EFFECT OF THE AQUEOUS EXTRACT OF *Glycine max* ON THE TESTIS OF POST NATAL MALE WISTAR RATS.

BY

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DEPARTMENT OF ANATOMY,

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MAY, 2024.

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A PROJECT SUBMITTED TO THE DEPARTMENT OF ANATOMY,

SCHOOL OF BASIC MEDICAL SCIENCES,

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UNIVERSITY OF BENIN,

BENIN CITY.

IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF BACHELOR OF SCIENCE DEGREE(B.Sc.) IN ANATOMY

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CERTIFICATION

This is to hereby certify that this research was carried out by, **OKOYE, CHIOMA RITA** (Matriculation Number: **BMS1902068**) in the Department of Anatomy, School of Basic Medical Sciences, University of Benin, Benin City, Nigeria in partial fulfilment of the requirement for the award of Bachelor of Science Degree (B.Sc.) in Anatomy under the supervision of Mrs. Akporobo Ejeguo and any assistance given was duly acknowledged. All literatures and other sources of information consulted, cited or used in this research have been acknowledged and properly referenced.

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DEDICATION

This work is dedicated to God and the B.V. Mary

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I extend my deepest gratitude to a circle of individuals whose unwavering support has been the bedrock of my journey through this academic scheme. My Parents, Mr. and Mrs. Julius Okoye, have provided a foundation of love and sacrifice that has shaped my world. My siblings, Kingsley, Benedicta, Julius, Dorothy and Leonard, have offered endless encouragement, while Aunty Paulina's prayers and my entire family's support in all forms have been a source of collective strength and refuge.

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ABSTRACT

This study comprehensively examines the phytochemical composition and physiological effects of Glycine Max extract on Wistar rats, shedding light on its potential therapeutic applications. Through qualitative analysis, the extract was found to contain a diverse array of phytochemicals, including reducing sugars, saponins, flavonoids, phenolics, eugenols, terpenoids, alkaloids, and proteins. Three (3) groups (A-C) of six (6) each were created from the eighteen (18) juvenile Wistar rats (PND 42) that ranged in weight from 35g to 69.5g and were the offspring of DAMS. Group A served as the reference point (control) while B and C were the therapy groups, 250 mg/kg and 500 mg/kg of Glycine max aqueous extract, respectively, were administered to them as treatment. With the aid of an orogastric tube, the DAMS was fed orally for the duration of its four-week treatment. At the conclusion of six (6) weeks, the juveniles were fed, weighed, and slaughtered.

Detailed physiological assessments, including animal weights and organ weights, were conducted, revealing dose-dependent changes in body mass and relative organ weights. Histological analysis of tissue samples provided insights into the extract's impact on tissue morphology and integrity. Furthermore, antioxidant activity was evaluated through assays measuring catalase, superoxide dismutase, glutathione peroxidase, and malondialdehyde levels, indicating the extract's potential to modulate oxidative stress pathways. The findings underscore the multifaceted biochemical and physiological effects of Glycine Max extract, suggesting its promising role as a therapeutic agent. Further investigation is warranted to elucidate its mechanisms of action and explore its clinical applications in treating various pathological conditions.

CHAPTER 1

1.0 INTRODUCTION

1.1 BACKGROUNG OF STUDY

Soybeans *(Glycine max (L.) Merrill.*) hold a prominent role among agricultural crops as the primary source of concentrated proteins of high quality and vegetable oil. For many generations, people have utilized soybean seeds to make a wide range of fresh, fermented, and dried cuisines in Asia and other regions of the world (Probst and Judd 1973). Nutritious food items made from soy, like the fermented natto, tempeh, miso, and soy sauce (including tamari). (Richard *et al*.,1995), the non-fermented foods, protein bars, fortified cereals, whole dry soybeans, soynuts, soy sprouts, soymilk, tofu, okara, yuba, and fresh green soybeans (edamame), have been created for human consumption, soybeans are particularly high in proteins, dietary fiber, iron, manganese, phosphorus, and numerous B vitamins, including folate. For vegans and vegetarians, as well as those who wish to eat less meat, soy is a great source of protein (Rizzo *et al.*, 2018). Because soy delivers a complete protein profile and can be processed into meat and milk substitutes, unlike some other beans, oil-extracted soy meal is also utilized as a wholesome animal feed as it can be an excellent alternative for animal feeds.. In addition to being used domestically, soy oil has numerous applications in the manufacturing of plastics, pharmaceuticals, papers, inks, paints, varnishes, insecticides, and cosmetics. Locally, infant food manufactures in the country use soybeans because of its high nutritional value.(Bhatia *et al.*, 2008).

The Impact of Soybeans on Medicine

Consuming soybeans contributes to maintaining controlled blood sugar levels, particularly during pregnancy, blood glucose levels are kept within a normal range by the isoflavones included in soy products, which are thought to regulate glucose metabolism (Zhuang *et al.* 2020). Soybeans contain compounds called saponins, which may have anti-cancer properties. Soybeans may provide cancer prevention in addition to other health advantages (MacDonald *et al.*, 2005). Soy beans are rich in minerals including calcium, magnesium, phosphorus, vitamin D, and proteins that help to strengthen your bones. Consuming foods made from soybeans may lower the risk of heart disease, stroke, and coronary heart disease, among other conditions. (Yan et *al.* 2017), there is evidence that soy consumption and soy-based meals are associated with fewer and milder hot flashes in women who are perimenopausal and postmenopausal.

Soybean has numerous health-benefits but also has side effects on excess consumption such as constipation, diarrhoea, or in severe cases thyroid function abnormalities on thyroid deficient individuals (American College of Allergy, 2022). Also, there are those who has allergy to soybean. Therefore, prior to

consuming soybeans in large quantities it is advised to receive a medical consult from your dietitian or nutritionist.

The usage of soy oil for biodiesel has expanded recently an additional option for industrial utilization of renewable energy sources. Because it is a legume crop, soybeans may use atmospheric nitrogen through a process called biological nitrogen fixation, which reduces their reliance on artificial nitrogen fertilizers. Given its numerous benefits, there is good reason for its significant participation in global crop development initiatives. Soybeans are members of the Papilionaceous subfamily and the Leguminosae family. In 1948, Ricker and Morse recommended that the correctly identified soybean should be *G. max (L.*) Merrill (Gazzoni 1994). The growth of soybeans is extremely susceptible to changes in the environment, and one main component that significantly affects their production is water. Additionally, temperature and photoperiod have a significant role on determining how soybeans are cultivated across space as well as time. Over the years, this wonder crop has experienced amazing growth despite a number of biotic and abiotic challenges as well as production limitations.

1.2 AIM OF STUDY

The aim of the study is to investigate the effects of the ethanolic extract of Soybeans (*Glycine max)* on the testis of male wistar rats.

1.3 SPECIFIC OBJECTIVES

The specific objectives are to investigate the effects of ethanolic extract of *Glycine max* on

- The body weight of treated wistar rats
- Testicular weight of treated wistar rats
- Histology of testis of treated wistar rats

1.4 JUSTIFICATION OF STUDY

Humanity has struggled with reproduction and fertility issues for generations, and these issues persist today. The reproductive system's viability determines

the criteria for fertility. Testis examination can be used to compute this fertility index. The morphology and physiology of human and Wistar rat testicles are remarkably similar. There are many health benefits associated with *soybeans (Glycine max),* which includes prevention of breast cancer, hot flushes during menopause, and improved nutrition. Therefore, in order to clarify any potential antifertility, it is essential to investigate its effect on the development of the testis.

CHAPTER 2

2.0 LITERATURE REVIEW

2.1.0 PLANT STUDY: *GLYCINE MAX*

Glycine max, often known as soybeans, are an edible seed and an annual legume in the *Fabaceae* family of plants (Emily Rodriguez, 2023). With millions of people obtaining vegetable protein from soybeans and hundreds of chemical goods made from them, soybeans are the most important bean in the world economy.

