frozen if not analysed immediately (Machado *et al.* 2009). The analysis was carried out in the Department of Pharmacology and Toxicology Animal house and laboratory, University of Nigeria, Nsukka.

2.12 Thyroid Hormone Analysis

The kits (for T_3, T_4, and TSH) were those produced by Monobind Inc., 100 North Pointe Drive, Lake Forest, California, USA. Sera from the experimental rats were analysed for thyroid hormone levels using the enzyme-linked immunosorbent assay (ELISA) method (Schuurs and Van-Weeman, 1977; Soos and Siddle, 1982). Before proceeding with the assay, all reagents, serum references and controls were brought to room temperature (20-27ºC). The microplates' wells for each serum calibrator, control, and specimen to be assayed were formatted in duplicate. 25µl of the appropriate serum reference, control or specimen was pipetted into the assigned well for tT_4, 50µl for tT_3 and 50µl for TSH. Then 50µl of working reagent A, tT_4 or tT_3 – enzyme conjugate solution was added to the appropriate well. For TSH, 100µl of TSH was added and thoroughly mixed for 30 seconds. 50µl of biotinylated tT_4 or tT_3 – antibody conjugate solution was added to the appropriate wells. The microplate was swirled gently for 20-30 seconds to mix; it was then covered and incubated for 60 minutes at room temperature. Then, the incubation mixture was removed by flicking plate contents into a waste container. The microplate wells were rinsed and flicked 5 times with distilled water. The wells were then struck sharply onto absorbent paper or paper towels to remove all

residual water droplets. 100µl of working substrate solution was added to all wells and incubated at room temperature for 15 minutes. This was followed by addition of 50µl of stop solution to each and gently mixed for 15 – 20 minutes.

Within 30 minutes of adding the stop solution, the absorbance in each well was then read at 450nm in a microplate reader. The result obtained was used to plot the absorbance for each duplicate serum reference against the corresponding tT_4 in pmol/L, tT_3 concentration in pmol/L and TSH in mU/L on linear graph paper. The points are then connected with a best-fit curve to determine the concentration of tT_4, tT_3, and TSH.

2.13 Lipid Profile Analysis

2.13.1 Total cholesterol

Total plasma cholesterol was determined according to the method described by Kishi *et al.* (2002) using commercially available kit. This was predicated on the principle that cholesterol esters are hydrolyzed by cholesterol esterase into free cholesterol and fatty acids. Free cholesterol is then oxidized to cholest-4-en-3-on and hydrogen peroxide (H_2O_2). Hydrogen peroxide in the presence of phenol and amino-antipyrin forms a complex of red colour that absorbs between 500 – 550nm. The assay protocol carried out according to the table below:

Solution/SN	Blank	Standard	Test
Reagent 1+2	1ml	1ml	1ml
Cholesterol standard	-	20µl	-
Sample	-	-	20µl
Distilled water	20µl	-	-

The tubes were shaken and then incubated for 5 minutes at 37ºC. Absorbance was read using a spectrophotometer at 550 nm. Total cholesterol was calculated as:

$$\text{Total cholesterol (mg/dL)} = \frac{\text{Absorbance of test sample}}{\text{Absorbance of standard}} \times \text{concentration of standard}$$

2.13.2 Lipoprotein analysis

2.13.2.1 High density lipoprotein cholesterol

The High density lipoprotein cholesterol content was determined by using the methodology of Trinder (1969). The High density lipoprotein cholesterol (HDL-C) was, first of all, precipitated in the presence of phosphotungistic acid and magnesium chloride.

The separation procedure involved adding 500µl of the plasma sample into marked Eppendorf tubes followed by 50µl of precipitating reagent which was pipetted into the same tube. These were mixed together and centrifuged at 10,000 rpm for 15 minutes. The clear separation was pipetted into a tube and HDL-C content was then determined as shown below:

Solution/SN	Blank	Standard	Test
Reagent 1+2	1ml	1ml	1ml
Cholesterol standard	-	50µl	-
Sample	-	-	50µl
Distilled water	50µl	-	-

The colour that developed was read at 550 nm using a spectrophotometer. Serum high density lipoprotein was calculated as:

$$\text{HDL (mg/dL)} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \text{concentration of standard}$$

2.13.2.2 Low density lipoprotein cholesterol

Low density lipoprotein concentration was determined using the method of Friedwald *et al.* (1972) as:

$$\text{LDL}\,((\text{mg/dL}) = \text{Total cholesterol} - \left(\text{HDL} + \frac{\text{Total triglyceride}}{5}\right)$$

2.13.2.3 Total triglyceride

Total triglyceride was determined by the enzymatic method described by Buccolo and David (1973) using commercially available kit. This was based on the principle that triglyceride was first hydrolyzed to glycerol and free fatty acids. Glycerol was then phosphorylated by adenosine-5-triphosphate (ATP) to form glycerol-1-phosphate (G-1-P) and adenosine-5`-diphosphate (ADP) in the reaction catalysed by glycerol kinase.

The triglyceride working reagent was prepared according to the manufacturer's instructions while the spectrophotometer was set at 550nm. After warming the working reagent to assay temperature, a series of cuvets labeled blank, standard, and sample was set up. 1 ml of the triglyceride working reagent was pipetted into each cuvet. Then 10 µl of water, glycerol standard and sample were added into corresponding cuvets. The mixture was homogenized by inversion and then incubated for 5 minutes at 37^0C. The optical density or absorbency (A) of blank, standard, and sample was read at 550nm and total triglyceride was calculated as:

Total triglyceride (mg/dL) =

$$\frac{\text{Absorbance of sample} - \text{Absorbance of blank}}{\text{Absorbance of standard} - \text{Absorbance of blank}}\ \text{X Concentration of standard}$$

2.14 Histopathology of the Thyroid Gland of Hyperthyroid Rats.

The histopathological studies were carried out following the methodology of Ejere and Adegoke (2005) with minor modification.

2.14.1 Sources of tissue samples and tissue fixation

Before anaesthetizing the rats for dissection, a wide-mounted container with screw-capped cover was placed nearby. The container was sizeable enough to accommodate the excised tissues freely and ensured that unnecessary pressures were not put on the tissues (Carleton, 1967). The anaesthetized rats were placed on the dissecting board and the thyroid glands of the rats were dissected out.

The dissected tissue samples were transferred into 10% neutrally buffered formalin fixative immediately after collection and allowed to stand for 24 – 48 hours. This was carried out in order to arrest metabolic activities in the tissues, avoid autolysis and protein precipitation thus preventing enzymatic digestion of dead tissues. After fixation, specimens were trimmed using a scalpel to enable them to fit into an appropriately labeled tissue cassette. The filled tissue cassettes were then stored in formalin until processing begins.

2.13.2 Tissue dehydration and clearing

Tissue dehydration was done to remove water from the biological tissues since paraffin wax is immiscible with water. The fixed tissues were passed through several changes of alcohol, 70% alcohol for 24 hours and 90% alcohol for 12 hours and through absolute alcohol to remove water from the fixed tissues and allow complete infiltration of tissues by paraffin. Following removal of water from the collected specimen during dehydration, the

tissues were cleared. The tissues were then passed through xylene for 3 hours to prevent shrinkage and tissue brittleness in paraffin.

