EVALUATION OF THE EFFECT OF ETHANOLIC EXTRACT OF *Sesamum indicum* ON KETOCONAZOLE INDUCED TESTICULAR DAMAGE IN MALE WISTAR RATS

BY

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1.0 INTRODUCTION

BACKGROUND INFORMATION

Toxins such as drugs, chemicals, endocrine disruptors, biogenetically engineered western diets and life style among other factors are known to affect normal body functions and most especially reproductive health (Izegbu *et al.,* 2005). One of such toxins (Drugs) which posses adverse health hazard despite it`s advantages is Ketoconazole an antifungal drug used in the treatment of fungal infections such as blastomycosis, histoplasmosis, coccidioidomycosis and para coccidioidomycosis (Amir, 2008).

Ketoconazole is packaged pharmaceutically in the form of tablets, Creams and injection and has been reported to inhibit the stimulation of testicular testosterone production by Human Chorionic Gonadotropine in a dose dependent manner (Richard *et al.,* 2005). Steroids such as dihydrotestosterone and estradiol are equally, selectively displaced from serum-binding globulin by ketokonazole (Grosso *et al.,* 2005). The suppression of testicular testosterone synthesis and displacement of oestrogen from sex hormone binding globulin may decrease the androgen/oestrogen ration of the blood and contributes to the development of gynaecomastia that has being reported in some ketoconazole treated patients (Boyden *et al.,* 2005).Administration of ketoconazole alters sperm indices and causes severe histopathological lesion such as depletion of germ cells, degeneration of the seminiferous tubules, marked oxidative damage to testicular lipids and alteration of natural anti oxidants(Amin,2008).

Over the years, there has being increased scientific research to minimize the health hazards potentiated by some toxin and this was done on phytochemical being extracted from plant species (Bankole *et al.,* 2007). In an effort to stem the contraindications of some of these

synthesized drugs, Plant medicine (phyto-medicine) has being used as alternatives in many parts of Africa and the rest of the world (Elujoba *et al.,* 2005).

Effective health cannot be achieved in Africa, unless orthodox medicine is complemented with traditional medicine (Elujoba *et al.,* 2005).At least 80% of Africans depends on plant medicine for their health care (Sofowora, 1993).Fruit and vegetables have being recognized as natural source of various bioactive compounds (Penington and Fisher, 2010). The bioactive property of fruits and vegetables could be attributed to their phyto-constituents such as flavanoids, anthocyanins, vitamins C and E, phenolic compounds, dietary fiber and carotinoids (Gonz, Aguilar, *et al.,* 2008).One of such vegetable plant is Sesame *indicum* whose taxonomic O*rder* is *lamiales,* Family is *pedialiaceae,* Genus is *sesamum* and Specie is *indicum.* Sesame seeds exist in brown, black and yellow forms and are called " *ridi" in* Hausa, " Ekuku" in Yoruba, " Isasa" in Ibo and " *go-ri-go"* in Ebira languages.

Sesame seed has being reported to contain high level of unsaturated fatty acids which are Oleic acid-38.84%, linolenic acid-46.26 % (Nzioku *et al.,* 2009). Trace elements such as Calcium, Iron, Magnesium, Zinc, Copper and Phosphorous are also reported to be contained in it (Obiajunwa *et al.,* 2005). Sesame seed is also reported to be rich in phyto estrogenic lignans which is an important phytochemical known to man (Thompson *et al.,* 1991).

Sesame seed oil has being used as a healing oil for thousands of years it naturally posses antibacterial property for common skin pathogen such as staphylococcus and streptococcus it also posses anti fungal, antiviral and anti inflammatory effects (Amir, 2008). The entire sesame plant are very valuable and serve as staple food in most ethnic groups in north central and south

western part of Nigeria and this may be attributed to the high level of fecundity among adult males in these regions (Akpan *et al* .,2006).

2.0 CHAPTER TWO (LITERATURE REVIEW)

Studies on Testicular damages have drawn the attention of considerable number of people especially amongst Basic Medical Scientists and Clinicians over the past few years (Skakkebaek, 2003; Shittu, 2006, 2010; Shittu et al., 2005, Shittu et al., 2006a).

The prevalence of testicular damages worldwide has been attributed to exposure to environmental toxin/endocrine disruptors and the infiltration of biogenetically engineered western diets and lifestyles among other factors into the developing worlds (Izegbu et al., 2005; Shittu, 2006, 2010; Shittu et al., 2007a, Shittu et al., 2008a).

More disturbing is the declining sperm quality of human and wildlife species due to environmental exposures to these endocrine disruptor pollutants and chemicals that are believed to cause gene mutations as noticed over some years (Duty et al., 2003, Sharpe & Irvine, 2004; Sharpe & Shakkebeak, 1993; Shittu, 2006, 2010; Shittu et al., 2008a; Vos et al., 2000; Zuping et al., 2006).Fungal infections such as histoplasmosis and coccidioidomycosis has being ranked as one of the leading causes of testicular damages especially in Africa (WHO, 1987; WHO, 1991) Attempt to tackle this, has generated problems of numerous adverse effects of the synthetic chemical/hormonal agents (antibiotics, and steroids etc) available as treatment options.(Shittu, 2006, 2010; Shittu et al., 2009).

Intake of ketoconazole induces reproductive toxicity such as clear reduction of weight of the testes and epididymis, alteration of sperm indices like serum testosterone and severe

histopathological lession such as degeneration of the seminiferous tubules and depletion of germ cells.

Similarly, marked oxidative damages to testicular lipid and alteration of biomarkers of lipid peroxidation such as Catalase and Super Oxide Dismutase (SOD) were reported in association with ketoconazole intake (Amir, 2008).

Ketoconazole (kee" toe kon' a zole) is an imidazole derivative and fungicidal agent which is believed to work by several mechanisms, including inhibition of the fungal 1,4α-ergosterol demethylase which is responsible for converting lanosterol to ergosterol and which blocks cell membrane synthesis(United States National Library of Medicine, 2015)

Ketoconazole may also inhibit fungal triglyceride and phospholipid synthesis and fungal oxidative and peroxidative enzyme activity, causing accumulation of hydrogen peroxide, contributing to deterioration of organelles. Ketoconazole is used in the treatment of a many fungal infections including blastomycosis, candidiasis, coccidiomycosis, tinea pityriasis versicolor and histoplasmosis. Ketaconazole has been used as adjuvant therapy of prostate cancer, because of its effects in lowering androgen production by both the testes and adrenal glands. Ketoconazole was approved for use in the United States in 1981, but has largely been replaced by other antifungal agents that have fewer side effects and wider range of activity. Current indications include systemic fungal infections due to candida, blastomycosis, coccidiomycosis, histoplasmosis, chromomycosis and paracoccidioidomycosis.(United States National Library of Medicine, 2015)

Concerted effort is continuously made leading to an increased intensive study on extracts and other active phytochemicals extracted from plant species that can be used to minimize these testicular damages (Bankole et al., 2007; Hanilyn, 1998; Nascimento et al., 2000; Shittu, 2010; Shittu et al., 2006b; Shittu et al., 2007b).

Moreover, these phytochemicals are equally recognized by the World Health Organization to have proven potential of treating microbial infections, diabetes, cancers and infertility/ subfertility among other known chronic medical conditions (Bankole et al., 2007; Shittu, 2010; Shittu et al., 2006b; Shittu et al., 2007a; Shittu et al., 2008a; Shittu et al., 2009).

As at today, natural resources have been used to produce various bioactive compounds such as alkaloids, ethanol, organic acids, immunomodulator and vitamins that could cause testicular enhancement. (www.intechopen.com)

Improved Fertility Potential and Antimicrobial Activities of Sesame Leaves Phytochemicals and polysaccharides etc that are of medicinal values in food, medical, chemical and biochemical industries have being of research concern to many. (Hanilyn,1998; Shittu, 2006, 2010).

Hence, expressed worldwide is the search for an ideal folkloric phyto-chemical medicinal agent with a broad spectrum and proven potential of treating testicular damages and it`s related condition including microbial infection among the other causes with minimal or no

side effects as compared to synthetic drugs (Bankole et al, 2007; Shittu, 2006, 2010; Shittu et al, 2006b; Shittu et al, 2007, 2007b, 2007c; Shittu et al, 2008a, Shittu et al, 2009; Shittu et al, 2010).