2.1.1 BRIEF HISTORY

In East Asia, soybeans were an important crop long before written records were kept. (William *et al*., 2013). There is still disagreement in science over the origins of soybean farming. *Glycine soja* (formerly known as *G. ussuriensis*), a legume indigenous to central China, is the closest surviving ancestor of the soybean. (Nelson *et al*., 2006). Research indicates that domestication of soybeans occurred in China between 7000 and 6600 BC, Japan between 5000 and 3000 BC, and Korea approximately 1000 BC (Xinxiang *et al*., 2011).

At the Daundong site in Korea during the Mumun period, the first clearly domesticated, cultigen-sized soybean was found (Miriam et al 2008) Soy was revered for its role in crop rotation and was consumed on its own, in bean curd, and in soy milk before fermentation products like fermented black soybeans (douchi), jiang (Chinese miso), soy sauce, tempeh, natto, and miso were created.

In the Malay Archipelago, soybeans were first brought to Java in the 13th century, and possibly much earlier. Portuguese, Spanish, and Dutch traders brought soy beans and their products to Asia by the 17th century, whence they arrived on the Indian Subcontinent. Soybeans were brought from China to America and Europe by the eighteenth century. China brought soy to Africa in the late 1800s, and it has since expanded widely throughout the continent.

Although they are most likely much older, the cultivateans first appeared in the eastern side of northern China about 2000 BC (Denis.J *et al*., 2007). The blackened plant remnants of the wild soybean found in the Jiahu provide the oldest known proof of the use of glycine of any type. A Neolithic site in China's Henan province was inhabited between 9000 and 7800 B.C. years in calendar months (Xuexiang *et al*., 2011). In and around this area, a large number of the

archaeologically burnt soybean specimens have been discovered. (Zhao et al., 2004).

Five plants are considered sacred: soybeans, rice, wheat, barley, and millet. This was declared by the mythical Emperor Shennong of China in 2853 BC. (History of soybeans *et al*., 2012). Soybeans were a gift from Southeast China and the Yangtze River delta, according to ancient Chinese annals. According to the Great Soviet Encyclopaedia, soybeans were first cultivated in China over 5,000 years ago. Certain academics propose that soybeans were first domesticated around 3500 BC in China. But according to recent studies, wild forms began to seed early in East Asia—before 5000 BC—in a number of different areas (Mohammad Rafiq *et al*., 2001).

The earliest preserved soybeans that were found in resembled present kinds in terms of size and shape. In Korean archaeological sites dating back to approximately 1000 BC. (Stark and Miriam T et al., 2005). Soybean cultivation as a food crop dates back to approximately 1000–900 BC, according to radiocarbon dating of materials recovered through flotation during excavations at the early Mumun era Okbana site in Korea. (Miriam *et al.*, 2005). Jomon-era soybeans, which date back to 3000 BC in Japan, are likewise noticeably bigger than wild counterparts (Akika *et al*., 2012).

By the Zhou era (c. 1046-256 BC) in China, soybeans had become a significant crop. It is unclear, nevertheless, exactly when, when, and under what conditions soybeans grew to form intimate relationships with humans. Before the Han era, soybeans were unknown in South China (Xuexiang *et al*., 2011). In South and Southeast Asia, soybeans were introduced during the first century AD and the Age of Discovery (15–16th century). This spread was brought about by the creation of commerce routes via land and sea. The ancient Kojiki (Records of Ancient Matters), finished in AD 712, is the first known Japanese literature that mentions soybeans.

In 1857, the soybean made its way to Africa. (Shurtleff *et al*., 2009). The Ewe people of Southeast Ghana and Southern Togo create Soya Meme, also known as Baked Soya, in the settlement of Bame Awudome, which is close to Ho, the capital of the Volta Region of Ghana.

Fig 2.1; *Glycine max* (Fotolia., 2011)

2.1.2 TAXONOMY:

Table 2.1 showing the taxonomy of *Glycine max*

Taxon	Scientific name and common name
Kingdom	*Plantae (plants)*
Subkingdom	*Tracheobionta (vascular plants)*
Super division	*Spermatophyta (seed plants)*
Division	*Magnoliophyta (flowering plant)*
Class	*Magnoliopsida (dicotyledon)*
Subclass	*Rosidae*
Order	*Fabales*
Family	*Fabaceae/Leguminosae (Legume or Pea)*
Tribe	*Phaseoleaei*
Genus	*Glycine wild. (soybean)*
Species	*Glycine max (L.) Merr. (soybean)*

(Germplasm Resources Information Network (GRIN-Taxonomy) [2020], (ITIS [Integrated Taxonomic Information System [2020]).

2.1.3 DESCRIPTION:

Glycine max (L.) Merr., or soybean, is an erect leguminous plant that can grow to a height of one meter. Although it originated in Asia, this herbaceous annual grows quickly nowadays and is cultivated all over the world. In ideal soil circumstances, its tap-root can reach a depth of up to 2 meters, while its secondary roots can reach the top 15-20 centimetres of the soil. Bradyrhizobium japonicum infections typically cause nodules on the roots. (Ecoport *et al*., 2010),

commercial cultivars of this species often have large, oval-to-lanceolate leaflets, these leaves themselves are trifoliate. (Giller *et al*., 2007), the corolla of papilionaceous flowers is 5-7 mm long and can be white, pink, purple, or blueish. Fruits are two or three-seeded pods that contain spherical, yellow seeds that can range in colour from yellow to black on the hilum (Koivisto *et al*., 2006). Generally speaking, soybeans are seeds with 20% or less oil content. The major producers of soybeans are the United States, Brazil, Argentina, and China, with 231 million tons produced in 2008 making it the largest oil-seed crop (FAO *et al*., 2010). An abundant protein cake is produced during oil extraction, which can then be processed into a range of feed and food items. A prominent feed commodity and the primary source of protein in many animal diets is soybean meal, which is one of these items. Whole (or full-fat) soybeans can also be used. Whereas the demand for soybean meal and feed, which together account for about two-thirds of the crop's value, is currently driving the crop's increase, soybeans were once generally farmed for their oil in recent years (FAO *et al*., 2006).

In tropical Asia and Africa, soybeans are consumed as food. Food made from soy, such as tofu, soybean milk, other unusual dishes, is relatively new to the West. Making flour, milk tofu, and tofu-like items is beneficial. It can be fermented to create tempeh, miso, yuba, and soy sauce, or it can be roasted and consumed as a snack. Bean sprouts and immature soy beans are also consumed as vegetables (Giller *et al*., 2007). Hay can be made by gazedding, ensileing, or drying the stem and leaves of soybeans. Cattle find the leaf to be exceedingly appetizing, highly digestible, and rich in nutrients (Koivisto *et al*., 2006). The average yield of soybeans worldwide is 2.25 t/ha, however the average production in Africa yields (Giller *et al*., 2007) are 0.5 t/ha. Two of the main soybean products used in feed are soybean oil and soybean meal, which is a by-product of oil extraction and whole soybeans, also referred to as "full-fat" soybeans; the latter are typically heat-processed to eliminate the anti-nutritional component and increase feed value. These goods can be flaked, powered, pelletized, or ground. Additional products include soybean flour, which is very low-fibre powdered and screened soybean meal; soybean protein isolates and concentrates, which are essentially pure protein; soybean molasses and souffles, which are made from washing soybean flakes and flour; soybean mill feed, which is a by-product of making soybean flour; and soybean mill run, which is a by-product of making soybean meal.

2.1.4 DISTRIBUTION

Asia is where soybeans originated. Almost all continents between 53°N and 53 S, as well as sea level and elevations up to 2000, produce it today after

domestication in China 3000 years ago. USA, Brazil, Argentina, China, and India are the top generating nations.

Ideal growth conditions include average daily temperatures of about 30´C, 850 mm of annual rainfall, at least 500 mm of water throughout the growing season, and a pH range of 5.5 to 7.5 in well-drained soil. Acidity of the soil and toxicity of aluminium affect soya. (Ecoport *et al*., 2010), it can tolerate brief intervals of waterlogging and brief droughts.

2.1.5 ENVIRONMENTAL IMPACT

--Soil improver and nitrogen-fixing legume

N-fixing legumes include soybeans. It can be grown as a rotation crop with cotton, maize, and sorghum, or as green manure. Soya needs to be weeded within the first 6–8 weeks following seeding. Its quick growth can help lessen weeds after that. According to reports, it lessens the highly harmful parasitic weed Striga hermonthica to crops in Africa (Giller *et al*., 2007).