2.13.3 Tissues embedding

Prior to sectioning, the tissue blocks were infiltrated with paraffin which acts as a support during the sectioning process. Tissues were embedded or blocked out using the leukhand embedded mould. The L pieces were arranged on an aluminium base to form a rectangle. The molten paraffin was then poured into the moulds and the selected surfaces of the tissues embedded with the aid of a pair of blunt end forceps and allowed to set. The embedded tissues were separated into different blocks and then attached to wooden blocked with the aid of an electric spatula.

2.13.4 Tissue sectioning

The blocks were then trimmed using a rotary microtome and knife. At the end of each trimming, the blocks were arranged on ice trays in order to cut thin sections using the rotary microtone at a thickness of 5 micron. Sections were then collected with the help of a camel hair brush and then placed on the slide. Sections were flood picked with 20% alcohol in other to spread out fold on the sections and then floated out on a water bath with a temperature 5^0C - 10^0C below the melting point of the wax used. The sections were picked and floated on a water bath and then picked with a pre-labelled slide. The slides were dried on a hot plate at a temperature of 5^0C - 10^0C above the melting point of wax used. These were left on the hot plate for 15 minutes.

2.13.5 Tissue staining

The staining methods employed in staining the sections were haematoxylin and eosin method to demonstrate general tissue structure as follows. They were dewaxed in two bathes

of xylene for 2 minutes each and dehydrated in descending grades of alcohol, absolute: 90%, 70% for 2 minutes each and then wash in water. They were then stained with haematoxylin for 15minute after which excess stains were washed with tap water and differentiated in 1% acid alcohol briefly. Washing in running tap water and blueing for 10 minutes was done followed by counter staining in 1% aqueous eosin for 3 minutes before washing off in water. Dehydration through 70% alcohol, 90% alcohol and absolute for 2 minutes each was followed by clearing in 2 baths of xylene for 2 minutes.

2.13.4 Analysis of sections

The slides were then mounted in cover slips with DPX devoid of air bubbles. The stained sections on the slides were examined using X10, then X20, and X40 objective microscope. Then, photomicrographs of the slides were captured using a Moticam Images Plus 2.0 digital camera (Motic China Group Ltd. 1999–2004).

Statistical Analysis

The statistical package for social sciences (SPSS), version 20, was used for the statistical analysis. Data obtained were recorded as mean ± SEM. The mean values of the data collected were subjected to two way analysis of variance (ANOVA) while means of the treatment group were separated using Duncan's Multiple Range Test (DMRT). Level of significance was set at $P < 0.05$.

CHAPTER THREE

RESULTS

3.1 Qualitative and Quantitative Phytochemical Composition of Aqueous *Dennettia tripetala* Leaf Extract

Tables 2 and 3 show the result of the qualitative and quantitative phytochemical compositions of the aqueous *Dennettia tripetala* leaf extract respectively. Qualitatively, it was observed that the aqueous *D. tripetala* leaf extract possess tannin in high quantity. Similarly, the aqueous *D. tripetala* leaf extract was found to possess alkaloids, saponins, flavonoids, reducing sugars, oil and fats, carbohydrate and cardiac glycosides in moderate quantities while acidic compound, protein, steroids, phenols, and terpenoids were detected in low quantity and resins was absent.

Table 2: Qualitative phytochemical composition of aqueous *Dennettia tripetala* leaf extract

Phytochemicals	Bioavailability
Alkaloids	++
Tannins	+++
Saponins	++
Acidic compounds	+
Reducing substance	++
Protein	+
Oil and fats	++
Carbohydrates	++
Resins	-
Flavonoids	++
Cardiac glycosides	++
Steroids	+
Terpenoids	+
Phenol	+

Keys: +++ = Detected in high quantity

++ = Detected in moderate quantity

+ = Detected in low quantity

- = Absent

Table 3: Quantiitative phytochemical composition of aqueous *Dennettia tripetala* leaf extract

Phytochemicals	**Composition (%)**
Alkaloids	24
Tannins	58.42
Saponins	33.82
Flavonoids	42.02
Cardiac glycosides	32
Phenol	0.33

3.2 Acute Toxicity Test

There was no mortality at different doses (10, 100 and 1000mg/kg) of the aqueous *D. tripetala* leaf extract after 24 hours. Also, at dose levels of 1600 mg/kg, 2900 mg/kg and 500 mg/kg, there was no death recorded although, elated mood and hyperactivity were observed in the animals as shown in Table 4.

Table 4: Acute toxicity test of the ethanol extract of aqueous *Dennettia tripetala* leaf extract

Groups	The dose of extract administered (mg/kg b.w.)	Number of deaths	Behavioural expression
	PHASE 1		
Group A	10	0/3	Normal effect
Group B	100	0/3	Normal effect
Group C	1000	0/3	Normal effect
	PHASE 2		
Group D	1600	0/3	Elated mood for 26 mins
Group E	2900	0/3	Elated mood for 26 mins
Group F	5000	0/3	Hyperactivity for 1.12minutes

n = 3

3.3 Effects of Different Doses of Aqueous *Dennettia tripetala* Leaf Extract on the Body Weights (BWs) of Hyperthyroid Male Albino Rats

The weekly effects of aqueous *D. tripetala* leaf extract on the body weights (BWs) of hyperthyroid male albino rats were shown in Table 5. The baseline results (Week 0) were statistically similar ($P > 0.05$) among the groups except with the normal control group. At week 1, the group that received 500mg/kg body weight of *D. tripetala* showed the highest significant ($P < 0.05$) body weight while the hyperthyroid control group recorded the least value (87±1.92). There was no significant difference ($P > 0.05$) in the body weight of the animals among the groups at week 2, although, there were variations. At week 3, the group dosed with 100mg/kg body weight of *D. tripetala* recorded the highest significant ($P < 0.05$) body weight value when compared with other groups. Statistically similar ($P > 0.05$) body weight results were observed at week 4 among the groups. There was no dose-dependent difference in body weight at weeks 1 and 2 while weeks 1 and 4 showed a dose-dependent decrease in weight. It was observed that the weights of the extract dosed groups were higher than the control groups at weeks 2 and 3.

Conclusively, whereas the normal control group showed a gradual non-significant ($P > 0.05$) duration dependent increase in weight, the other control groups and the extract treated groups showed a progressive significant ($P < 0.05$) duration dependent increase in the body weight from weeks 0 to 4.