The phyto-estrogens appeared to be one of such natural estrogenic agents that have attracted so much attention in the last decade in view of their reported health benefits and they include four broad classes of phytochemicals namely the lignans (sesame seed and flaxseed), isoflavonoids (soybeans), stillbenes and coumestrol (Adlercreutz *et al.*, 1986; Murkies, 1998; Shittu, 2006, 2010).

These agents mimic endogenous estrogens and depending on their concentrations, they either act agonistically or antagonistically by displacing the endogenous estrogens from the binding sites on the estrogens receptors among its other mechanisms of actions (Kuiper et al, 1997; Shittu, 2006, 2010).

Based on the fact that sesame are consumed in large amounts in the diet, the metabolic effects noticed are usually that of anti estrogenic, thus, compete with the much more potent endogenous estradiol for the estrogens receptor (ER1 & ER2) binding sites and ultimately blocked their estrogenic activity (Martin *et al.,* 1978; Whitten & Naftolin, 1991).

Sesame plant is one of the richest food sources of phyto-estrogenic lignans, a valuable phyto-chemical known to man since the dawn of civilization (Thompson et al, 1991)

Sesame seed is one of the oldest oilseed crops known, domesticated well over 3000 years ago. It was a major summer crop in the Middle East for thousands of years, (Raghav *et al*.,1990)Sesame is highly tolerant to drought like conditions, making it suitable to grow where other crops may fail (Raghav *et al.,* 1990) Sesame has one of the highest oil contents of any seed. With a rich nutty flavor, it is a common ingredient in cuisines across the world like other nuts and foods; it can trigger allergic reactions in some people. (Raghave *et al.,* 1990)

Sesame fruit is a capsule, normally pubescent, rectangular in section and typically grooved with a short triangular beak. (Oplinger *et al.,*1990). The length of the fruit capsule varies from 2 to 8 cm; its width varies between 0.5 to 2 cm, and the number of loculi from 4 to 12. (Oplinger *et al*., 1990) The fruit naturally splits open (dehisces) to release the seeds by splitting along the septa from top to bottom or by means of two apical pores, depending on the varietal cultivar. The degree of dehiscence is of importance in breeding for mechanized harvesting as is the insertion height of the first capsule (Oplinger *et al.,* 1990).

Sesame seeds are small. The size, form and colours vary with the thousands of varieties now known. Typically, the seeds are about 3 to 4 millimeters long by 2 millimeters wide and 1 millimeter thick. The seeds are ovate, slightly flattened and somewhat thinner at the eye of the seed (hilum) than at the opposite end. The weight of the seeds is between 20 and 40 milligrams. The seed coat (testa) may be smooth or ribbed (Oplinger et al.,1990).

Sesame is very drought-tolerant, in part due to its extensive root system. However, it requires adequate moisture for germination and early growth. While the crop survives drought as well as presence of excess water, the yields are significantly lower in either condition. Moisture

levels before planting and flowering impact yield photoperiod also impacts the oil content in sesame seed; increased photoperiod increases oil content. The oil content of the seed is inversely proportional to its protein content (Oplinger *et al.,* 1990)

Sesame varieties have adapted to many soil types. The high yielding crops thrive best on well-drained, fertile soils of medium texture and neutral pH. However, these have low tolerance for soils with high salt and water-logged conditions. Commercial sesame crops require 90 to 120 frost free days. Warm conditions above 23 °C (73 °F) favor growth and yields. While sesame crops can grow in poor soils, the best yields come from properly fertilized farms (Oplinger *et al.,* 1990)

Sesame has one of the highest oil content of any seed, some varietals exceeding 50% oil content compared to soybean's 20 %. Sesame oil is one of the most stable vegetable oils, with long shelf life, because of the high level of natural antioxidants such as sesamin, sesamolin, and sesamol (Oplinger 2002 and Ray 2011)

Oil from the seed is used to prepare salad oils and margarine, and contains about 47 % oleic and 39% linoleic acid.(Oplinger 2002 and Ray 2011)

Sesame seed oil, like sunflower seed oil, is rich in Omega 6 fatty acids, but lacks Omega 3 fatty acids.(Oplinger 2002 and Ray 2011) Sesame seed is also rich in protein, at 25% by weight. The flour that remains after oil extraction is between 35 to 50 % protein, has good effective carbohydrates, and contains water-soluble antioxidants like sesaminol and glucosides that provide added shelf-life to many products.(Oplinger 2002 and Ray 2011) This flour, also called sesame meal, is an excellent high-protein feed for poultry and livestock. The addition of sesame to high lysine meal of soybean produces a well balanced

animal feed. Sesame seeds contain the lignans pinoresinol and lariciresinol (Ivon, *et al.*, 2008).

Sesame seeds and sesame oil are a serious allergen to some people including infants. In Australia the occurrence of allergy to sesame seed was estimated to be 0.42% among all children, while in the United Kingdom the allergic reaction was found to affect 0.04 % of adults. The occurrence of allergy with intake of sesame in patients with some form of food allergy was found to be much higher than in the general population, ranging from 0.5% in Switzerland to 8.5% in Australia. In other words, allergy arising from sesame affects a small percentage of overall human population, but sesame allergy is highers in people who already show symptoms of allergy to other foods (Clare.,2011)

The symptoms of sesame seed allergy can be classified into Systemic reactions; which is indicative of anaphylaxis(Charlene *et al*., 2009). characterized by symptoms including hives (urticaria), lip and eyelid swelling (angioedema) sneezing, nasal itching, congestion, rhinorrhea, wheezing, cough, tightness of throat, hoarse voice, difficulty in breathing, abdominal pain, unconsciousness, shock with drop of blood pressure. In the systemic reactions can also be included severe reactions like dizziness, drowsiness, chills and collapse as has been reported in patients after ingestion of a falafel burger.

Other symptoms include facial or generalized redness (“flushing”), hives (urticaria) on smaller or larger parts of the body, swelling of the eyelids, lips or other parts of the face, itching of the eyes or of the skin in general, hay fever symptoms in the eyes and eczema. Respiratory symptoms observed include hay fever, asthma, cough, wheeze, or difficulty in

breathing. Gastrointestinal symptoms: Itching in the mouth and/or tongue soon after chewing and ingesting (Oral allergy syndrome) and abdominal pain.

An amounts as low as 100 mg of sesame seeds or flour and 3 ml of oil can trigger allergic reactions in severe cases of sesame allergic individuals. Most patients, however, show allergic reactions after consuming 2–10 grams of sesame seeds or flour. The onset of the symptoms may occur within a few minutes up to 90 minutes after ingestion of a sesame seed product. Most patients had other allergic diseases such as asthma, hay fever, and eczema, and most patients also had a relative with an allergic disease. More than two thirds of the patients with sesame allergy also had food allergic reactions to other foods (Clare.,2011).

Prevalence of sesame allergy varies per country. While it is one of the three most common allergens in Israel (Aoronov *et al.,* 2008) sesame allergy prevalence is considered small relative to other allergens in the United States (Ben Shoshan, Harrigson and Soller *et al*.,2010)Some experts consider sesame allergies to have "increased more than any other type of food allergy over the past 10 to 20 years" in the United States (Charlene *et al*., 2009). Such increasing prevalence led Canada to issue regulations that require food labels to note the presence of sesame.(Health Canada., 2015)

In addition to products derived from sesame such as tahini and sesame oil (Charlain *et al*.,2009). Persons with sesame allergies are warned to stay away from a broad assortment of processed foods including baked goods, tempeh, and generic "vegetable oil."(Canadian food Inspection Agency,2014) In addition to possible food sources, individuals allergic to sesame have been warned that a variety of non-food sources may also trigger a reaction to sesame, including adhesive bandages, cosmetics, hair care products, perfumes, soaps and sunscreens,

drugs, some fungicides and insecticides, lubricants, ointments and topical oils, and pet food.(Canadian Food Inspection Agency,2014)

At least one study found that "standard skin and blood testing for food allergies doesn't predict whether a child has true sesame allergy (Permaul, *et al.,* 2009) In which case, a food challenge under the direction of a physician may be required to properly diagnose a sesame allergy.

There appears to be cross-reactivity between sesame allergens and peanut, rye, kiwifruit, poppy seed, and various tree nuts such as hazelnut, black walnut, cashew, macadamia and pistachios (Permaul *et al.,* 2009)

Sesame seed is now increasingly being incorporated into human diet worldwide because of their reported health benefits (Shittu, 2006, 2010).