-- Loss Of Bio-Diversity

In Brazil and Argentina, the intense monoculture of soybeans has a detrimental impact on biodiversity and habitats. Mechanical weeding causes more soil erosion, and heavy farming severely depletes the fertility of the soil. Massive deforestation in Brazil, Argentina, and Paraguay is also a result of soybean farming. (Steinfield & Associates, 2006)

-- Genetically modified soybeans

It is outside the purview of this datasheet to fully address the complicated debate surrounding the environmental effects of genetically modified crops. Although glyphosate-resistant soybeans have been introduced, there is disagreement over the real reduction in herbicide use. Thanks to the vast regions planted to soybeans, the 10% decline reported by certain authors may be deemed either too little (or related to variables other than GM soybean) or significant enough. Some authors argue that by encouraging farmers to use no-till or conservation tillage techniques that lessen soil erosion and fuel consumption, herbicide-tolerant soybeans can indirectly assist the environment (Edwards *et al*., 2009). There has been evidence of spontaneous pollen-mediated gene flow, however it is thought to be too rare to be a problem. On the other hand, gene flow through seed is quite likely. Although it is conceivable, transgene introgression into wild soybeans in China and Korea is nevertheless thought to be too rare to represent a significant worry (Owen *et al*., 2009).

2.1.6 MORPHOLOGY

Habitat: Tolerates extreme heat and harsh winters; grows on a variety of soil types; best suited for regions with hot, humid summers.
Habit: Erect, bushy, pubescent annual with grey hairs all over; some have a tendency to twine and be prostrate; determinate cultivars create terminal inflorescence, while indeterminate cultivars display axillary inflorescence.
Roots: Taproot, tiny spherical, occasionally lobed nodules.
Stem: branched; cotyledon axils contain buds. Unless the tip is destroyed, the primary leaves typically do not form.
Leaves: stipules are short lanceolate, stipels are minute, petiole is long, slender, and cylindrical; foliage is alternate, trifoliate, and sometimes five-foliate. most cultivars drop their leaves as the pods open; leaflets are oval to lanceolate, usually palea green in colour, with a rounded base, acute or obtuse apex, and frequently slightly slanted lateral leaflets start to mature.
Inflorescence: A short, axillary raceme with clusters that is terminal if the type is known.
Flower: Little flowers with two oval, sharp bracteoles.
Calyx: Having two upper and three lower lobes, the calyx is half-length joined and hairy.
Corolla: White or lilac; oblong in shape, always emarginated (notched at the extremity); wings: narrow and oblong; keel: shorter than wings, not fused along upper surface.
Androecium: Anthers are uniform and globose, whereas stamens are Monadelphous and vexillary, free at the base.
Gynoecium: glabrous, curve-shaped style, few ovules, sessile hair, and capitate stigma.
Fruit: Pods, which are borne in clusters on short stalks and are slightly curved and pale yellow, grey, or black.
Seed: Testa straw yellow, green, brown, or black, or a blotched and mottled combination of these colours; hilum tiny; cotyledon yellow or green. Globose.
Pollination: It is generally the case that self-pollination occurs. Flowers begin to bloom in the early, pollen falls straight upon the stigma in the morning, either right before or right after it opens. While cross-pollination occurs when bees and other insects visit flowers, it typically occurs in less than 1% of cases.

2.2 PHYSICAL DESCRIPTION AND CULTIVATION

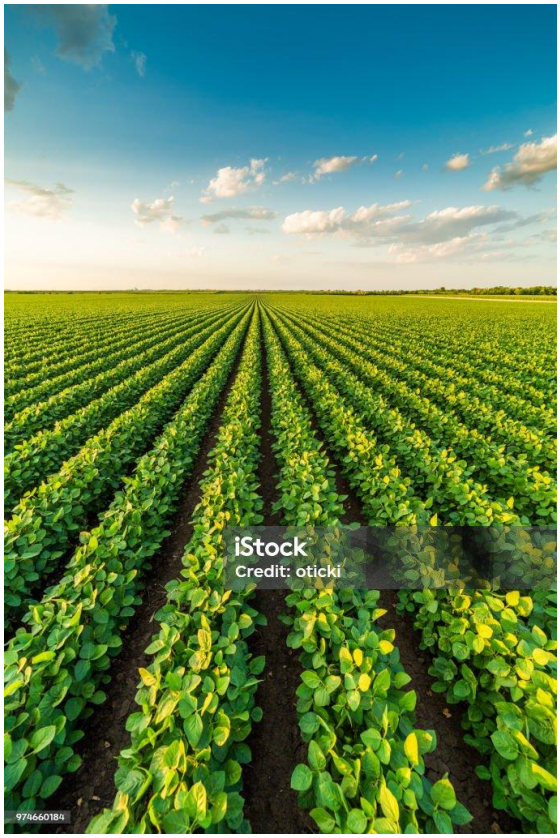


Fig 2.2; Green ripening *Glycine max* field, agricultural landscape (oticki., 2018)

Soybean is a hairy annual with a large tap root system, the majority of which is located in the top 15 cm of soil. The tap root can develop as deep as two meters, while adventitious roots emerge from the hypocotyls (Chaturvedi *et al.* 2011). Cultivated soybeans grow erect, yet procumbency is not rare in germplasm resources (Burton 1997). Modern soybean cultivars are mainly erect, bushy, and 20-180 cm tall, with a few primary branches but no secondary branches. Exceptionally prostrate and freely branched forms are also found, particularly in types intended for foraging. The leaves are trifoliate and alternating, with long petioles, short stipules, and stipples; the leaflets are oblong to lanceolate, with a mucronated tip. In soybeans, photoperiod significantly affects flowering and maturity. The flowers are typical papilionaceous, white or pale purple, with a tubular calyx of five unequal sepal lobes and a five-member corolla consisting of a posterior standard petal, two lateral wing petals, and two anterior keel petals (Guard, 1931). The androecium is diadelphous, with a 9 + 1 configuration. The solitary pistil is unicarpellate and contains 1-4 campylotropous ovules (Palmer 2001). The style curls back towards the posterior stamen and is encircled by a knob-like stigma (Carlson and Lersten 1987). Each flower is supported by two bracteoles and has a hairy calyx with five pointed sepals that are joined for half their length. The pods have short stalks and come in bunches of 3-15, 3-7 cm long and hairy, light brown when mature, and slightly constricted between the seeds. The seeds vary significantly in shape, size, and

colour, but are often spherical and yellowish, brown, or black, with epigeal germination. Soybeans are largely self-pollinated, with natural cross-pollination rates ranging from 0.03 to 1.14% (Culter 1934; Caviness 1966). The wild annual soybean, G. soja, is primarily self-pollinated, whereas the perennial wild relative, G. argyrea (Ting.), and its closely related species, G. clandestine (Wendl.), have both self-fertilized cleistogamous and chasmogamous flowers on the same plant (Brown et al. 1986; Schoen and Brown 1991; Palmer et al. 2001). Chasmogamous flowers attract insect pollinators, leading to cross-pollination. Small insects, such as thrips and honeybees are primarily responsible for natural outcrossing in soybeans, however other insects have been documented feeding on soybean blossoms. Palmer et al. (2001) went into detail into insect-mediated cross pollination in soybeans. Self-pollinating soybean flowers contain 3-4 ovules that mature before to anthesis (Stelly and Palmer 1985). Flowers open and typically self-pollinate during anthesis. To arrange controlled pollination, carefully remove the sepals and petals from young, unopened flowers. This is followed by the removal of anthers using forceps or tweezers, albeit this is not always necessary (Stelly and Palmer 1985). The remaining flowers from the inflorescence are likewise removed. Pollination is completed the following morning. For this, the flowers that are about to open Should be taken. The first sepals are removed with forceps, followed by the standard, wing, and keel petals to expose the anthers. The anthers are then gently stroked onto the stigma until the pollen is clearly visible. A tiny pod is typically visible in 6-7 days.