Table 5: Weekly effects of different treatments of aqueous *D. tripetala* leaf extract on the body weight, BW (g) of hyperthyroid male albino rats

Groups	Duration				
	Week 0	Week 1	Week 2	Week 3	Week 4
Normal Control	103.92 ± 4.58^{b1}	104.42 ± 10.00^{ab1}	111.00 ± 7.82^{a1}	119.88 ± 10.31^{ab1}	130.61 ± 19.39^{a1}
Hyperthyroid Control	73.30 ± 3.51^{a1}	87.14 ± 1.92^{a2}	107.03 ± 2.68^{a3}	124.76 ± 7.49^{ab4}	149.75 ± 1.52^{a5}
Standard Control	79.22 ± 2.80^{a1}	98.02 ± 8.40^{ab2}	113.30 ± 5.03^{a3}	113.67 ± 0.90^{a3}	152.68 ± 0.55^{a4}
100mg/kg	74.75 ± 2.40^{a1}	110.21 ± 5.20^{b2}	125.39 ± 8.61^{a23}	141.75 ± 6.15^{b34}	155.48 ± 5.81^{a4}
250mg/kg	79.76 ± 1.60^{a1}	97.76 ± 1.20^{ab1}	115.41 ± 8.33^{a12}	139.04 ± 12.55^{ab23}	154.95 ± 19.73^{a3}
500mg/kg	78.43 ± 6.05^{a1}	115.31 ± 2.61^{b12}	131.19 ± 19.93^{a2}	137.03 ± 6.48^{ab2}	143.91 ± 18.82^{a2}

Mean values in a column with different letters as superscript are significantly different at $p < 0.05$

Mean values in a row with different numbers as superscript are significantly different at $p < 0.05$

3.4 Effects of Different Doses of Aqueous *Dennettia tripetala* Leaf Extract on the Triiodothyronine (T_3) Level of Hyperthyroid Male Albino Rats

The weekly effects of aqueous *D. tripetala* leaf extract on the T_3 level in hyperthyroid male albino rats were shown in Table 6. The baseline results (Week 0) showed that the T_3 level of the normal control group was significantly lower ($P < 0.05$) when compared with the other groups. The dose-dependent analysis showed that at weeks 1, 2 and 4, the groups dosed with 250mg/kg and 500mg/kg *D. tripetala* leaf extract recorded no significant difference ($P > 0.05$) when compared with the normal control and standard control groups, but varied significantly ($P < 0.05$) when compared with those of the hyperthyroid control and the 100mg/kg extract dosed groups. However, whereas there was no significant difference ($P > 0.05$) in the serum T_3 levels of the 100mg/kg extract dosed group when compared with that of the hyperthyroid control group at week 1, a statistically significant difference ($P < 0.05$) was observed in comparison with that of the hyperthyroid control group at weeks 2 and 4. At week 3, there was no significant difference ($P > 0.05$) in the serum T_3 levels of the extract dosed groups when compared with those of the normal control and standard control groups, however, a statistically significant difference ($P < 0.05$) was observe in comparison with that of the hyperthyroid control group. Furthermore, the extract dosed groups recorded a dose-dependent decrease in the serum T_3 levels from week 1 to 4.

The duration-dependent analysis revealed that the serum T_3 levels of the animals fluctuated significantly ($P < 0.05$) in all the groups but insignificantly ($P > 0.05$) in normal control group. In 100mg/kg extract dosed group, the T_3 levels at weeks 1 and 2 had no significant difference ($P > 0.05$) when compared to week 0 but was significantly lower at weeks 3 and 4 when compared to week 0. In 250mg/kg and 500mg/kg extract dosed groups, the T_3 levels at weeks 1, 2, 3 and 4 were significantly lower ($P < 0.05$) when compared to that of week 0.

Table 6: Weekly effects of different treatments of aqueous *D. tripetala* leaf extract on the T_3 levels (pmol/L) of hyperthyroid male albino rats

Groups	Duration				
	Week 0	Week 1	Week 2	Week 3	Week 4
Normal Control	3.33 ± 0.23^{a1}	3.25 ± 0.11^{a1}	3.20 ± 0.34^{a1}	3.32 ± 0.22^{a1}	3.11 ± 0.11^{a1}
Hyperthyroid Control	6.47 ± 0.90^{b1}	7.97 ± 1.46^{b12}	10.18 ± 0.56^{c2}	8.70 ± 0.53^{b12}	8.42 ± 0.41^{c12}
Standard Control	7.33 ± 1.15^{b2}	4.35 ± 0.39^{a1}	4.39 ± 0.47^{a1}	3.45 ± 0.22^{a1}	2.77 ± 0.21^{a1}
100mg/kg	7.33 ± 1.11^{b2}	6.61 ± 0.38^{b2}	6.89 ± 1.21^{b2}	3.78 ± 0.29^{a1}	5.11 ± 0.11^{b12}
250mg/kg	5.22 ± 0.07^{ab4}	4.41 ± 0.35^{a23}	4.94 ± 0.14^{a34}	3.74 ± 0.29^{a12}	3.20 ± 0.10^{a1}
500mg/kg	6.16 ± 1.19^{ab2}	3.86 ± 0.32^{a1}	4.49 ± 0.35^{a12}	3.09 ± 0.12^{a1}	3.14 ± 0.10^{a1}

Mean values in a column with different letters as superscript are significantly different at $p < 0.05$

Mean values in a row with different numbers as superscript are significantly different at $p < 0.05$

3.5 Effects of Different Doses of Aqueous *Dennettia tripetala* Leaf Extract on the Thyroxine (T_4) Levels of Hyperthyroid Male Albino Rats

The weekly effects of aqueous *D. tripetala* leaf extract on the T_4 level in hyperthyroid male albino rats were shown in Table 7. The baseline results (week 0) showed that the serum T_4 level of the normal control group was significantly lower ($P < 0.05$) when compared with that of the other groups. The week 1 results revealed that there was no significant difference ($P > 0.05$) in the serum T_4 levels among the groups except with that of the normal control group. At week 2, the T_4 level varied significantly ($P < 0.05$) among the groups. The hyperthyroid control group showed the highest serum T_4 value while the normal control group recorded the least value (14.19±0.62). However at week 3, whereas the extract dosed groups recorded no significant difference ($P > 0.05$) when compared with the standard control groups, a statistical significant difference ($P < 0.05$) was observed in comparison with those of the normal control and hyperthyroid control groups. At week 4, the groups dosed with 100mg/kg and 250mg/kg *D. tripetala* leaf extract varied significantly ($P < 0.05$) when compared with the control groups. On the other hand, the 500mg/kg *D. tripetala* leaf extract dosed group was insignificantly ($P > 0.05$) higher than the standard control but varied significantly ($P < 0.05$) when compared with the normal control and the hyperthyroid control groups. Furthermore, it was observed that there was no dose-dependent difference in T_4 levels at weeks 1, 3 and 4 while week 2 showed a dose-dependent decrease in T_4 levels.