The plant is rich in trace elements/minerals such as calcium, iron, magnesium, zinc, copper and phosphorus (Obiajunwa et al, 2005; Shittu, 2006, 2010; Shittu et al., 2006b, Shittu et al, 2009).

All parts of the sesame plant such as the seed, oil and leaves are also useful and are locally consumed as a staple food by subsistence farmers in the Northern, South-west and Middle - belt regions of Nigeria and are used in folkloric medicine in Asia and Africa (Akpan – Iwo et al, 2006; Shittu et al, 2006b; Shittu, 2010). It`s androgenic value may account for the high fecundity among the adult males population in these particular areas (Shittu, 2006; Shittu, 2010). The local names of the plants are" ekuku–gogoro" in Yoruba, "ridi" in Hausa, "go-ri-

go" in Ebira and "beni" (Tiv/Idoma and English) or gingelly (English) (Gill, 1992; Shittu, 2006 2010; Shittu et al, 2009).

Recent study on bioavailability of sesame plants consumption in humans have shown that the lignans usually undergo extensive metabolism in the intestine depending on the characteristics of the individual intestinal microflora to produce mammalian lignans enterolactone especially (Penalvo et al., 2005; Shittu et al, 2009). Moreover, plasma lignans concentrations showed a linear correlation with urinary excretion of lignans (Penalvo et al., 2005; Shittu et al, 2009).

In this study, Tab. Provirone(Mesterolone) is adopted and compaired with ethanolic extract of *Sesamum indicum* as the standared drug. Mesterolone is an androgenic drug that enhances testosterone production (Bayer, 2010). Mesterolone is used in the treatment of potency disturbances such as reduced efficiency in middle and advanced age, complains that are attributed to androgen deficiency like reduced efficiency, fatigue, lack of concentration, weak memory, disturbances of libido, potency, irritability, disturbances of sleep and depressive moods (Bayer, 2010) Mesterolone is also known to enhance growth, development and functioning of androgen dependant target organs by stimulating them (Bayer, 2010). It is also known to help promote growth the development of male secondary sex characteristics especially in the case of prepuberal androgen deficiency (Bayer, 2010). Mesterolone is also used in the treatment of oligozoospermia and deficient leydig cell secretion (Bayer, 2010) with mesterolone, sperm count and sperm quality as well as the fructose concentration in the ejaculate can be improved or normalized, thus increasing the chances of procreation(Bayer, 2010).

Mesterolone acts by binding to serum proteins such as albumin and sex hormone binding globulin leading to generation of maximum serum drug level of 3.1±1.1ng/ml after 1.6±0.6hours(Bayer, 2010) mesterolone is rapidly inactivated by metabolism with a metabolic clearance rate of 4.4±1.6ml.min-1.kg-1(Bayer,2010) the main metabolite for mesterolone metabolism is 1α methyl androsterone(Bayer,2010)

The suppliments added to mesterolone are lactose monohydrate, maize starch, polyvidone-25,000, methyl parahydrobenzoate, propyl parahydrbenzoate and magnesium stearate(Bayer,2010)

Mesterolone is package pharmaceutically with each tablet containing 25mg and the recommended dose is one tablet two- three times daily for for weeks or months depending on the individual diagnoses (Bayer,2010)

3.0 CHAPTER THREE (RESEARCH METHODOLOGY)

3.1 RESEARCH PROBLEM

Few researches have centered on drugs that can reduce the risk associated with ketoconazole treatment particularly on the testes. For this reason, there is the need to conduct research on plants that can ameliorate these effects as they will form an alternative source of treatment. Testicular damage have been reported or linked to medication with ketoconazole; an anti fugal drug used in the treatment of fungal infections such as histoplasmosis, coccidioidomycosis and paracoccidioidomycosis (Amir, 2008).

The adverse effects of this drug are most times regrettably unnoticed by majority of its users.Intake of ketoconazole induces reproductive toxicity such as clear reduction of weight of the testis and epididymis, alteration of sperm indices like serum testosterone and severe histopathological lession such as degeneration of the seminiferous tubules and depletion of germ cells.

Similarly, marked oxidative damages to testicular lipid and alteration of natural anti oxidant Catalase and Super Oxide Dismutase (SOD) were reported in association with ketoconazole intake (Amir, 2008).

3.12 JUSTIFICATION OF THE STUDY.

The need to conduct research on plants that can ameliorate the toxic effects associated with ketoconazole intake is necessary as they may serve as alternative source of treatment.

3.13 SIGNIFICANCE OF THE STUDY

The study will add to the needed literature required for further research in this area of study.

The study may also be useful in the minimizing the testicular damages caused by ketoconazole.

3.14 AIM OF THE STUDY

The aim of the study is to evaluate the effects of ethanolic extract of *Sesamum indicum* on ketoconazole induced testicular damages in male Wister rats.

3.15 THE OBJECTIVES OF THE STUDY ARE

To evaluate sperm cells parameters (sperm cell count, sperm cell motility, sperm cell viability and sperm cell morphology) before and after administration of ketoconazole and ethanolic extract of sesame seed in male Wistar Rats.

To evaluate the histology of the testes following the administration of ketoconazole and ethanolic extract of sesame seed using Heamatoxylin and Eousin stains for routine histological techniques and periodic Acid Schiff (PAS) to demonstrate the prescence of glycoproteins of the testis in male Wistar Rats.

To investigate the role of *Sesamum indicum* seed on biochemical parameters such as Catalase (CAT), Super Oxide Desmutase (SOD), and Malondialdehyde (MDA), level using a spectrophotometer

To determine the testis protein concentration using Bradford reagent.

3.16 MATERIALS

3.17 ANIMALS

A total number of forty two adult male Wistar rats were used for the experiment, they were procured from the Department of Human Anatomy, ABU Zaria and kept under standard laboratory conditions and allowed free access to food and water.

3.18 SEED

Sesamum indicum seed was obtained from samaru market- zaria and authenticated in the herbarium of the Department of Biological Sciences, ABU Zaria.The sesame seed was macerated using 95% ethanol to obtain its extract at the Department of Pharmacology, ABU Zaria and preserved in an air tight container at standard temperature and pressure.

3.19 PROCEDURE FOR MACERATION OF SESAME SEED

470g of sesame seed was macerated having determined the weight using a weighing balance.

A scale pan of 25×75 cm2 was initially weighed and the pan filled with sesame seed. The weight of the sesame seed was determined from the formula below:

Weight of extract+ weight of scale pan - weight of scale pan, the sesame seed was poured into 2 ml maceration apparatus (Pyrex). The macerated seed was later transferred into a beaker .

3.20 APPLICATION OF ETHANOL

3.5 L of 95% v/v of ethanol was used to mix the macerated seed to cause the removal of flavanoids and lignans, it was stirred and kept for six hours to allow the supernatant to collect

above the surface of the ethanol. The supernatant was sieved off using a metallic sieve, while the infranatant was collected into a separate beaker for else use.

3.21 DRYING

The supernatant was collected into an evaporating dish using a spatula and placed on a water bath and heated to a temperature of 60˚C to completely remove the ethanol. The extract was left for eighteen hours to allow for evaporation to take place.

3.22 PACKAGING OF EXTRACT AND WEIHGT

A spatula was used to collect the extract into a 20 ml weighed beaker (pyrex), covered with a foil paper and preserved at normal room temperature.

3.23 WEIGHT OF EXTRACT

The following calculation was used to determine the weight of the extract:

Weight of (extract+ Beaker) – weight of beaker

3.24 DRUGS AND CHEMICALS

200 mg of ketoconazole tablet and 25mg of Tab. Provirone (Mesterolone) with dispatched No. 82339737 (Bayer- 2014) was used.