2.2.1 NUTRITIONAL PROFILE OF SOYBEANS

Soy is a premium source of protein. It is one of the few plant foods that is known to contain every essential amino acid, including those found in meat, along with amaranth seed and, to a lesser extent, quinoa (Megan., 2023). The soybeans are; protein, high in fibre, low in saturated fat, devoid of cholesterol, lactose, excellent source of omega-3 fatty acids, antioxidants, phytoestrogens etc. Soybeans come in many colours, including: Green soybeans: Young green soybeans are also called edamame. People can steam them and eat them out of the pod as an appetizer. Shelled edamame is also available in salads, stir-fries, and soups.

Yellow soybeans: Producers typically use yellow soybeans to make soy milk, tofu, tempeh, and tamari. They also play a role in the production of soy flour for baking.

Black soybeans: Several Asian food cultures use simmered or fermented black soybeans in traditional dishes. Soy milk and cheese are also options for those looking to replace dairy in the diet.

2.2.2 SOY PRODUCTS

There are various methods to ingest soybeans.
There are two types of soybean-based foods: fermented and unfermented. Soy products that have undergone fermentation include miso, tempeh, natto, and soy sauce, whereas unfermented foods consist of tofu, soymilk, edamame, soy nuts, and sprouts. (Kumar et al., 2020)
Some soy products, like tempeh or tofu (prepared with a calcium coagulant) and soy drinks fortified with calcium, are sources of both calcium and iron.
"Second generation" soy food is another category of food made from soy. This comprises soymilk yoghurts and cheeses, soy breads, soy pasta, and tofu sausages and burgers. Products containing soy or substances derived from soy, such as lecithin, which is present in several baked goods and chocolate, are also included in this category. (Kumar et al., 2020)
Green tea and soybeans
Hormone-like compounds are found in soybeans, termed phytoestrogens, which are organic substances that are naturally present in plants (the word "Phyto" means "plant"). These substances mimic the effects of the female hormone oestrogen when the correct circumstances are met, albeit they have a 1,000-fold lower potency.
Isoflavones is one kind of phytoestrogen among many others. Strong antioxidants, isoflavones can imitate the actions of oestrogen (Zhuang *et al.*, 2020), but further research is needed to fully understand their physiological effects. The most prevalent food source of isoflavones is soybeans. However, the kind, brand, and preparation technique of soy cuisine all affect how much isoflavones are present. (Li *et al*., 2020). Soy flour and soy nuts are among the best sources of isoflavones.

2.2.3 BENEFITS OF SOYBEAN FOR HEALTH

Studies indicate that soy products and soybeans food made from soy has several health advantages.

--Soy and coronary heart disease
Consuming a lot of soy products is associated with a decreased risk of cardiovascular illness, which includes coronary heart disease and stroke.
During the reproductive years, estrogen may shield women from heart disease; however, heart disease risks rise after menopause. (Yan *et al.*, 2017)

--Soybean and Cholesterol
It has been demonstrated that soybeans reduce LDL and total cholesterol, two major heart disease risk factors.

Based on a review of clinical trials, 14g to 50g of soy protein can considerably raise HDL (good) cholesterol levels while significantly lowering triglycerides, LDL (bad) cholesterol, and total blood cholesterol.
The Food and Drug Administration of the United States also discovered that adults who consume at least 25g of soy protein (about 4 meals of soy) every day on a diet low in cholesterol and saturated fat can cut LDL cholesterol by roughly 3 to 4%. (Blanco *et al.*, 2019)
Processed soy products are less effective at lowering cholesterol than whole soy products, such as soymilk, soybeans, and soy nuts.

How this occurs is unknown; soy proteins or phytoestrogens may be involved alone or in combination. There may be more factors at work, such as the high fibre and low saturated fat content of soy.
Another explanation would be a decrease in the consumption of animal protein, which includes cholesterol and saturated fat, which would subsequently lower blood cholesterol levels.
But research also reveals that isoflavone supplementation and consuming soy protein without them reduce cholesterol, but not significantly alone don't do anything to decrease cholesterol.

--Menopause and soybeans
Soy is believed to help lessen menopausal symptoms because of its phytoestrogen concentration (such as hot flushes). This is based on findings of Asian women, who generally have diets high in soy and experience fewer hot flushes during menopause than women whose diets are high in meat. (Matsumura *et al*., 2012). It seems that the phytoestrogens in soy function something like menopausal hormone treatment (MHT). But in order to reap the same health benefits as standard MHT, regular soy eating would need to last for over a year. (Chen *et al*., 2015), soybeans may help some women control menopausal hot flushes, even if only slightly.

Avoid taking supplements containing highly processed soy if you are at high risk of breast cancer, or you have survived breast cancer. Consuming whole soy foods in moderation is more beneficial.

--Additional health advantages of soy
Other potential health advantages of soy-based diets include:
Reduced blood pressure; enhancements to blood arteries, such as increased artery wall elasticity; better bone health; and defense against some cancers, such as breast cancer
Enhanced mental clarity and spatial recollection.

Some examples of foods with an average isoflavone concentration are; A half-cup of soybeans contains 40–75 mg of isoflavones, Half a cup of soy flour contains 45–69 mg of isoflavones, A single 250ml glass of Soy beverage: 15–60 mg of isoflavones, 115g of tofu contains 13–43 mg of isoflavones, A 110g tempeh block with 41mg isoflavones, One soy yogurt container has 26 milligrams of isoflavones, Two soy bread slices contain 7 to 15 mg of isoflavones, 0.4 to 2.2 milligrams of isoflavones per teaspoon of soy sauce. Typically, 30 to 50 mg of isoflavones is sufficient to provide health advantages.

According to certain research, traditional soy foods typically have greater positive health impacts than "second generation" soy foods, to enhance your soy consumption, pick complete soy foods such as tempeh, soymilk, soy bread, and tofu, verify the ingredient lists of the soy-based items you the goods you purchase aren't made of soy isolates but rather entire soybeans, verify that goods (like cereals) have soy protein and not only isoflavone added to it, remember to read food labels; a lot of fermented soy products, such as soy sauce, are unhealthy and contain a lot of salt. Some additional foods high in phytoestrogens other than soy are; wholegrain cereals, such as buckwheat, rye, corn, oats, and wheat, nuts and seeds, including sesame, sunflower, pumpkin, linseed, flaxseed, and almonds, alfalfa sprouts, extra virgin olive oil is one type of oil. Other legumes include kidney beans, lentils, and chickpeas.

2.2.4 RISK OF SOY TO BABIES

In general, eating soy products as part of a regular diet has more advantages than disadvantages. Research indicates that heavy soy consumption is not harmful, despite worries that it may be for certain males and those with thyroid issues, research has also revealed that there doesn't seem to be any damage associated with using soy infant formula in healthy, full-term new-borns but it's advised to stay away from new born soy formula as it might be dangerous for premature babies. (Alina Petre et al., 2020). Consult your physician or maternity and child health nurse.

--ITEMS MADE FROM GENETICALLY MODIFIED SOY

There are many who refuse to consume genetically modified (GM) food. The majority of genetically modified substances found in food sold in Australia come from soy products imported from the United States. While some soybean crops have undergone genetic modification to make them herbicide-resistant, non-GM soybeans are genetically similar to these crops, in addition to core soy products like tofu or soy flour, genetically modified soy can also be found in a variety of other foods including additionally demonstrated that there does not seem to be any risk associated with the use of soy new-born formula in healthy,

full-term babies. On the other hand, baby soy formula, such as chocolates, bread, margarine, mayonnaise, and potato chips. In Australia, the term "genetically modified" must always be listed on approved genetically modified foods and products that include unique DNA or unique protein.

--ALLERGIES TO SOY

Some people can develop a soy allergy as a result of their immune system responding inappropriately to soy protein.(American College of Allergy et al., 2022). Allergies to soy are frequent in young children and neonates. Among the symptoms are; Tingling and itching in the lips and mouth, skin flushing, nausea, diarrhoea, shortness of breath, hives, disorientation, confusion. A reaction won't always occur from soy products since fermented soy products have less allergens than raw soybeans, many individuals with soy allergies can safely consume soy lecithin, soya bean oil, and several other forms of fermented soy. Consult your physician for guidance if you have a soy allergy. Read food labels carefully at all times, and stay clear, you can go to your primary care physician or your obstetrical paediatric nurse for assistance.