The duration-dependent analysis revealed that the normal control recorded non-significant ($P > 0.05$) fluctuation in the T_4 level of the animals while other groups except the 500mg/kg *D. tripetala* leaf extract group fluctuated significantly ($P < 0.05$). The 500mg/kg *D. tripetala* leaf extract group recorded a progressive significant ($P < 0.05$) duration dependent decrease in T_4 level from week 0 to 4. Furthermore, in the extract dosed groups,

Table 7: Weekly effects of different treatments of aqueous *D. tripetala* leaf extract on the T_4 levels (pmol/L) of hyperthyroid male albino rats

Groups	Duration				
	Week 0	Week 1	Week 2	Week 3	Week 4
Normal Control	12.82 ± 0.82^{a1}	13.39 ± 0.80^{a1}	14.19 ± 0.62^{a1}	13.36 ± 0.37^{a1}	13.01 ± 0.63^{a1}
Hyperthyroid Control	29.55 ± 1.86^{bc12}	22.70 ± 3.26^{b1}	33.22 ± 0.47^{d2}	29.26 ± 0.91^{c12}	27.27 ± 3.52^{c12}
Standard Control	31.91 ± 1.65^{c3}	21.08 ± 1.46^{b2}	19.54 ± 1.13^{b2}	17.06 ± 1.43^{b12}	14.81 ± 0.54^{ab1}
100mg/kg	26.38 ± 1.29^{b3}	21.68 ± 2.11^{b123}	23.77 ± 1.76^{c23}	19.16 ± 1.07^{b12}	18.48 ± 0.48^{b1}
250mg/kg	28.71 ± 2.15^{bc2}	21.57 ± 3.41^{b1}	21.36 ± 0.38^{bc1}	18.07 ± 0.59^{b1}	18.92 ± 0.75^{b1}
500mg/kg	28.83 ± 1.58^{bc3}	23.06 ± 2.66^{b2}	20.51 ± 0.52^{b2}	18.20 ± 1.10^{b12}	15.39 ± 0.26^{ab1}

Mean values in a column with different letters as superscript are significantly different at $p < 0.05$

Mean values in a row with different numbers as superscript are significantly different at $p < 0.05$

the serum T_4 levels decreased significantly ($P < 0.05$) at weeks 1, 2, 3 and 4 when compared with that of week 0.

3.6 Effects of Different Doses of Aqueous *Dennettia tripetala* Leaf Extract on the Thyroid Stimulating Hormone (TSH) Levels of Hyperthyroid Male Albino Rats

The weekly effects of aqueous *D. tripetala* leaf extract on the TSH level in hyperthyroid male albino rats were shown in Table 8. At week 0 there was no significant difference ($P > 0.05$) among the extract dosed groups when compared with the hyperthyroid control and standard control groups while the normal control group recorded the highest significant difference ($P < 0.05$) in the TSH level. At week 1, the 100mg/kg extract dosed group recorded no significant difference ($P > 0.05$) when compared with the normal control and the standard control groups but varied significantly ($P < 0.05$) from the other extract dosed groups and the hyperthyroid control group. However in week 2, whereas there was no significant difference ($P > 0.05$) in the serum TSH levels of the 100mg/kg and 250mg/kg extract dosed groups when compared with the standard control group, a statistical significant difference ($P < 0.05$) was observed in comparison with those of the normal control, hyperthyroid control and 500mg/kg extract dosed groups. Furthermore, it was observed that the 500mg/kg extract dosed group recorded the highest significant difference ($P < 0.05$) in the TSH levels at this week 2. At weeks 3 and 4, the extract dosed groups revealed no significant difference ($P > 0.05$) in the TSH levels when compared with the normal control and standard control groups. It was observed that the hyperthyroid group recorded the lowest in TSH serum values from weeks 0 to 4. Similarly, whereas there was no dose-dependent significant difference in the TSH levels in weeks 3 and 4, week 1 showed a significant dose-dependent decrease in the TSH level, while week 2 recorded a progressive significant dose-dependent

Table 8: Weekly effects of different treatments of aqueous *D. tripetala* leaf extract on the TSH (mU/L) level of hyperthyroid male albino rats

Groups	Duration				
	Day 0	Week 1	Week 2	Week 3	Week 4
Normal Control	2.67 ± 0.30^{b1}	2.71 ± 0.45^{b1}	2.63 ± 0.42^{b1}	3.12 ± 0.07^{b1}	2.82 ± 0.55^{b1}
Hyperthyroid Control	0.10 ± 0.02^{a1}	1.08 ± 0.08^{a2}	0.34 ± 0.22^{a1}	0.09 ± 0.02^{a1}	0.09 ± 0.01^{a1}
Standard Control	0.13 ± 0.03^{a1}	2.81 ± 0.31^{b2}	3.42 ± 0.35^{bc2}	3.17 ± 0.23^{b2}	3.20 ± 0.44^{b2}
100mg/kg	0.14 ± 0.07^{a1}	2.58 ± 0.33^{b2}	2.84 ± 0.32^{bc23}	3.51 ± 0.44^{b23}	3.69 ± 0.30^{b3}
250mg/kg	0.12 ± 0.05^{a1}	2.19 ± 0.57^{ab2}	3.19 ± 0.07^{bc23}	3.67 ± 0.34^{b3}	3.70 ± 0.26^{b3}
500mg/kg	0.12 ± 0.03^{a1}	2.15 ± 0.57^{ab2}	3.76 ± 0.34^{c3}	3.63 ± 0.35^{b3}	3.29 ± 0.29^{b3}

Mean values in a column with different letters as superscript are significantly different at $p < 0.05$

Mean values in a row with different numbers as superscript are significantly different at $p < 0.05$

increase ($P < 0.05$) in its level. It was also observed that the TSH levels of the extract dosed groups were higher than the control groups at weeks 3 and 4.

The duration-dependent analysis revealed that the 100mg/kg and 250mg/kg extract dosed groups recorded a progressive significant increase ($P < 0.05$) in TSH level from weeks 0 to 4 while the 500mg/kg dosed group and the control groups showed no duration-dependent difference in the TSH levels.

3.7 Effects of Different Doses of Aqueous *Dennettia tripetala* Leaf Extract on the Total Cholesterol (TCH) Levels in Hyperthyroid Male Albino Rats

The weekly effects of aqueous *D. tripetala* leaf extract on the TCH levels of hyperthyroid male albino rats were shown in Table 9. The baseline results (Week 0) were statistically similar ($P > 0.05$) among the groups. At week 1, the TCH levels of the 100mg/kg extract dosed group was statistically similar ($P > 0.05$) to the hyperthyroid group but varied significantly ($P < 0.05$) from other groups. The TCH levels of the 250mg/kg and 500mg/kg extract dosed groups were statistically similar when compared to the standard control group but were significantly higher ($P < 0.05$) than the normal control group. The normal control group had the least TCH value (58.13±60). However, at week 2, whereas there was no significant difference ($P > 0.05$) in the serum TCH levels of the 250mg/kg and 500mg/kg extract dosed groups when compared to the standard control group, a statistical significant difference ($P < 0.05$) was observed in comparison with those of the other groups. Also, the TCH levels of the 100mg/kg extract dosed group were significantly higher when compared with the hyperthyroid control group but significantly lower when compared to that of the normal control group ($P < 0.05$). The hyperthyroid control group had the least TCH value (42.29±0.75). Moreover, week 3 result revealed that the TCH levels of the 100mg/kg extract

dosed group was statistically similar ($P > 0.05$) to that of the hyperthyroid group, however, a statistical significant difference was observed when compared with those of the other extract dosed groups (250mg/kg and 500mg/kg extract dosed groups) and the other control groups (normal control and standard control groups). The TCH levels of the 500mg/kg extract dosed group were significantly similar ($P > 0.05$) to that of the standard control group but were significantly higher ($P < 0.05$) when compared to the other extract dosed groups (100mg/kg and 250mg/kg extract dosed groups) and other control groups (normal control and hyperthyroid control groups). Similarly, whereas the week 4 result recorded no significant difference ($P > 0.05$) in TCH levels of the 250mg/kg extract dosed group when compared with that of the 500mg/kg extract dosed group, a statistical significant difference ($P < 0.05$) was observed in comparison with the control and 100mg/kg extract dosed groups. The standard control group recorded the highest TCH value (112.70±14.84) while the hyperthyroid control group recorded the least TCH value (47.50±5.85). The difference was significance ($P < 0.05$).