3.25 EQUIPMENTS. The equipments used for the study included light microscope (Olympus), micro centrifuge, micropippet, spectrophotometer, ocular micrometer,digital weighing balance, dissecting set, orogastric tube, normal saline and fixatives,

3.26 EXPERIMENTAL DESIGN

The animals were randomly distributed into eight groups of six rats each (A-H)

3.27 METHOD

Table 1: Groups of the Animals/Administrations

S/N	GROUPS	ADMINISTRATION(mg)	DECAPITATION DAY
1	A(Normal Control)	Normal Saline daily throughout the experimental period.	Day 17
2	B(Abnormal Control)	Ketoconazole (100mg) once daily for five (5) days (commencing from 13^{th} -18^{th} day of experiment).	Day 17
3	C (Prophylactic)	500mg of ethanolic extract of *sesame indicum*+100mg of ketoconazole. Once daily for twelve and half $(12)_{1/2}$ days and five (5) days respectively.	Day 17
4	D(Therapeutic Low dose)	100mg of Ketoconazole+250mg of ethanolic extract of sesame *indicum* once daily for five (5) days and twelve and half $(12_{1/2})$ days respectively.	Day 17
5	E(Therapeutic	100mg of Ketoconazole+500mg of ethanolic	Day 17

	High dose)	extract of sesame *indicum* once daily for five (5) days and tweive and half days ($12_{1/2}$) respectively.	
6	F(Standard Drug and Ketoconazole)	Ketoconazole (100mg) once daily for five days+0.125mg of mesterolone twice daily for two weeks.	Day 17
7	G (Sesame seed extract only)	500mg of ethanolic extract of *Sesamum indicum* seed only for Twelve and half days($12_{1/2}$) days only	Day 17
8	H(Standard drug only)	0.125mg of Mesterolone twice daily for two weeks	Day 17

3.28 EXPERIMENTAL PROCEDURE

The weight of the animal shall be taken weekly throughout the experimental period and the mean weight of each group shall be calculated to enable us derive a statistical data for the effects of the extracts and ketoconazole on the weight of the wistar Rats.

The following calculation shall be used for deriving the doses of ethanolic extract of sesame seed for the various groups.

An LD_{50} of ethanolic extract of sesamum *indicum* in Wister rats was conducted and was found to be above 5000mg as described below:

3.2.8 .0 METHOD

3.2.8.1 DETERMINATION OF THE MEDIAN LETHAL DOSE

The median lethal dose of the ethanolic extract of sesamumindicum seed was determined using Lorke`s method (1983)

3.2.8.2 EXPERIMENTAL PROCEDURE

The animals were categorized into two phases ofI and II.Phase I consisted three (3) groups of three (3) animals each. While phase II consisted three (3) groups of one (1) animal each. The phase I groups were orogastrically intubated with (10, 100 and 1000)mg of ethanolic extract of sesamumindicum seed respectively with the aid of a calibrated 2ml syringe with an intubation needle 0f size 24 mounted on it. The animals in phase II were similarly administered (1600, 2900 and 5000) mg of the extract respectively.

3.2.8.3 DETERMINATION OF THE EQUIVALENT DOSE OF SESAMUM INDICUM INMILILITRES

The total weight of the extract for the research was obtained by summing up all the administrable dose based on lorke`s method which is calculated as 10×3 + 100×3 + 1,000×3 + 1,600 + 2,900+ 5,000= 1,283mg.

1,283mg was measured using a chemical balance and 1ml of tween -80 (polysorbate) was added to decrease the viscosity or enhance the vehicular movement of the extract considering it`s lipid nature. The concentration obtained was 1,283mg/ml which is equal to 13.297ml when measured using a measuring cylinder.

The equivalent dose of 10mg in ml was measured and the value 0.0104ml was obtained. Similar measurement was made for other doses and the values (0.104, 1.04, 1.66, 3.00 and 5.20) ml was obtained respectively.

3.2.8.4 RESULT

No mortality was recorded in any of the groups, no physical signs of toxicity was equally recorded however, some of the general observationsmade are tabulated below.

3.2.8.5 OBSERVATIONS ON INTAKE OF ETHANOLIC EXTRACT OF *SESAMUM INDICUM*

Table 1

NO. of Animals	Dose (mg)	General observation	Physical signs of toxicity	No. of mortality.
3	10	Apparently healthy and active.	No observable signs of toxicity.	Nil
3	100	Apparently healthy and active.	No observable sign of toxicity.	Nil
3	1000	Apparently healthy and partially inactive.	No observable sign of toxicity	Nil
1	1,600	Apparently healthy, inactive and frigid.	No observable signs of toxicity	Nil
1	2,900	Apparently healthy and partially in active.	No observable sign of toxicity	Nil

1	5000	Apparently healthy and presence of goose hair	No observable signs of toxicity	Nil

3.2.8.6 DISCUSSION

There were no observed physical signs of toxicity at any stage of the experiment; no mortality was equally recordedon administration of all the doses.

3.2.8.7 CONCLUSSION

An LD could not be determined due to record of no mortality in any of the rats. It can be concluded that at a dose of 5000mg, the ethanolic extract of sesamumindicum is safe for consumption in wistar rats and an arbitrarily value may be chosen as a high or low dose for a research on the extract.

The average weight of the rat proposed for the experiment is (130-178) g. Since the primary aim of the study is to evaluate the effects of ethanolic extract of sesame seed on the testes, a dose that is expected to exert an influence, is postulated be 10% and 5% of the maximal limit dose which is equivalent to500mg and 250mg as the high dose and low dose respectively and in the ratio 2:1 This is because, on administering a dose of 5000mg of sesame seed extract, a non of the Rats died.

By simplification, 10% of maximum limit dose (5000) = 500mg. while 5% of the maximal limit dose is 250mg

By extension, the value for group E is 500mg. Similar calculation was made for groups D and C which are the therapeutic low and high dose groups and the values (500 and 250) mg were obtained for both respectively. Group B will be administered 100mg/kbw of ketoconazole while group A will be administered 1ml of normal saline throughout the experimental period to keep the rats in normal physiologic state. Group F will be administered 100mgof ketoconazole and 0.125mg/kbw of Mesterolone (testosterone) to serve as the standard group. Group G will be administered 500mg of ethanolic extract of sesame seed to verify Shitus` work on sesame. Group H will be administered 0.125mg of mesterolone to equally verify the effect of mesterolone in the testes of Wister rats.

The equivalent doses of the drugs and the sesame seed extract; in ml (milliliters) will be derived using the formulae stated below:

$$\text{Volume (ml)} = \frac{\text{Dose} \times \text{weight(kg)}}{\text{Stock concentration}}$$

After humanely sacrificing the animals as specified in table one (1), the testes will be excised, and weighed after being washed in normal saline solution. While the right testis will be fixed in Phosphate Buffered Solution (PBS) to enable us determine the testis protein concentration, the left testis will be fixed in Boiun`s fluid for routine histological analysis.

The epididymis of the left testis will be removed and immersed in 5ml of formal saline for determination of sperm parameter. Blood sample shall be collected from the heart via cardiac puncture into a plain tube for biochemical studies.

3.29 MORPHOLOGICAL STUDIES

3.30 ORGAN WEIGHT

The changes in mean weights of the body alongside the testicular weight will be determined using a weighing balance.

3.31 HISTOLOGICAL STUDIES.

(i) The fixed testis will be processed using routine histological techniques, staining will be done with H and E and periodic Acid Schiff (PAS) will be used to determine the glycoprotein present in the sperm.

(ii) Sperm parameter will be determined by conducting semen analyses

(iii) The vertical and horizontal diameters of the lumen of the semininеferous tubules will be measured using an ocular micrometer.

(iv) Osmium tetra oxide will equally be used to demonstrate the steroid secreting cell and studied underlight microscopes at a magnification of ×400mg.

(v) The height of the epithelium of each seminiferous tubule will be determined using ocular micrometer and the mean for each group will be obtained.

3.32 BIOCHEMICAL STUDIES.

A section of the testis from each group will be suspended into 100microlitre of lysis buffer and homogenized on ice to obtain tissue lysate.

The protein concentration in the lysate of the testis will be determined using Bradford reagent.

5ml of collected blood sample from each of the groups will be centrifuged at a speed of 5000rpm to obtain it`s serum.

The serum obtained from the blood will be used to determine the level of superoxide dismutase (SOD), catalase (CAT) and malondialdehyde (MDA).

4.0 RESULT

The procedure for the biochemical parameters of the samples are outlined as follows

4.1 REDUCED GLUTATHIONE CONCENTRATION

Reduced glutathione (GSH) concentration measurement was done according to Ellman (1959) as described by bRajagopalan *et al* . (2004). The principle is based on the reaction of 5, 5`- dithiobis nitro benzoic acid (DNTB) and reduced Glutathione (GSH).