2.3 ORGAN STUDY; THE TESTIS

The testes are male sex glands having both an endocrine and exocrine functions. The testicles, or testes, are important for the production and storage of sperm until they’re mature enough for ejaculation. They also produce testosterone, which is responsible for sex drive, fertility, and the development of muscle and bone mass. The testes are oval-shaped reproductive structures that are found in a sac of skin called the scrotum and are separated by the scrotal septum. The scrotum hangs outside the body in front of the pelvic region near the upper thighs and separated by the scrotal septum. The testis has a bean-shape and measures 3-5 cm in length and 2-3 cm in width. When palpated through the scrotum, the testes are smooth and soft. (Ertekin *et al.*, 2008) The spermatic cord suspends the superior aspect of the testes. Inferiorly, the testes are attached to the scrotum by the scrotal ligament which is a remnant of the gubernaculum. In general, the left testis is affixed slightly lower than the right testis.

The double-layered tunica vaginalis envelop the testes except at the posterior and superior borders where the epididymis and spermatic cord are attached. The visceral or inner layer of the tunica vaginalis is close to the epididymis, testes and vas deferens. On the posterior lateral surface of the testes, there is a small space between the testes and body of the epididymis which is known as the sinus of the epididymis. Deep to the tunica vaginalis is located the tunica albuginea, which is a durable fibrous covering of the testes. (Svingen *et al*., 2013). The epididymis is a tiny, elongated, curving structure that is firmly compressed and extremely convoluted. Its length, when open in a straight line, is thought to be around 20 feet. The three components of the epididymis are the

head (caput), body (corpora), and tail (Cauda). It is located on the posterior border of the testis. (Altay *et al.*, 2008). The epididymis head is located at the upper pole of the testes and receives seminal fluid from the testicular ducts. After that, it allows sperm to enter the distal section of the epididymis. The epididymal ducts are long and provide plenty of room for sperm to develop and store.

2.3.1 GROSS ANATOMY

Situated in the scrotum, the testis is an ovoid, paired male reproductive organ that is divided from its partner by a scrotal septum. The adult testis has an average volume of about 25 millilitres, and some people have compared its form and size to that of a large olive or small plum. Its dimensions are usually 3.5–5 cm in length, 2.5–3 cm in width, and 3 cm in depth (anteroposterior diameter). (Snell *et a*l. 2000). The testis is palpably smooth and obliquely positioned, with a small anterior and lateral slope to the superior pole and its long axis nearly vertical. The left testis often sits lower than the right testis because it is suspended superiorly by the spermatic cord. The testis is attached to the scrotum inferiorly by the gubernaculum's remnant, the scrotal ligament (Saunders Elsevier *et al*., 2006). The testis is covered in a double layer by the tunica vaginalis testis, a relic of the processus vaginalis, with the exception of the superior and posterior borders, where the testes are adhered to by the spermatic cord and epididymis. (McGraw-Hill *et al.,* 2004)

The testis, epididymis, and ductus deferens are all directly covered by the visceral layer of the tunica vaginalis testis. This layer fills the sinus of epididymis, a slit-like recess between the testis and the body of the epididymis on the posterolateral surface of the testis. (Moore *et al*., 2006). Adjacent to the internal spermatic fascia, the parietal layer of the tunica vaginalis is larger and reaches superiorly into the distal a section of the neural cord. The testis's hard, fibrous outer layer is called the tunica albuginea, and it extends deep beneath the tunica vaginalis. It is reflected inward on the posterior surface to form the mediastinum testis, an incomplete vertical septum. The testis's mediastinum stretches from the gland's superior to almost inferior region. As it moves inferiorly, its width gets smaller. Many incomplete septa are released anteriorly and laterally, radiating to the surface of the glands and attaching to the tunica albuginea. These create many, cone-shaped openings that widen at the gland's surface and get smaller as they converge to the mediastinum, dividing the inside of the testis. Within these areas, the many glandular structure lobules (the tiny yet seminiferous tubules long, tightly wound tubes) are kept. The ducts and veins that travel to and from the glandular material are supported by the mediastinum (Moore *et al*., 2006).. Sperm and nutritional fluid are produced by the germ cells lining the seminiferous tubules. The contents of these tubules are

released into an anastomosing duct network, which finally empties into the epididymis.

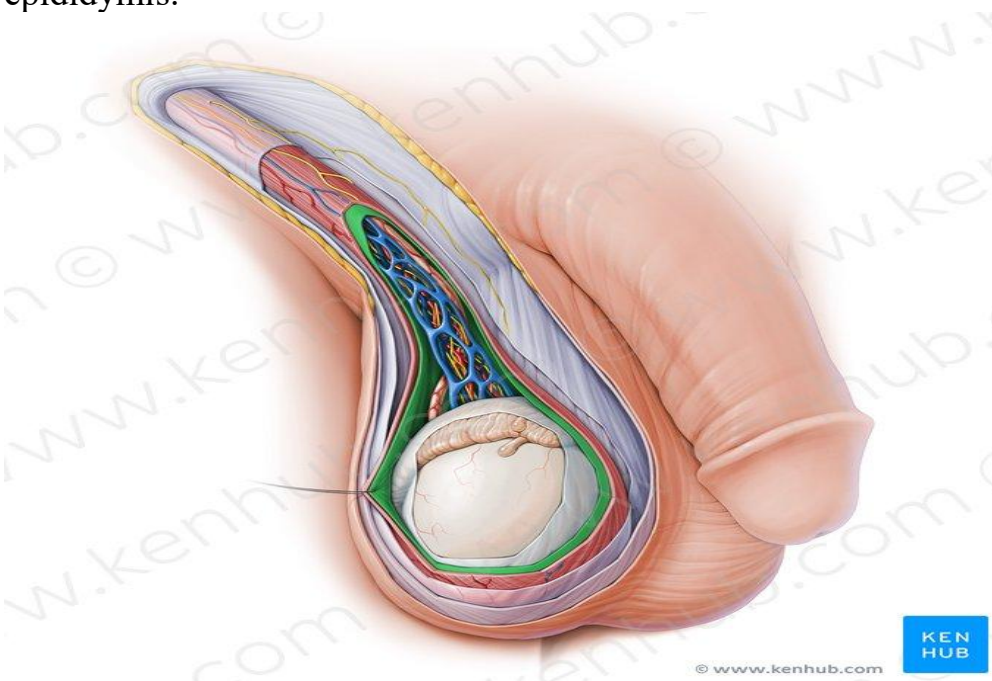


Fig 2.3.1; the testis (Jana Vaskovic., 2023)

2.3.2 HISTOLOGY

The glandular tubules that make up the testis are lobulated. There are sporadic clusters of "interstitial cells," which contain Leydig cells, amidst the extremely convoluted tubules that are kept together by loose connective tissue. A basement membrane made of laminated connective tissue with many elastic fibres and flattened cells in between the layers makes up each tubule. An outer layer of flattened epithelioid cells covers the basement membrane. The epithelial cells that make up the basement membrane are stacked in multiple irregular layers. At the periphery, these cells can be further divided into germ cells and various spermatogenesis cells, which eventually mature into mature sperm cells as they move toward the lumen. Sertoli cells are also scattered throughout the layer; they help the growing sperm cells. (Nickel *et al.*, 2007).

Less convoluted tubules converge into 20–30 bigger, straight ducts (tubuli recti) at the apices of the lobules. This fibrous stroma, which is bordered with flattened epithelium (rete testis), is where these ducts join to form anastomosing tubes. There are about fifteen ducts that emerge from the tubes, all of which are originally straight (Nickel *et al*., 2007). They grow and get more twisted after puncturing the tunica albuginea at the superior mediastinum. Fibrous bands and delicate areolar tissue hold these convolutions together. Known as the conic vasculosi, they are a group of conical masses. The epididymis is composed of

this series. Columnar epithelium with ciliated edges lines these larger channels. The muscle tissue located beneath this epithelium is primarily shaped like a circle. While the ductus deferens and the epididymis tail join at this point. The microscopic anatomy shows that the duct has expanded, grown in diameter, and retained its ciliated columnar epithelial lining.