Minimal fluctuation occurred in the mean weekly TCH levels of the rats in the extract dosed groups. Time-dependent analysis showed that in the extract dosed groups, the TCH level increased significantly ($P < 0.05$) at weeks 1, 2, 3 and 4 when compared to week 0. In the same way, the TCH level in the normal control and standard control groups increased significantly ($P < 0.05$) at weeks 1, 2, 3 and 4 when compared to week 0. However, in the hyperthyroid group, the TCH levels increased significantly ($P < 0.05$) at week 1, decreased insignificantly at weeks 2 and 3 ($P > 0.05$), and increased insignificantly at week 4 ($P > 0.05$). In addition, the extract dosed groups recorded no time-dependent difference from week 1 to 4.

Table 9: Weekly effects of different treatments of aqueous *D. tripetala* leaf extract on the Total Cholesterol level (mg/dL) of hyperthyroid male albino rats

Groups	Duration				
	Week 0	Week 1	Week 2	Week 3	Week 4
Normal Control	46.47 ± 3.27^{a1}	58.13 ± 1.60^{a12}	58.57 ± 6.56^{b12}	70.73 ± 7.78^{ab23}	79.17 ± 2.96^{b3}
Hyperthyroid Control	46.20 ± 2.08^{a1}	78.20 ± 2.17^{b2}	42.97 ± 0.75^{a1}	46.20 ± 1.68^{a1}	47.50 ± 5.85^{a1}
Standard Control	48.17 ± 2.92^{a1}	68.07 ± 4.62^{ab12}	81.60 ± 5.54^{c2}	109.43 ± 10.96^{c3}	112.70 ± 14.84^{c3}
100mg/kg	48.83 ± 2.67^{a1}	73.80 ± 3.73^{b2}	73.00 ± 3.86^{bc2}	56.50 ± 4.10^{a12}	72.20 ± 8.95^{ab2}
250mg/kg	50.23 ± 2.03^{a1}	69.63 ± 5.74^{ab12}	77.47 ± 5.34^{c12}	99.50 ± 14.59^{bc2}	97.37 ± 13.71^{bc2}
500mg/kg	53.43 ± 8.14^{a1}	72.60 ± 6.49^{ab12}	85.97 ± 5.12^{c2}	119.23 ± 11.40^{c3}	98.40 ± 10.64^{bc23}

Mean values in a column with different letters as superscript are significantly different at $p < 0.05$

Mean values in a row with different numbers as superscript are significantly different at $p < 0.05$

3.8 Effects of Different Doses of Aqueous *Dennettia tripetala* Leaf Extract on the High Density Lipoprotein Cholesterol (HDL-C) Level in Hyperthyroid Male Albino Rats

The weekly effects of aqueous *D. tripetala* leaf extract on the HDL-C levels of hyperthyroid male albino rats were shown in Table 10. The baseline result (week 0) recorded no significant difference ($P > 0.05$) among the groups. At week 1, the extract dosed groups revealed no significant difference ($P > 0.05$) when compared with the standard control group but varied significantly from normal control and hyperthyroid control groups ($P < 0.05$). The hyperthyroid control group had the highest HDL-C value while the normal control group had the least value. The difference was significant ($P < 0.05$). At week 2, whereas the 250mg/kg extract dosed group recorded no significant difference ($P > 0.05$) when compared with the normal control and standard control groups, a statistical significant difference ($P < 0.05$) was observed in comparison with the other extract dosed (100mg/kg and 500mg/kg) and hyperthyroid groups. Furthermore, week 3 result showed no significance difference ($P > 0.05$) in the HDL-C levels of the 100mg/kg and 250mg/kg extract dosed groups when compared with the standard control group, however, a statistical significant difference ($P < 0.05$) was observed in comparison with the other control groups (normal control and hyperthyroid control groups) and the 500mg/kg extract dosed group. Moreover, at week 4, the extract dosed groups showed no significant difference ($P > 0.05$) when compared with the normal control and standard control groups but varied significantly from the hyperthyroid control group ($P < 0.05$).

The time-dependent analysis showed that significant decreases ($P < 0.05$) occurred among the rats in the treatment groups. In the extract dosed groups, the HDL levels decreased significantly ($P < 0.05$) at weeks 1, 2, 3 and 4 when compared to week 0.

Table 10: Weekly effects of different treatments of aqueous *D. tripetala* leaf extract on the HDL-C levels (mg/dL) of hyperthyroid male albino rats

Groups	Duration				
	Week 0	Week 1	Week 2	Week 3	Week 4
Normal Control	43.83±2.94^{a2}	26.07±1.09^{a1}	27.10±2.06^{ab1}	31.10±1.59^{b1}	29.60±0.92^{a1}
Hyperthyroid Control	43.93±6.08^{a1}	42.53±2.20^{c1}	49.63±2.74^{c1}	39.70±4.74^{c1}	51.23±1.99^{b1}
Standard Control	45.33±4.10^{a4}	32.33±1.02^{b23}	28.10±1.26^{ab12}	23.10±1.12^{ab1}	35.93±2.25^{a3}
100mg/kg	45.87±6.67^{a2}	33.60±1.81^{b1}	31.43±0.58^{b1}	27.50±1.36^{ab1}	35.77±1.42^{a12}
250mg/kg	37.50±4.45^{a2}	33.33±0.35^{b12}	28.17±1.45^{ab1}	27.83±3.01^{ab1}	36.43±1.39^{a12}
500mg/kg	37.87±4.54^{a3}	30.80±0.43^{b23}	24.07±1.27^{a12}	19.90±1.18^{a1}	32.63±4.43^{a23}

Mean values in a column with different letters as superscript are significantly different at $p < 0.05$

Mean values in a row with different numbers as superscript are significantly different at $p < 0.05$

3.9 Effects of Different Doses of Aqueous *Dennettia tripetala* Leaf Extract on the Low Density Lipoprotein Cholesterol (LDL-C) Level in Hyperthyroid Male Albino Rats

The weekly effects of aqueous *D. tripetala* leaf extract on the LDL-C level of hyperthyroid male albino rats were shown in Table 11. At week 0, the LDL-C level of the extract dosed groups had no significant difference ($P > 0.05$) when compared to the hyperthyroid control and standard control groups but varied significantly when compared to the normal control group. At week 1, where as there was no significant difference ($P > 0.05$) in the LDL-C levels of the 100mk/kg extract dosed group when compared with the normal control group, a statistical significant difference ($P < 0.05$) was observed in comparison with the other extract dosed groups (250mg/kg and 500mg/kg) and other control groups (hyperthyroid control and standard control groups). Furthermore, week 2 result recoded that the LDL-C level of the 500mg/kg extract dosed group had no significant difference in comparison with the standard control group, however, a statistical significant difference was observed when compared to the other extract dosed groups (100mg/kg and 250mg/kg extract dosed groups) and other control groups (normal control and hyperthyroid control groups). Moreover, the extract dosed groups varied significantly ($P < 0.05$) in comparison to the control groups in weeks 3 and 4. In addition, it is noteworthy that there was no dose-dependent difference in the LDL-C levels in the extract dosed groups at week 1, however, a significant dose-dependent increase in the LDL-C levels was observed in weeks 2, 3 and 4.