4.1.1 REAGENTS:

0.2 M phosphate buffer: 8.40g of NaH2P04 and 9.94g of Na2HPO4 were dissolved in distilled water and made up to 1000ml mark in a volumetric flask. The buffer was adjusted to a PH of 8.0. 10% Trichloro-acetic (TCA) : 10g of TCA was dissolved in distilled water and made up to 100l in the volumetric flask.

Ellman`s reagent is composed of 19.8mg of 5, 5`- dithiobis (nitro benzoic) (DTNB) in 100ml of 0.1% sodium nitrate

4.1.2 PROCEDURE:

1.5ml of 10% TCA was added to 150µl of serum or tissue homogenate (in phosphate-saline at a pH of 7.4) and centrifuged at 4000rpm for 5min. 1 ml of the supernatant was treated with 0.5ml of Ellman`s reagent and 3ml of phosphate buffer (0.2M, pH 8.0). The absorbance read at 412nm. The quantity of the GSH was derived from the graph of the GSH standard curve as shown below.

Type of Analysis: Oxidative Stress Parameters

Work sheet: Reduced Glutathione Concentration (GSH)

Method: Ellman`s (1959) described by Rajagopalan *et al* (2004)

Sample Size: 48

Comment: Mr. Kabiru Yusuf

Date and Time: November, 19 2015, 12:46

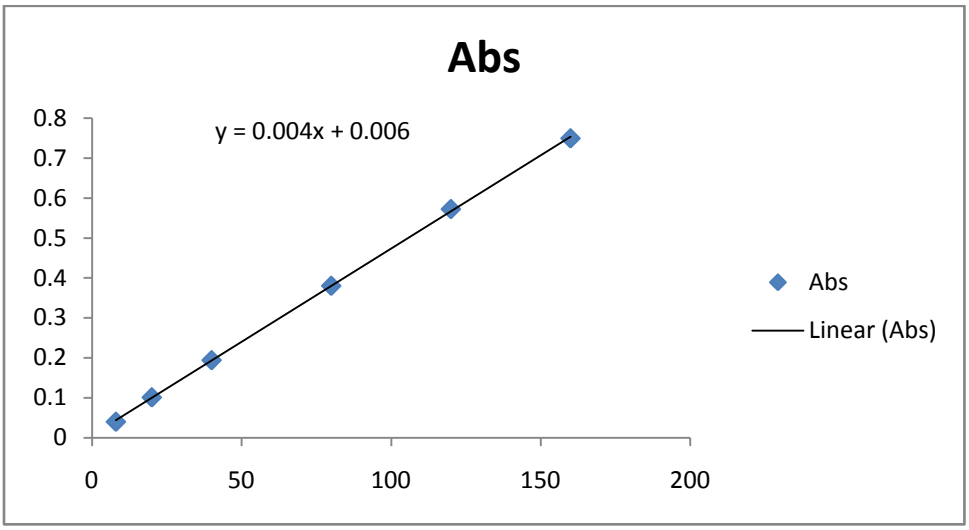
GROUPS.	GSH SAMPLE	DRUG/DOSE	CONC. (µg/ml)	MEAN CONC.	SD (±)
GROUP 1	- CTR1	1ml of Normal saline	231.67	178.61	78.091

	- CTR2		66.67		
	- CTR3		233.33		
	- CTR4		75.00		
	- CTR5		230.00		
	-CTR6	100mg of ketoconazole	235.00		
GROUP 2	+CTR1		138.33	133.88	40.43
	+ CTR2		118.33		
	+ CTR3		141.67		
	+ CTR4		133.33		
	+ CTR5		136.67		
	+ CTR6		135.00		
GROUP 3	PRLT1	90mg of *Sesamum indicum* seed and100mg of ketoconazole	108.33	139.17	33.28
	PRLT2		186.67		
	PRLT3		93.33		
	PRLT4		166.67		
	PRLT5		185.00		
	PRLT6		95.00		
GROUP 4	TLD1	100mg of ketoconazole and 45mg/kgbwt of *Sesamum indicum* seed.	56.67	66.39	7.27
	TLD2		80.00		
	TLD3		58.33		
	TLD4		63.33		
	TLD5		76.67		
	TLD6		63.33		
GROUP 5	THD1	100mg of ketoconazole and 90mg/kgbwt of *Sesamum indicum* seed.	68.33	59.45	6.14
	THD2		61.67		
	THD3		71.67		
	THD4		45.00		
	THD5		51.67		
	THD6		58.33		
GROUP 6	(KT+SD)1	100mg of ketoconazole and 4.5mg of Tab. Mesterolone	48.33	48.88	32.92
	(KT+SD)2		51.67		
	(KT+SD)3		58.33		
	(KT+SD)4		50.00		

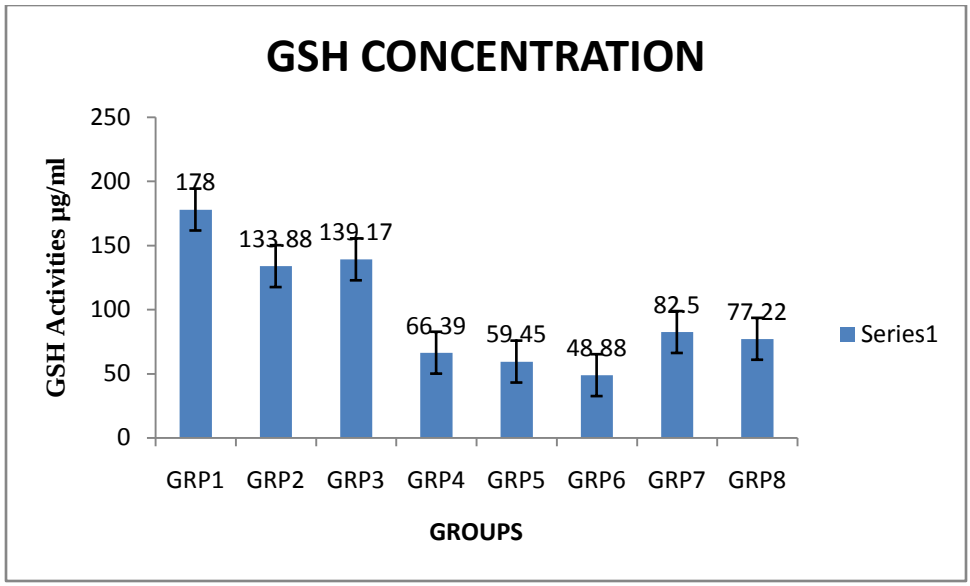
	(KT+SD)5		43.33		
	(KT+SD)6		41.67		
GROUP 7		Tab. Mesterolone 4.5mg		82.5	9.56
	SD1		105.00		
	SD2		85.00		
	SD3		88.33		
	SD4		63.33		
	SD5		81.67		
	SD6		71.67		15.77
GROUP 8		*Sesamum indicum* seed, 90mg/kgbwt.		77.22	
	SS1		71.67		
	SS2		75.00		
	SS3		93.33		
	SS4		55.00		
	SS5		76.67		
	SS6		91.67		

std µg/ml	**Abs**
8	0.04

20	0.101
40	0.194
80	0.38
120	0.572
160	0.749



GROUPS	MEAN CONC.(µg/ml	SD±
GRP1	178	78.09
GRP2	133.88	40.43
GRP3	139.17	33.28
GRP4	66.39	7.27
GRP5	59.45	6.14
GRP6	48.88	32.92
GRP7	82.5	9.56
GRP8	77.22	15.76



4.2 CATALASE (CAT) ACTIVITY

Catalase (Cat) activity was measurd using Abei`s method(1974). 10µl of serum was added to test tube containing 2.8ml of 50mM potassium Phosphate (buffer pH7.0). the reaction was initiated by adding 0.1ml of freshly prepared 30 mM H_2O_2 and the decomposition rate of H_2O_2 was

measured at 240 nm for 5 minutes on a spectrophotometer. Amolar extinction coefficient (E) of 0.041Mm-1 cm-1 was used to calculate the catalase Activity.

Catalase activity was calculated as $\frac{\text{Absorbance of Sample}}{\text{Molar extinction coefficient(E)}} \times$ Protein concentration (mg/ml)

One unit is the amount of catalase that decomposes 1 Umol of H_2O_2 at pH of 7.0

Catalase concentration = $\frac{\text{Absorbance of sample}}{\text{Molar extinction coefficient.}}$

The catalse concentration are calculated and tabulated in the table below.