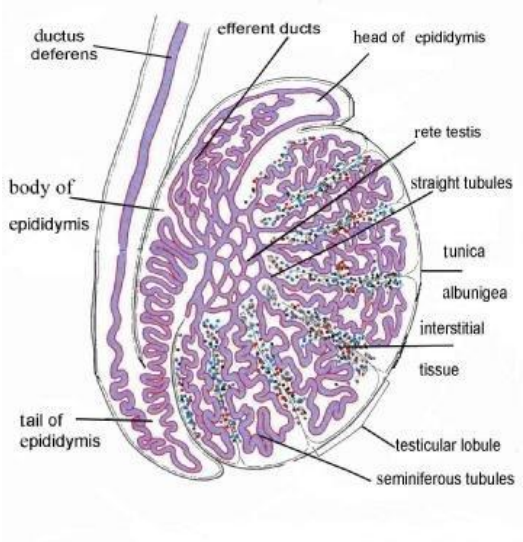


Fig 2.3.2; histology of the testis (Abd-Elmaksoud Ahmed., 2009)

2.3.3 SEMINIFEROUS TUBULES;

The testis's seminiferous tubules open into the rete testis, a continuous chamber of flattened, irregularly shaped anastomosing channels. The rete is located next to the tunica albuginea, the connective tissue capsule that covers the testis, at one pole of the testis (hilus). Additionally, blood veins enter the capsule at the hilus. Newly released spermatozoa and seminiferous fluids from testicular seminiferous tubules enter and exit the body through the rete testis. Many efferent ductules that are responsible for carrying fluids and sperm into the extremely convoluted tubular structure called the epididymis—which rests on the rear of the testis—emerge from the rete. The epididymis is where sperm mature and are stored. The rete testis is located in rodents primarily beside the tunica albuginea, it extends roughly 1/3–1/2 into the testis proper in humans and large mammals (Hess, *et al*., 2018). When it comes to endocytosis, the squamous to short cuboidal epithelium is very active. Lysosomes take in and break down various proteins that are derived from the lumen of seminiferous tubules. Certain proteins are secreted by Sertoli cells and may have an effect on sperm during their formation in the testis. However, their functional significance diminishes as the sperm go towards the epididymis. Certain proteins originating from the testis seem to be cleared by the rete. Therefore, while appearing to be a passive channel, the rete plays crucial functions in

adjusting the lumen to allow sperm to enter the ducts that efferent. It might also help efferent duct blockages to widen and stop the blood-testis barrier from being disrupted (Hess, *et al*., 2018).. In order to repair a diminished or insufficient spermatogenesis, stem cells have been injected into the lumen of seminiferous tubules using the rete testis, a procedure that has gained therapeutic significance in the last ten years.

2.3.4 SPERMATOGENESIS

The process by which sperm cells within the male reproductive organs—the testes—develop and begin is known as spermatogenesis. Seminiferous tubules, which are made up of many thin, tightly coiled tubules, make up the testes. Sperm cells are created inside the tubule walls. Numerous sporadically distributed cells known as sertoli cells are also found within the walls of the tubules. These cells serve to sustain and nourish the immature sperm cells by providing them with blood products and nutrients. (Aakasha *et al.*, 2023). From the outside surface of the seminiferous tubule to the central channel of the tubule, the growing germ cells are transported by the Sertoli cells. The testes continuously produce sperm cells, but not all seminiferous tubules simultaneously generate sperm cells (Gloria *et al*., 2023). It can take a single immature germ cell up to 74 days to complete full maturation, and there are periodic resting intervals in between this growing process.

The stem cells found on the outer wall of the seminiferous tubules are the source of all immature cells, or spermatogonia. Nearly all of the material in the stem cells is nuclear. (The area of the cell that houses the chromosomes is called the nucleus.) The process of mitosis, which is the duplication of cells, is how stem cells start their life. From this first crop, half of the new cells become future sperm cells and the other half stay as stem cells so that there's always more germ cells available (Grace Young, 2016) Primary sperm cells are spermatogonia that will eventually mature into mature sperm cells. These travel to a more central area of the seminiferous tubule from the outer part and adhere to the Sertoli cells. After then, the main sperm cells undergo some development as a result of an increase in the quantity of cytoplasm, or material outside the nucleus, and organelles, which are structures found in the cytoplasm. The primary cells split into what is known as a secondary sperm cell after entering a resting phase. This cell division results in the nuclear material separating. There are 46 chromosomes in the nucleus of the original sperm cells, but only 23 chromosomes in each of the secondary sperm cells as in the egg, there are only 23 chromosomes. The traits of both individuals mix as the sperm and egg meet and share chromosomes, and the new organism begins to grow. Before fertilizing an egg, the secondary sperm cell must still mature, which involves several modifications to its form and structure. The nuclear material becomes

more oval-shaped and compacted, and the sperm head grows in this region. A cap known as the acrosome covers a portion of the head and is crucial for facilitating sperm entrance into the egg. (Young Grace, 2016). The tailpiece is fastened to the head's other end. The secondary sperm cell provides the source of the tail cytoplasm. The mature sperm are propelled by their undulating movement, which is caused by a long, narrow bundle of filaments. After reaching maturity, the sperm is moved via the lengthy seminiferous tubules and kept in the testes' epididymis until it is time for it to exit the male body.

2.3.5 SPERMIOGENESIS

The process by which spermatids mature into mature spermatozoa is known as spermiogenesis, which is the last step of spermatogenesis. The spermatid is a roughly round cell with a nucleus, centrilobe, Golgi apparatus, and mitochondria at the start of the stage. By the time it is finished, it has drastically changed into an elongated spermatozoon, complete with a head, midpiece, and tail.

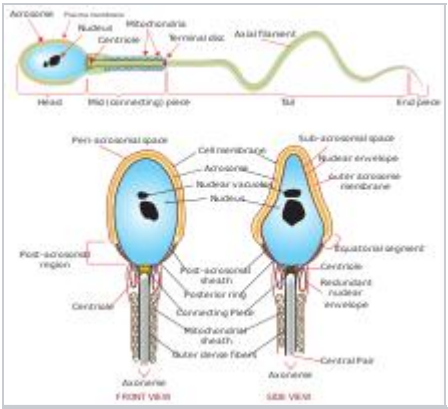


Fig 2.3.3; phases of spermiogenesis. (Mariana Ruiz., 2018)

Four phases are often recognized in the process of spermiogenesis: the Golgi phase, the cap phase, the tail formation stage, and the maturation stage.

-- Golgi stage

The spermatids start to acquire polarity; hitherto, they had been primarily radially symmetrical. The Golgi apparatus produces the enzymes that eventually form the acrosome at one end of the head (O'Donnell 2014). The distal centriole starts to produce an axoneme at the other end, where it develops a thicker midpiece where the mitochondria congregate. Additionally packaged and highly condensed is the DNA of spermatids. Certain nucleus basic proteins are first used to bundle the DNA; during spermatid elongation, these proteins are then replaced with prostamines. Transcriptional inactivity exists in the resulting densely packed chromatin. (O'Donnell, 2014)

-- Acrosome/cap stage
Encircling the condensed nucleus, the Golgi apparatus transforms into the acrosomal cap. (O'Donnell, 2014)
development of the tail. The sperm tail is formed by the elongation of one of the cell's centrioles. An intermediary framework known as the "manchette" facilitates this extension. (O'Donnell, 2014). The developing spermatozoa align themselves during this phase such that their tails point away from the epithelium and toward the lumen's centre.
-- Phase of Maturation
Around Sertoli cells in the testes, the extra cytoplasm is phagocytosed and referred to as the residual body of Regaud.(O'Donnell, 2014)
-- SEPARATION
After the mature spermatozoa are freed from the protective Sertoli cells and enter the seminiferous tubule lumen, a process known as spermiation occurs, which eliminates any leftover extraneous cytoplasm and organelles.(O'Brian Moira *et al.*, 2011).Now when they are mature, the resultant spermatozoa are sterile since they are not motile. With the help of peristaltic contraction, the non-motile spermatozoa are moved to the epididymis in testicular fluid secreted by the Sertoli cells.
They develop motility in the epididymis. Nevertheless, rather of using the spermatozoon's motility, muscular contraction is used to move the mature spermatozoa through the rest of the male reproductive system. Before the sperm pass through the male and female reproductive systems, they are prevented from fertilizing the egg by a glycoprotein coat covering the acrosome (Nicholls, *et al*., 2011). The enzymes heparin (made in the female reproductive system) and FPP (fertilization promoting peptide, generated in the prostate gland) capitulate the sperm lets the sperm attach to the egg by removing its covering.