Conclusively, whereas the duration-dependent analysis revealed minimal fluctuation in the LDL-C of the rats in the control group, a progressive significant increase ($P < 0.05$) in the LDL-C was observed in the other groups from week 2 to 4.

Table 11: Weekly effects of different treatments of aqueous *D. tripetala* leaf extract on the LDL-C levels (mg/dL) of hyperthyroid male albino rats

Groups	Duration				
	Day 0	Week 1	Week 2	Week 3	Week 4
Normal Control	31.33 ± 8.35^{b2}	19.00 ± 0.58^{ab12}	18.33 ± 0.67^{a12}	17.67 ± 0.33^{a1}	18.67 ± 2.03^{a12}
Hyperthyroid Control	18.33 ± 1.20^{a1}	22.00 ± 1.15^{abc1}	27.33 ± 2.91^{ab12}	25.67 ± 3.28^{a12}	34.33 ± 6.81^{ab2}
Standard Control	18.33 ± 1.20^{a1}	23.00 ± 0.58^{bc1}	48.00 ± 2.00^{d2}	64.33 ± 6.39^{cd2}	67.00 ± 12.70^{de2}
100mg/kg	17.67 ± 0.33^{a1}	19.67 ± 1.20^{ab1}	33.67 ± 3.18^{bc12}	45.33 ± 7.31^{b2}	45.67 ± 7.6^{bc2}
250mg/kg	16.67 ± 0.88^{a1}	25.33 ± 0.88^{c2}	40.00 ± 1.15^{cd3}	54.00 ± 5.20^{bc4}	56.67 ± 1.76^{cd4}
500mg/kg	16.33 ± 1.20^{a1}	18.33 ± 2.33^{a1}	46.67 ± 5.55^{d2}	75.67 ± 4.18^{d3}	82.00 ± 3.21^{e3}

Mean values in a column with different letters as superscript are significantly different at $p < 0.05$

Mean values in a row with different numbers as superscript are significantly different at $p < 0.05$

3.10 Effects of Different Doses of Aqueous *Dennettia tripetala* Leaf Extract on the Total Triglyceride (TG) Level in Hyperthyroid Male Albino Rats

Table 12 shows the weekly effects of aqueous *D. tripetala* leaf extract on the TG level in hyperthyroid male albino rats. At week 0 (baseline), 250mg/kg and 500mg/kg extract dosed groups recorded no significant difference ($P > 0.05$) when compared with the normal control and standard control groups. Week 1 result recorded there was no significant difference ($P > 0.05$) in the TG levels of the 500mg/kg extract dosed group when compared with the hyperthyroid control and standard control groups, however, these varied significantly ($P < 0.05$) when compared with the normal control and other extract dosed groups (100mg/kg and 250mg/kg extract dosed groups). In addition, the 250mg/kg extract dosed group showed no significant difference ($P > 0.05$) in comparison with the normal control group while 100mg/kg extract dosed group recorded the least TG value (46.63±0.67). However in week 2, whereas the extract dosed groups recorded no significant difference ($P > 0.05$) when compared with the normal control groups, a statistical significant difference ($P < 0.05$) was observed in comparison with the hyperthyroid control and standard control groups. It is also noteworthy that the standard control group recorded the highest TG value while the hyperthyroid control group recorded the lowest value. The difference was significant ($P < 0.05$). Furthermore, the 100mg/kg extract dosed group had no significant difference ($P > 0.05$) when compared to the hyperthyroid control group but was significantly lower ($P < 0.05$) in comparison with the other groups in week 3. Also, 250mg/kg and 500mg/kg extract dosed groups were significantly higher when compared to the control groups and the 100mg/kg extract dosed groups. Moreover, the 500mg/kg extract dosed group was statistically similar ($P > 0.05$) to the hyperthyroid control group but was significantly higher ($P < 0.05$) when compared to the other groups at week 4. The 250mg/kg extract dosed group

Table 12: Weekly effects of different treatments of aqueous *D. tripetala* leaf extract on the TG levels (mg/dL) of hyperthyroid male albino rats

Groups	Duration				
	Week 0	Week 1	Week 2	Week 3	Week 4
Normal Control	49.07 ± 4.68^{ab1}	63.17 ± 4.44^{ab12}	66.53 ± 3.28^{ab12}	72.40 ± 10.19^{ab2}	60.47 ± 3.77^{bc12}
Hyperthyroid Control	40.73 ± 1.79^{a1}	70.73 ± 6.23^{b2}	38.70 ± 4.39^{a1}	31.37 ± 1.42^{a1}	33.23 ± 1.90^{a1}
Standard Control	44.27 ± 5.00^{ab1}	65.90 ± 7.21^{b1}	79.00 ± 5.47^{b12}	64.63 ± 26.34^{ab1}	111.20 ± 10.35^{d2}
100mg/kg	52.27 ± 3.04^{b1}	46.63 ± 0.67^{a1}	49.97 ± 22.90^{ab1}	53.87 ± 3.14^{a1}	54.50 ± 4.43^{b1}
250mg/kg	46.47 ± 1.56^{ab1}	62.37 ± 11.71^{ab12}	68.23 ± 6.05^{ab12}	98.57 ± 6.47^{b3}	77.30 ± 11.02^{c23}
500mg/kg	45.23 ± 1.07^{ab1}	72.47 ± 3.26^{b2}	69.13 ± 8.01^{ab2}	105.77 ± 9.12^{b3}	106.53 ± 4.42^{d3}

Mean values in a column with different letters as superscript are significantly different at $p < 0.05$

Mean values in a row with different numbers as superscript are significantly different at $p < 0.05$

was significantly higher ($P < 0.05$) when compared with the normal control and hyperthyroid control groups while the 100mg/kg extract dosed group was significantly lower in comparison with the normal control group but significant higher ($P < 0.05$) when compared to the hyperthyroid control group. Additionally, a significant dose-dependent increase in the TG levels was observed in weeks 1, 3 and 4 while insignificant dose dependent increase was observed in week 2.