Type of Analysis: Oxidative Stress Parameters

Work sheet: Catalase

Method: Abei`s method (1974)

Sample Size: 48

Comment: Mr. Kabiru Yusuf

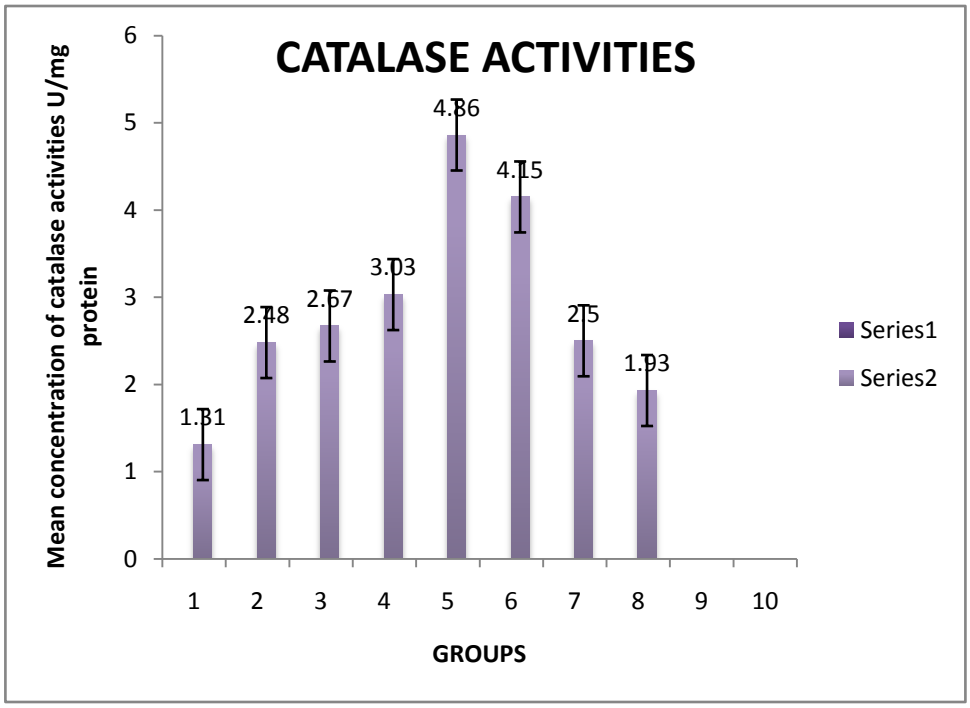
Date and Time: November, 19 2015, 12:46

GROUPS.	CAT. SAMPLE	DRUG/DOSE	CONC. U/mg protein	MEAN CONC.	SD (±)
GROUP 1	- CTR1	1ml of Normal saline	1.5718	1.31	0.53
	- CTR2		0.6228		
	- CTR3		1.7519		
	- CTR4		0.534		
	- CTR5		1.7867		
	-CTR6		1.6078		
GROUP 2	+CTR1	100mg of ketoconazole	2.49	2.48	0.23
	+ CTR2		2.082		
	+ CTR3		2.7491		
	+ CTR4		2.3171		
	+ CTR5		2.5402		
	+ CTR6		2.6975		
GROUP 3	PRLT1	90mg of *Sesamum indicum* seed and100mg of ketoconazole	5.4331	2.67	1.30
	PRLT2		2.4993		
	PRLT3		2.0148		
	PRLT4		1.364		
	PRLT5		2.633		
	PRLT6		2.0596		
GROUP 4	TLD1	100mg of ketoconazole and 45mg/kgbwt of *Sesamum indicum* seed.	2.7842	3.03	1.23
	TLD2		2.7384		
	TLD3		2.7987		
	TLD4		0.8969		
	TLD5		4.7939		
	TLD6		4.1613		
GROUP 5	THD1	100mg of ketoconazole and 90mg/kgbwt of *Sesamum indicum*	6.9512	4.86	2.88
	THD2		8.0325		

	THD3	seed.	1.9082		
	THD4		2.5157		
	THD5		1.6282		
	THD6		8.1098		
GROUP 6		100mg of ketoconazole and 4.5mg of Tab. Mesterolone		4.15	0.52
	(KT+SD)1		4.1758		
	(KT+SD)2		3.6884		
	(KT+SD)3		4.7419		
	(KT+SD)4		3.8282		
	(KT+SD)5		4.9501		
	(KT+SD)6		3.6043		
GROUP 7		Tab. Mesterolone 4.5mg			
	SD1		4.0163	2.50	1.21
	SD2		2.1317		
	SD3		0.5528		
	SD4		1.6095		
	SD5		3.685		
	SD6		3.0518		
GROUP 8					
	SS1	*Sesamum indicum* seed, 90mg/kgbwt.	1.5658	1.92	1.33
	SS2		0.5498		
	SS3		1.503		
	SS4		3.9981		
	SS5		0.5127		
	SS6		3.3865		

GROUPS	MEAN CONC(µg/ml	SD±
GRP1	1.31	0.53
GRP2	2.48	0.23
GRP3	2.67	1.3
GRP4	3.03	1.23
GRP5	4.86	2.88
GRP6	4.15	0.52
GRP7	2.5	1.21

GRP8 1.93 1.33



4.3 LIPID PEROXIDATION

Lipid Peroxidation as an evidence by the formation of TBARS was measured by the modified method of Niehaus & Samauelson (1968) and described by Akanji *et al.*(2009)

The principle is based on the fact that lipid peroxidation generates peroxide intermediate which upon cleavage release malondialdehyde, a product which reacts with thiobarbituric acid. The product of the reaction is a coloured complex which absorbs light at 535 and can be measured

Reagents :

15% Trichloro acetic Acid (TCA) solution, 0.37% Thiobarbituric Acid (TBA) and Hydrochloric Acid solution.150µl of serum and tissue homogenate were treated with 2ml of TBA-TCA-HCL reagents(1:1:1) and placed in water bath at 90 ˚c for 60 min. the mixture was cooled and centrifuged at 4000rpm for 5 minutes malonaldehyde complex was then measured at 535 nm. Malonaldehyde formed was then calculated using the molar extinction coefficient of 1.56×10-5 cm-1M-1

$$\text{MDA Conc.} = \frac{\text{Absorbance of sample}}{1.56\times10\text{-}5\text{cm-}1\text{M-}1}$$

$$\text{TBARS Conc.nmols/mg protein} = \frac{\text{Absorbance of sample}}{1.56\times10\text{-}5\times\text{protein Conc.(mg)}}$$

The value of the lipid perioxidation is tabulated below:

Type of Analysis: Oxidative Stress Parameters

Work sheet: Lipid peroxidation(MDA)

Method: Okhawa *et al* (1979) modified by Atawodi *et al* (2011)