2.3.6 LAMINA PROPRIA

The seminiferous tubules' lamina propria is ideally positioned between the spermatogonia and Sertoli cell membranes and the systemic circulation. It is composed of cellular and acellular zones that are well delineated in close proximity to the seminiferous, the basement membrane, a thin layer of extracellular matrix that surrounds the epithelium, has been demonstrated by electron microscopy (EM) to comprise the basal and reticular layers, lamina. There is a transparent area outside the basal lamina where type I collagen fibrils are present in different orientation. There is a layer of flattened cells called the peritubular myoid that surrounds this collagen zone or myofibroblast cells, which are subsequently layered with fibroblast and lymphatic endothelial cells. The structures surrounding the seminiferous tubules collectively are called the

lamina propria (Dym, 1994; Christl, 1990). Even so, the seminiferous tubules' lamina propria. In mature mammals, birds, and reptiles, this overall organizational plan (cellular and acellular zones), nevertheless, there is a significant variance in thickness, the basal lamina's homogeneity or lamellization, the different distribution or arrangement of collagen fibres, as well as the quantity and prominence of the myofibroblast layers within this lamina in the species under study at this time. Consequently, three categories have been identified (Christi., 1990). The lamina propria in the first kind is 1-3 μm thick and has a solitary layer of myoid tissue. It is composed of non-cellular lamellae on the inside and outside surrounding a solitary lamella of myoid cells and externally covered with of the layers of myofibroblasts of this lamina in the species so far studied. Therefore, three types have been recognized (Burgos *et al.*, 1970;). In the first type, the lamina propria is 1-3 μm thick and contains a single layer of myoid cells. It consists of an internal and external non-cellular lamella enclosing a single lamella of myoid cells and covered superficially by a layer of connective tissue cells. The internal non-cellular lamella is in turn made up of three layers: inner and outer homogenous layers and a middle one containing collagen. This type is found in rat, mouse, and hamster (Christl, 1990). In a second type, the components of the internal lamella are fused together and the myoid cells are in form of one, or possibly two layers with homogenous material and collagenous fibres in between and on the external surface. The lamina propria in this type is 2-3.5 μm thick and present in the guinea pig (Burgos *et al.* 1970). A third type with several layers of myofibroblast cells separated by varying amounts of extracellular glycosaminoglycans, proteoglycans, and collagen fibres has been described in other animals and man. The lamina propria in this type is 2.5-4.5 μm thick (Maekawa *et al*., 1996). The inner lamella (basal lamina) of this type of lamina propria may be a classical one with knob-like structure as in man, monkey, horse, dog, and cat (Bustos-Obregon, 1976) or be split into two layers as in rabbit and boar.

2.3.7 LEYDIG CELLS

Leydig cells are vital and important cells found in the male gonads' testes. Known as testicular interstitial cells, they are located in the space between seminiferous tubules, which house germ cells and Sertoli. These three cell types work together to regulate hormones, preserve spermatogenesis, and influence secondary sexual traits in males. (Axiotis *et al.*, 2007). Hormonal control of Leydig cells is provided by the hypothalamic-pituitary axis. These cells may be linked to malignant deformity or other pathologic alterations. Leydig cells can form groups of up to ten cells surrounding seminiferous tubules. Generally

speaking, they are characterized as polygonal cells with a big, spherical nucleus and eosinophilic cytoplasm. Due to the fact that these cells produce testosterone, they possess characteristics of cells that secrete steroids, such as a substantial number of mitochondria with tubulovesicular cristae, a large and well-developed smooth endoplasmic reticulum, and numerous huge lipid droplets. (Mahran *et al*., 2017). Liposomal accumulation in the form of several spherical irregular lumps, known as lipofuscin, is another characteristic of Leydig cells.
The cytoplasmic inclusions known as Reinke's crystalloids are unique to Leydig cells and have a pale stain. Usually organized in a linear pattern, these crystals have a cylindrical or rod-like form. These bodies have not yet been given a purpose, yet some research indicates that they are by-products of the testes' steroid metabolism and testosterone preparation. (Codesal, *et al.* 1991). Primary source of Leydig cells in men, testosterone or androgens. Their physiology enables them to be essential to numerous key physiological processes in males, including as spermatogenesis, the creation of sperm, the regulation of sexual development, and the maintenance of secondary sexual traits and behaviours. (Liu, *et al.,* 2019). The creation of androgen inside Leydig cells is the first of these vital processes, and it is mostly regulated and controlled by the hormone luteinizing hormone (LH), which is generated by the pituitary gland. The smooth endoplasmic reticulum and abundant lipid content of Leydig cells contribute to their increased steroid (androgen) production efficiency. (Liu *et al.,* 2019). Leydig cells generate androgens throughout the development of male embryos, which trigger the development of the Wolffian duct into male urogenital organs. Anti-Mullerian hormone is produced by sertoli cells, which stops the growth of the female vaginal system. Adult Leydig cells take the place of juvenile Leydig cells in adults, preserving androgen throughout life. (Behringer, Griswold, 2009)

Leydig cells are found in the interstitium outside seminiferous tubules, which is where spermatogenesis takes place, as was previously mentioned. Androgens work on germ and Sertoli cells in the seminiferous tubules, diffusing from the interstitium to sustain and stimulate sperm production. It is important to remember that the testes contain larger quantities of testosterone than the serum (Behringer, Griswold (2009). Reduced expression of vital proteins involved in the regulation of germ cells has been linked to abnormalities in spermatogenesis, which is correlated with lower intratesticular testosterone levels.

2.3.8 SERTOLI CELLS

The seminiferous tubules of the male gonads, the testes, contain sertoli cells. In 1865, they were first noticed by a young Italian doctor called Enrico Sertoli (Griswold, 2018). The other type of cells in the germinal epithelium is of the spermatogonia lineage, while sertoli cells make up the other type. Male sperm

generation heavily depends on sertoli cells, one of the most vital cell types. Near the basolateral section of the seminiferous tubule, they are frequently recognized as large, closely packed cells. These nursemaid cells of the initial spermatogonia are also referred to as sustainably occurring cells of Sertoli. In 2017, Duan P, Hu C, and others. The spermiogenesis process—which produces viable sperm—is aided by sertoli cells', Inhibin B, and Activin are only a few of the many essential chemicals secreted by sertoli cells. These mucous provide a hormonal negative feedback loop that can either directly or indirectly aid in spermatogenesis (Griswold, 1998).Alongside the nearby spermatogonia, sertoli cells initiate the process of spermatogenesis in response to pituitary hormones like follicle-stimulating hormone (FSH).The production of sperm is normal, but in mature males, the simple lack of sertoli cells in the testes might cause infertility due to their significance.(Zarzycka *et al.*, 2016).

A very important autosomal gene called SOX9 (SRY-Box Transcription Factor 9) is required for the formation and differentiation of Sertoli cells from their supporting cell progenitors in the genital ridge. Steroidogenic factor 1 (NR5A1) aids in the differentiation of Sertoli cells in addition to the sex-determining region of the Y chromosome (SRY).