Minimal fluctuation occurred in the mean weekly TG levels of the rats in the extract dosed groups. Time-dependent analysis showed that in 100mg/kg extract dosed group, the TG level decreased insignificantly ($P > 0.05$) at weeks 1 and 2 when compared to week 0, while the TG level at weeks 3 and 4 increased insignificantly ($P > 0.05$) when compared to week 0. In 250mg/kg and 500mg/kg extract dosed groups, the TG levels increased significantly ($P > 0.05$) at weeks 1, 2, 3 and 4 when compared to week 0.

3.11 Histopathological Features of the Thyroid Glands in Hyperthyroid and Control Rats

Figure 1 shows the photomicrographs of the histopathological sections of thyroid gland from the extract treated rats and control rats. The sections of the thyroid gland from the normal control, standard control and extract treated groups (groups 1, 3, 4, 5 and 6) revealed the well known histological picture of thyroid gland. The glands were composed of spherical follicles lined with single layer of tall columnar epithelial cells with rounded nuclei surrounding a lumen filled with gel-like viscous iodine-rich material called colloid exhibiting serrated or vacuolated peripheral edges. However, the section of the thyroid gland from the hyperthyroid control group (group 2) showed a marked change from the normal thyroid gland. The change observed involved the absence of colloid in some follicles (empty follicles-arrows). In addition, some follicles are lined with low collumnar epithelial cells.

Thus, the photomicrographs of the histopathological sections of thyroid gland of the extract treated groups showed no histological difference when compared to the normal control and standard control groups. However, the photomicrograph of section of the thyroid gland from the Hyperthyroid control group showed a histological difference when compared to the other control groups and extract treatment groups.

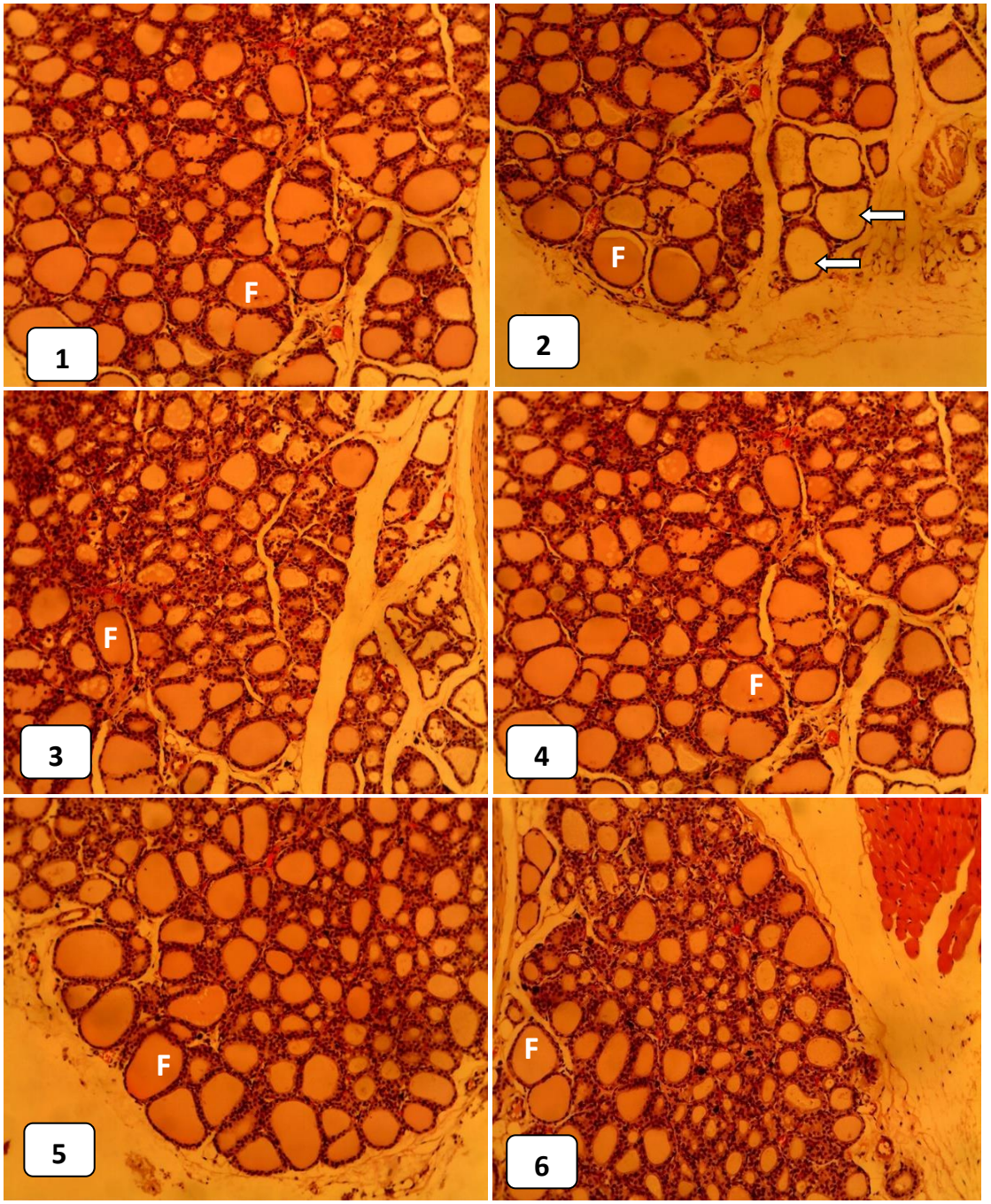

Figure 1. Photomicrograph of sections of the thyroid gland from experimental groups 1 (normal control group), 2 (hyperthyroid control group), 3 (standard control group), 4 (100mg/kg extract dosed group), 5 (250mg/kg extract dosed group) and 6 (500mg/kg extract dosed group) showing the follicles (F) containing colloids. See the white arrows showing absence of colloid in some follicles in group 2 (hyperthyroid group). There is no remarkable histologic differences in 1, 3, 4, 5 and 6. H and E x 400 Mag.

CHAPTER FOUR

DISCUSSION AND CONCLUSION

4.1 Discussion

Interest in medicinal plants is as a result of their richness in secondary metabolites such as alkaloids, glycosides, flavonoids, steroids, tannins, and saponins (Rohini and Padmini, 2016). The present study of the phytochemical screening of aqueous *D. tripetala* leaf extract revealed the presence of these secondary metabolites. This agrees with the work of Okoronkwo *et al.* (2015) who carried out studies on evaluation of chemical compositions and in vitro antimicrobial activity of extracts from *Dennettia tripetala* leaves; Osuagwu and Eme (2013) who examined phytochemical composition and antimicrobial activity of *Dialium guineense*, *Vitex doniana* and *Dennettia tripetala* leaves. These phytochemical constituents are known to exhibit medicinal as well as physiological activities (Ayoola *et al*., 2008).

The observation made in the present study that the aqueous *D. tripetala* leaf extract showed no lethal effect on the experimental animals at different doses administered, corroborate past report on this plant species. Anosike (2016) observed that the ethanol extract of *D. tripetala* seed extract were not toxic even at dose level of 5000mg/kg. This implies that *D. tripetala* extracts are of low toxicity.