Sample Size: 48

Comment: Mr. Kabiru Yusuf

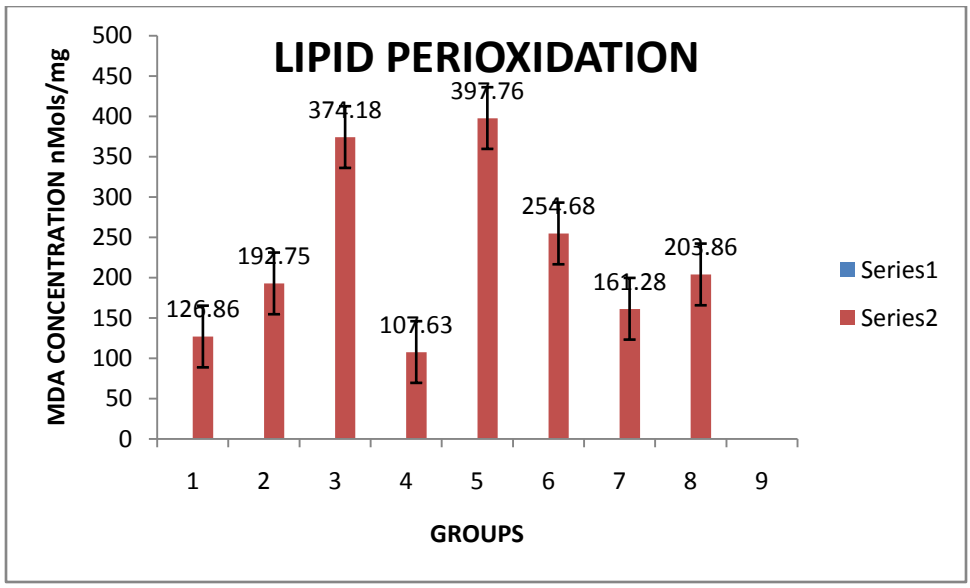
Date and Time: November, 19 2015, 12:46

GROUPS.	MDA SAMPLE	DRUG/DOSE	TBRSCONC. (nMols/mg protein)	MEAN CONC.	SD (±)
GROUP 1				126.86	53.35
	- CTR1	1ml of Normal saline	185.19		
	- CTR2		68.36		
	- CTR3		163.38		
	- CTR4		69.17		
	- CTR5		189.30		
	-CTR6		85.75		
GROUP 2	+CTR1	100mg of ketoconazole	192.69	192.75	13.07
	+ CTR2		183.57		
	+ CTR3		220.88		
	+ CTR4		189.86		
	+ CTR5		182.24		
	+ CTR6		187.24		
GROUP 3					
	PRLT1	90mg of *Sesamum indicum* seed and100mg of ketoconazole	1100.35	374.18	335.13
	PRLT2		298.33		
	PRLT3		173.99		
	PRLT4		113.06		
	PRLT5		365.38		
	PRLT6		193.97		
GROUP 4					
	TLD1	100mg of ketoconazole and 45mg/kgbwt of *Sesamum indicum* seed.	58.73	107.63	60.00
	TLD2		94.11		
	TLD3		56.36		
	TLD4		61.88		
	TLD5		215.59		
	TLD6		159.08		
GROUP 5					
	THD1	100mg of ketoconazole and	669.87		

	THD2 THD3 THD4 THD5 THD6	90mg/kgbwt of *Sesamum indicum* seed.	633.33 146.87 160.07 121.79 654.65	397.76	255.32
GROUP 6	(KT+SD)1 (KT+SD)2 (KT+SD)3 (KT+SD)4 (KT+SD)5 (KT+SD)6	100mg of ketoconazole and 4.5mg of Tab. Mesterolone	262.33 246.08 274.75 235.65 262.97 246.30	254.68	13.14
GROUP 7	SD1 SD2 SD3 SD4 SD5 SD6	Tab. Mesterolone 4.5mg	170.51 180.26 44.07 97.73 261.20 213.88	161.28	71.85
GROUP 8	SS1 SS2 SS3 SS4 SS5 SS6	*Sesamum indicum* seed, 90mg/kgbwt.	52.43 100.12 103.14 640.02 102.62 224.85	203.86	201.97

GROUPS	MEAN CONC	SD
GROUP1	126.86	53.35

GROUP2	192.75	13.07
GROUP3	374.18	335.13
GROUP4	107.63	60
GROUP5	397.76	255.32
GROUP6	254.68	13.14
GROUP7	161.28	71.85
GROUP8	203.86	201.97



4.4 Superoxide Dismutase (SOD)

Superoxide Dismutase was determined by a method described by *fridovich* (1989)

The pricinple behind the methodology is the ability of the superoxide (SOD) to inhibit auto oxidation of adrenaline at pH of 10.2. this forms the bases of the assay.

4.4.1 Reagents:

0.05M Carbonate buffer: 14.3g of NaCO3 and Na4.2gHCO3 was dissolved in distilled water and made up to 1000ml in a volumetric flask .the buffer was adjusted to a pH of 10.2

0.3Mm Adrenaline: 0.01g of adrenaline was dissolved in 17ml of distilled water. The solution was prepared fresh.

Procedure : Tissue homogenate of 0.1ml was diluted in 0.9ml of distilled water to make 1:10 dilution of microsome. An aliquant mixture of 0.2 ml of the diluted microsome was added to 2.5ml of 0.05M Carbonate buffer . The reaction was started with the addition of 0.3ml of 0.3Mm Adtrenaline. The reference mixture contained 2.5ml of 0.05 Carbonate buffer, 0.3ml of 0.3Mm Adrenaline and 0.2ml of distilled water. Absorbance was measured over 30s up to 150s at 480nM

Calculations

Increase in absorbance per minute = (A5-A1)/2.5

$$\% \text{ inhibition} = 100 - \frac{\text{increase in absorbance of substrate}}{\text{Increase in absorbance of blank}} \times 100$$

1 unit of sod activity is the quantity of SOD necessary to elicit 50% inhibition of the oxidation of adrenaline to adenochrome in 1 minute.

Type of Analysis: Oxidative Stress Parameters

Work sheet: Superoxide Dismutase (SOD)

Method: Fridovich (1989)

Sample Size: 48

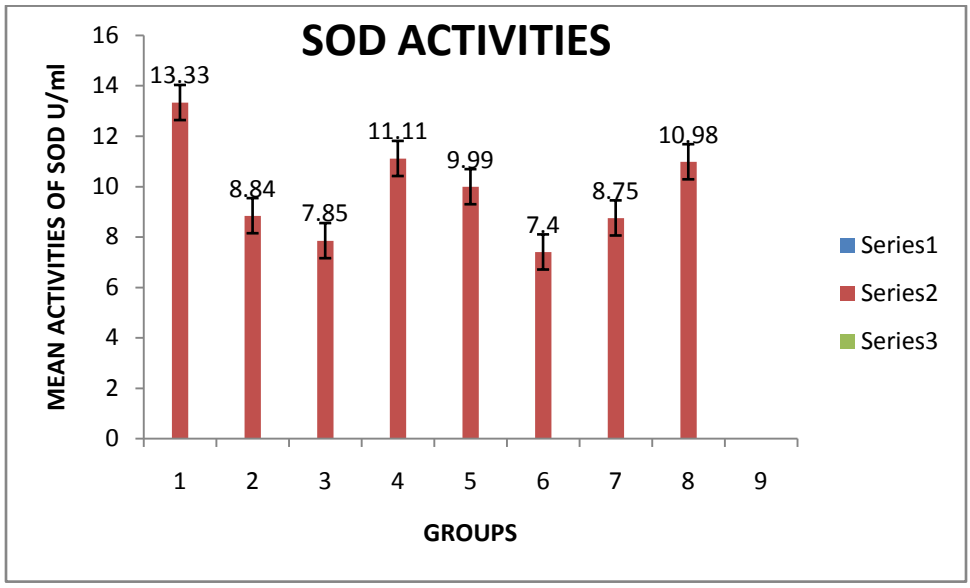
Comment: Mr. Kabiru Yusuf

Date and Time: November, 19 2015, 12:46

GROUPS.	SODSAMPLE	DRUG/DOSE	SOD ACTIVITY. U/ml	MEAN CONC.	SD (±)
GROUP 1	- CTR1 - CTR2 - CTR3 - CTR4 - CTR5 -CTR6	1ml of Normal saline	9.52 6.80 9.52 33.47 11.16 9.52	13.33	9.10
GROUP 2	+CTR1 + CTR2 + CTR3 + CTR4 + CTR5 + CTR6	100mg of ketoconazole	7.62 11.70 10.07 7.07 7.35 9.25	8.44	1.67
GROUP 3	PRLT1 PRLT2 PRLT3 PRLT4 PRLT5 PRLT6	90mg of *Sesamum indicum* seed and100mg of ketoconazole	7.89 5.99 6.80 7.62 11.16 7.62	7.85	1.61
GROUP 4	TLD1 TLD2 TLD3 TLD4 TLD5 TLD6	100mg of ketoconazole and 45mg/kgbwt of *Sesamum indicum* seed.	11.16 10.34 9.80 23.13 9.52 2.72	11.11	6.05
		100mg of			

GROUP 5	THD1 THD2 THD3 THD4 THD5 THD6	ketoconazole and 90mg/kgbwt of *Sesamum indicum* seed.	9.25 9.80 9.52 8.71 9.25 13.33	9.99	1.54
GROUP 6	(KT+SD)1 (KT+SD)2 (KT+SD)3 (KT+SD)4 (KT+SD)5 (KT+SD)6	100mg of ketoconazole and 4.5mg of Tab. Mesterolone	7.89 7.62 6.26 4.63 8.44 9.52	7.4	1.57
GROUP 7	SD1 SD2 SD3 SD4 SD5 SD6	Tab. Mesterolone 4.5mg	3.27 9.25 8.44 9.52 10.34 11.70	8.75	2.70
GROUP 8	SS1 SS2 SS3 SS4 SS5 SS6	*Sesamum indicum* seed, 90mg/kgbwt.	7.62 11.70 11.43 11.70 13.06 10.34	203.86	201.97

GROUPS	MEAN CONC.	SD±
GROUPS1	13.33	9.1
GROUPS2	8.84	1.67
GROUPS3	7.85	1.61
GROUPS4	11.11	6.05
GROUPS5	9.99	1.54
GROUPS6	7.4	1.57
GROUPS7	8.75	2.7
GROUPS8	10.98	1.7



Type of Analysis: Oxidative Stress Parameters

Work sheet: Assay of protein

Method: Sedmark JJ and Gross berg SE, A rapid Sensitive and versatile assay for protein (1977)

The Quick Start Bradford protein assay is a simple and accurate procedure for determining the concentration of protein in solution. It provides ready-to-use convenience by supplying the dye reagent at 1x concentration and two protein assay standards at seven prediluted concentrations. The prediluted standards are conveniently packaged in 2 ml screwcap vials, eliminating wasteful and sharp ampoules, and ensuring protein stability over the shelf life of the product.

1.1 Principle

The Bradford assay is a protein determination method that involves the binding of Coomassie Brilliant Blue G-250 dye to proteins (Bradford 1976). The dye exists in three forms: cationic (red), neutral (green), and anionic (blue) (Compton and Jones 1985). Under acidic conditions, the dye is predominantly in the doubly protonated red cationic form (Amax = 470 nm). However, when the dye binds to protein, it is converted to a stable unprotonated blue form (Amax = 595 nm) (Reisner et al. 1975, Fazekes de St. Groth et al. 1963, Sedmack and Grossberg 1977). It is this blue protein-dye form that is detected at 595 nm in the assay using a spectrophotometer or microplate reader. H+ H+ Cation « Neutral form « Anion

470 nm (red) 650 nm (green) 595 nm (blue) **2**The Quick Start Bradford protein assay is a simple and accurate procedure for determining the concentration of protein in solution. It provides ready-to-use convenience by supplying the dye

reagent at 1x concentration and two protein assay standards at seven prediluted concentrations. The prediluted standards are conveniently packaged in 2 ml screwcap vials, eliminating wasteful and sharp ampoules, and ensuring protein stability over the shelf life of the product.

Sample Size: 48

Comment: Mr. Kabiru Yusuf

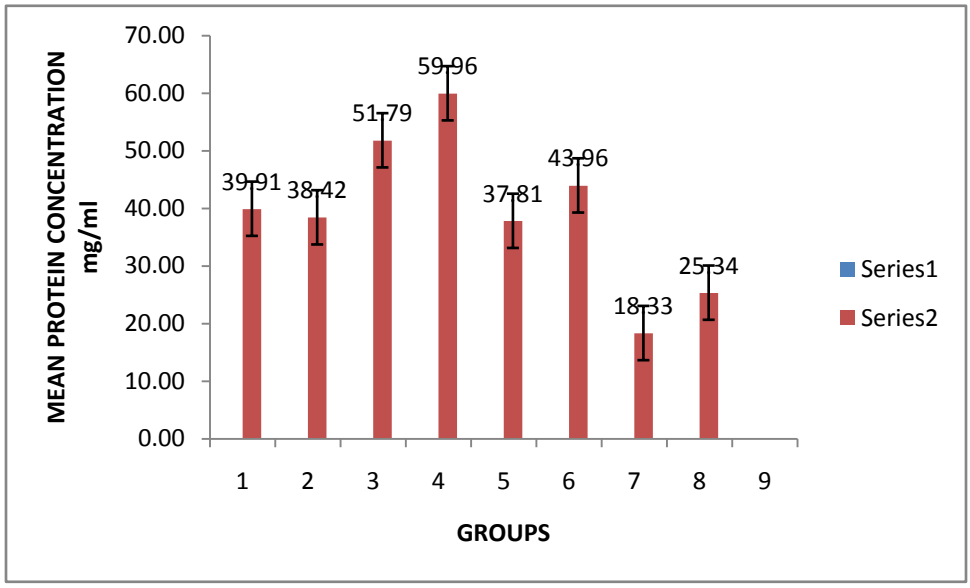
Date and Time: November, 19 2015, 12:46

GROUPS.	TISSUE SAMPLE	DRUG/DOSE	PROTEIN CONC. mg/ml	MEAN CONC.	SD (±)
GROUP 1	- CTR1 - CTR2 - CTR3 - CTR4 - CTR5 -CTR6	1ml of Normal saline	14.74 57.63 58.68 14.21 59.21 35.00	39.91	19.82
GROUP 2	+CTR1 + CTR2 + CTR3 + CTR4 + CTR5 + CTR6	100mg of ketoconazole	38.68 36.84 38.16 38.42 39.47 38.95	38.42	0.82
GROUP 3	PRLT1 PRLT2 PRLT3 PRLT4 PRLT5 PRLT6	90mg of *Sesamum indicum* seed and100mg of ketoconazole	53.68 48.68 41.32 64.21 54.74 48.16	51.79	7.06
GROUP 4	TLD1 TLD2 TLD3 TLD4 TLD5 TLD6	100mg of ketoconazole and 45mg/kgbwt of *Sesamum indicum* seed.	57.63 57.63 63.16 54.74 65.79 60.79	59.96	3.72
GROUP				37.81	

5	THD1 THD2 THD3 THD4 THD5 THD6	100mg of ketoconazole and 90mg/kgbwt of *Sesamum indicum* seed.	55.00 55.00 6.84 7.37 54.21 48.42		21.83
GROUP 6	(KT+SD)1 (KT+SD)2 (KT+SD)3 (KT+SD)4 (KT+SD)5 (KT+SD)6	100mg of ketoconazole and 4.5mg of Tab. Mesterolone	43.95 43.42 44.21 44.47 43.95 43.68	43.96	0.34
GROUP 7	SD1 SD2 SD3 SD4 SD5 SD6	Tab. Mesterolone 4.5mg	22.37 14.21 16.84 23.95 17.89 14.74	18.33	3.65
GROUP 8	SS1 SS2 SS3 SS4 SS5 SS6	*Sesamum indicum* seed, 90mg/kgbwt.	15.00 11.58 30.79 40.26 15.26 40.00	25.48	12.01

GROUPS	PROTEIN CONC	SD
GROUP1	39.91	19.82
GROUP2	38.42	0.82
GROUP3	51.79	7.06

GROUP4	59.96	3.72
GROUP5	37.81	21.83
GROUP6	43.96	0.34
GROUP7	18.33	3.65
GROUP8	25.34	12.01



3.33 STATISTICAL ANALYSIS

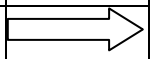
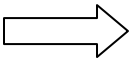
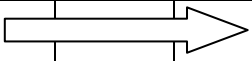
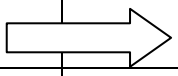
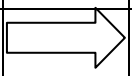
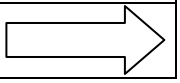
The data obtained from morphological and biochemical studies will be analyzed statistically. Data obtained will be expressed as mean $\pm$ (SEM) standard error of mean, one way analysis of variance (ANOVA) will be used to compare the mean difference between and within the groups and a P- value of ≤ 0.05 will be considered statistically significant.Statistical analysis will be carried out using statistical package for Social Sciences (SPSS) Version 2020.

RESEARCH BUDJET

S/N	**ITEMS**	**QUANTITY**	**RATE**	**Amount(N)**
1	Experimental Animals	42	500	15000
2	Vital feeds	10 bags	2000	20000
3	Cages	6	1000	7000
4	Bedding (saw dust)	2 bags	2000	4000
5	Sesamum *seed/extraction*	0.2kg	6000	6000
6	Drugs(ketoconazole, Mesterolone(Provirone)	2	10000	10000
7	Slides, stain and fixatives	lots	5000	5000
8	Biochemical analysis	150000	150000	150000
9	Statistical analysis	5000	5000	5000
10	Paper work/binding	6	10000	60000
11	Research assistant/logistics	2	50000	50000
	Total			337,000

WORK PLAN

YEAR 2015

ACTIVITY	**JUN**	**JULY**	**AUG**	**SEP**	**OCT**	**NOV**	**DEC**
Proposal presentation							
Purchase of Wister rats							
Laboratory work							
Thesis writing							
Internal defense							
External defense							

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