The present study revealed that hyperthyroid induced rats showed a decrease in body weight when compared to the normal rats. However, the body weight increased when treated with different doses of aqueous *D. tripetala* leaf extract (100mg/kg, 250mg/kg and 500mg/kg) and standard drug (1.35mg/kg Carbimazole) orally. This is in line with the work of Bhaigyabati *et al*. (2012) who used methanolic extract of sweet corn silk to revert the hyperthyroid condition in Swiss albino rats. Thyroid hormones stimulate the rate of

metabolism and heat production. Since the basal metabolic rate (BMR) in patients with hyperthyroidism is elevated, many patients with an overactive thyroid do, indeed, experience some weight loss. Surprisingly, the body weight of the rats in the hyperthyroid control group also increased. This could be attributed to the fact that the hyperthyroid rats increased the calories consumed to match the excess calories burned since those rats are allowed free access to water and feed.

Hyperthyroidism induced rats showed a decrease in TSH levels and increase in T_3 and T_4 levels. However, treatment with three different concentrations of aqueous *D. tripetala* leaf extract (100mg/kg, 250mg/kg and 500mg/kg body weight) showed a decrease in T_3 and T_4 levels and increase in TSH level, just as was observed with the standard drug. This agrees with the findings of Donzelli *et al*. (2016) who examined the effects of hypothyroidism and hyperthyroidism on tissue thyroid hormones in Wistar out-bred albino rats; Bhaigyabati *et al.* (2012) where methanolic extract of sweet corn silk revert the hyperthyroid condition in Swiss albino rats. The anti-thyroid properties of *D. tripetala* may be attributed to the presence of some phytochemicals in the extract. According to Sartelet *et al.* (1996), flavonoids extracted from fonio millet (*Digitaria exilis*) revealed potent anti-thyroid properties. Flavonoids not only inhibit thyroid peroxidase (TPO) but, acting on iodothyronine deiodinase enzymes, also inhibit the peripheral metabolism of thyroid hormones. Flavonoids also affect serum thyroid hormone binding and thyrotropin (TSH) regulation. Furthermore, polymers of the flevonoid phloretin interact with TSH, preventing its action on the thyroid cell (Cody *et al*., 1986).

Cholesterol is a lipid that combines with protein to form lipoprotein for adequate circulation in the blood. When lipoprotein has more protein than cholesterol, it is called high density lipoprotein cholesterol (HDL-C) and its circulation in the body till it gets to the liver is faster. However, when the lipoprotein has more cholesterol than protein, it is called low

density lipoprotein cholesterol (LDL-C) and its circulation in the body is slower leading to the obstruction of blood vessels (Nourooz-Zadeh *et al*., 1996). HDL-C is the good cholesterol and LDL-C is the bad cholesterol. The LDL-C is the major carrier of cholesterol in the blood. The role of LDL-C is to transport cholesterol to the peripheral tissues and regulate cholesterol synthesis at the sites (Mayes, 2000). When there is high cholesterol, the HDL-C and LDL-C levels are reversed making LDL-C level higher than HDL-C.

In this study, hyperthyroidism resulted in a decrease in the mean total cholesterol, LDL-C and triglyceride levels and an increase in HDL-C level in the experimental rats. After twenty eight days of treatment with standard drug and different concentrations of aqueous *D. tripetala* leaf extract (100mg/kg, 250mg/kg and 500mg/kg), TCH, LDL-C and TG levels increased while HDL-C decreased. This is in agreement with the work of Asvold *et al*. (2007) who reported that a linear positive association exists between thyroid-stimulating hormone (TSH) values and concentrations of total serum cholesterol, LDL cholesterol, non-HDL cholesterol and TG, and a linear negative association with HDL cholesterol. Similarly, Duntas (2002) maintained that hyperthyroidism exhibits an enhanced excretion of cholesterol and an increased turnover of LDL resulting in a decrease of total and LDL cholesterol levels. Decreased thyroid secretion greatly increases the plasma concentration of cholesterol because of decreased rate of cholesterol secretion in the bile and consequent diminished loss in the faeces due to the decreased number of low density lipoprotein receptors on liver cells (Guyton and Hall, 2000).

Thyroid hormones induce 3-hydroxy-3-methylglutarylcoenzyme A (HMG-CoA) reductase, which is the first step in cholesterol biosynthesis. Triiodothyronine (T_3) causes an up-regulation of LDL receptors, controls the sterol regulatory element-binding protein-2 (SREBP-2), which in turn regulates LDL receptor's gene expression and protects LDL from

oxidation (Rizos *et al*., 2011). Thyroid hormones influence HDL metabolism by increasing cholesteryl ester transfer protein (CETP) activity, which exchanges cholesteryl esters from HDL2 to the very low density lipoproteins (VLDL) and TGs to the opposite direction. It stimulates lipoprotein lipase (LPL), which catabolises the TG-rich lipoproteins, and the hepatic lipase (HL), which hydrolyzes HDL2 to HDL3 and contributes to the conversion of intermediate- density lipoproteins (IDL) to LDL and in turn LDL to sdLDL.

In addition, the result revealed that the total cholesterol and triglyceride levels in the extract dosed groups were significantly lower when compared to the standard control group while the HDL-C levels in the extract dosed groups were insignificantly higher when compared to the normal control groups. This could be attributed to the presence of some phytochemicals in the extract that have beneficial effects on blood cholesterol levels. According to Subramani and Casimir (2002), flavonoids prevent the oxidation of low-density lipoprotein, lowers the blood levels of cholesterol and triglycerides thereby reducing the risk for the development of atherosclerosis. Saponins have been reported to have beneficial effects on blood cholesterol levels. They bind with bile salt and cholesterol in the intestinal tract. This binding causes a reduction of blood cholesterol by preventing its re-absorption (Oyewole and Akingbala, 2011).

Histopathologies of the thyroid gland of the hyperthyroidism induced and treated rats were studied and were compared with the control rats. Photomicrograph of sections of the thyroid gland from experimental groups showed the absence of colloid in some follicles in hyperthyroid control. There was no remarkable histologic differences in normal control, standard control and extract dosed groups.

4.2 Conclusion

Conclusively, the aqueous *Dennettia tripetala* leaf extract has the potential to overcome hyperthyroidism in albino rat. Although, the mean total cholesterol, LDL cholesterol and triglyceride levels in the extract dosed groups were significantly increased when compared to the normal and hyperthyroid control groups, the mean total cholesterol and triglycerides were significantly decreased and HDL cholesterol increased in the extract dosed groups when compared to the standard control groups. The groups treated with 250mg/kg and 500mg/kg evidently showed a better result than that of the standard drug.

RECOMMENDATION

The consumption of *Dennettia tripetala* is highly recommended to all especially people with hyperthyroidism, at a dose of 250mg/kg to 500mg/kg of body weight continously for 28 days. However, it is also recommended that its consumption should be standardized to checkmate the extra significant effects observed on the lipid profile. Furthermore, the bioactive ingredients in the leaves should be investigated and properly documented.

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