Lack of these elements will cause the ovaries, or female gonads, to develop. When SRY and SOX9 levels approach critical levels, the gonads begin to morphologically resemble real testicles. The creation of testicular cords, the epithelization of Sertoli cells, the differentiation of Leydig and myoid cells, and the mitotic arrest of germ cells are some of these alterations. (Titi-Lartey *et al*., 2023)

The largest, irregularly shaped cells of the germinal epithelium in the seminiferous tubules are called sertoli cells. The most reliable technique to identify them is to look for the largest columnar cell that spans the apical lumen of the cross-sectioned seminiferous tubule and is still adhered to the basement membrane's basal lamina. They are frequently asymmetrical or pyramidal in form, but they are always oriented toward the in contrast to the secondary spermatogonia and spermatids that face the lumen on the basolateral side. It is possible to distinguish the Sertoli cells from the neighbouring main spermatogonia. In contrast to sertoli cells, which contain more pale nuclei in a bigger, irregularly shaped cell, primary spermatogonia frequently have darker distinct nuclei in a compact circular homogeneous cell (Suede *et al*. 2023)

It's crucial to understand that the shape of the Sertoli cell creates connective adhesion molecules and tight connections with nearby Sertoli cells to enable local testosterone sequestration. The blood-testes-barrier, which is the foundation of this junction, offers several prerequisites for proper spermatogenesis, such as immune system evasion, testosterone concentration, ion control, and barrier protection. Mature spermatogonia and spermatids are

inside the blood-testes-barrier of the seminiferous tubule because the seminiferous cell blood-testes-barrier is surrounded by the seminiferous cell epithelium, which is the only other outermost cellular structure.(Nistal *et al*., 1982)

Within the seminiferous tubules, sertoli cells perform a variety of jobs and functions. The ability to secrete a chemical is among its most crucial qualities named Mullerian Inhibiting Factor, which aids in stopping the development of female sex organs after the testes are determined embryologically. Additionally, sertoli cells release Inhibin B, which acts on the anterior pituitary to help control FSH. Sertoli cells secrete a material known as androgen binding protein, which helps to concentrate the amount of testosterone that is available. By controlling ions, amino acids, and eventually "nursing" the spermatogonia, sertoli cells help preserve ideal conditions for the initial spermatogonia and spermatogenesis process. Overall, the sertoli cell plays a critical role in spermatogenesis and is stimulated by the anterior pituitary through the FSH receptor. The Sertoli cells serve to break down any remaining cytoplasm during the last stage of spermatid maturation, known as spermiogenesis. It falls off the spermatid. (Nishimura *et al*., 2017)

Another remarkable structural function of the Sertoli cell is the preservation of the blood-testes barrier. Tight connections between neighbouring Sertoli cells, which are all positioned on the basement membrane of the seminiferous tubules, provide this barrier. The seminiferous tubules are divided into two functional layers by this configuration. The spermatogonia, both primary and secondary, and spermiogenesis are located inside the tight connections. Primitive spermatogonia and germinal epithelial cells are located outside the tight junction. Additionally, conditions that are able to store testosterone and raise local concentrations required for spermatogenesis are made possible by this barrier. Additionally, the blood-testes barrier aids in avoiding auto-immune illnesses and immune system surveillance. (Cheng. *et al*., 2004).

The role of sertoli cells is to support, shield, and nourish spermatogenic cells.(Hao, *et al.,* 2019), participate in the endocrine and paracrine regulation of spermatogenesis.(Shi *et al.,* 2018), during spermatogenesis, sertoli cells control the metabolism of cholesterol.(Titi-Lartey *et al.,* 2023), they secrete substances that facilitate the passage of spermatozoa.(Socorro*et al*., 2010), they engulf foreign objects, apoptotic cells, etc.(Arandjelovic, et al., 2015), under the influence of follicular stimulating hormone, they release the androgen-binding protein (FSH) (Khan *et al.,* 2023), lecretin B hormone, which inhibits the secretion of FSH (Carlsen *et al.,* 1999), tight connections between them form a blood-testes barrier.(Khan *et al.*, 2023)

2.3.9 GERM CELL

The only cells in an organism that pass on genes to their progeny are germ cells, highly specialized cells that give rise to gametes. In many animals, oogenesis (the creation of eggs) and spermatogenesis (the production of sperm) are sustained by germline stem cells (GSCs). The germ line is frequently referred to as immortal since the genetic information contained within germ cells is passed down from generation to generation. Thus, it's plausible that germ cells have evolved special defense mechanisms to preserve and pass on the genetic material that makes them who they are forever (Nayernia., 2008). However, research has demonstrated that germ cells are pluripotent at all developmental stages. We have successfully cultured mouse spermatogonial stem cells (SSCs) for an extended period of time, as have other groups. The cells shared phenotypic similarities with ES/embryonic germ cells, with the exception of their pattern of genomic imprinting. Under the same conditions that were utilized to trigger the differentiation of the ES cells, they differentiated into distinct types of somatic cells in vitro. When the SSCs were injected into blastocysts, they created germline chimeras. Moreover, we have demonstrated the ability of somatic stem cells to develop into germ cells. The in vitro production of male and female gametes presents new opportunities for the application of these cells toward the advancement of fundamental reproductive biology and, specifically, toward the development of therapeutic cloning, transgenic technologies, and infertility treatment. We started by configuring the in vitro gametogenesis systems utilizing three different techniques: 1) Germ derivation cells from somatic stem cells; 2) gametogenesis created from ES; and 3) in vitro gametogenesis based on cultivated spermatogonial stem cells. As stated by Nayernia (2008). Using embryonic stem cells, we devised a method for creating germline stem cell lines. These cells are functional, as demonstrated by fertilization following intracytoplasmic injection into mouse oocytes, and are capable of going through meiosis and producing haploid male gametes in vitro. Future studies should clarify the molecular and cellular processes that underlie the development of ES into functional gametes. Using a new method, we demonstrate the ability of bone marrow stem (BMS) cells to transdifferentiate into male germ cells. The recognized molecular markers of primordial germ cells were expressed by germ cells produced from BMS cells (Nayernia., 2008). We will review these facts and provide a hypothesis that some somatic stem cells may be direct descendants of the germ lineage based on these data as well as published evidence from other groups. The germ lineage forms soma, which serves as a "mother lineage" for all somatic stem cell lineages seen in the adult body, in order to pass genes on to the next generation.

2.3.10 PRIMARY FUNCTION

The male reproductive gland, or testis, is mostly in charge of creating androgens and sperm. The anterior pituitary gland releases Luteinizing Hormone (LH), which regulates testosterone levels; on the other hand, Follicle-Stimulating Hormone (FSH) levels regulate sperm production. (Pandey *et al.*, 2016)
Lobule tissues are responsible for the ovular form of the testes. Prior to the sperm becoming mature enough to ejaculate, the testicles, also known as the testes, are crucial for sperm production and storage. In addition, they create testosterone, which is necessary for fertility, sex desire, and the growth of bone and muscle mass. Producing and storing sperm is their primary duty. The production of testosterone and other androgen hormones, which are exclusive to men, depends on them as well.(Tim Jewell*.,* 2018)

2.3.11 EMBRYOLOGY

In the retroperitoneum, the testes begin as an undifferentiated gonad. The gonad differentiates into the testes due to the testis-determining factors (SRY gene) on the Y chromosome. Because the SRY gene is missing in females, the gonad develops into an ovary. The male sex hormone, testosterone, is produced by the testes when the foetus begins to develop. (Samppaio FJ, Favorito LA, 2014). The male genitalia can develop thanks to this sex hormone.
Through invagination, the tunica albuginea creates a connective tissue latery between the seminiferous tubules and the remainder of the testis. At eight to ten weeks, the Sertoli cells begin to produce Mullerian-inhibiting Substance (MIS), which forces the Mullerian ducts to regress. (Samppaio FJ, Favorito LA, 2014). In an adult male, the testicles and the prostatic utricle are the only areas where the Mullerian ducts are still present.
The testes, which are found in the belly, begin to descend throughout the third trimester of pregnancy and eventually enter the inguinal canal and scrotum. They travel via the inguinal canal, the abdominal wall, and the peritoneum. During development, the processus vaginalis, a structure that arises from the peritoneum, is contained in the inguinal canal (Gandhi *et al.*, 2017). It lets the testes descend, eventually going through apoptosis to produce the tunica vaginalis, which encircles a portion of the testis. Disruption of the processus vaginalis can result in issues like inguinal hernia and communicative hydrocele.

2.3.12 BLOOD SUPPLY AND LYMPHATICS

--Arterial supply;
The testes receive blood flow from the testicular arteries. Slightly below the renal artery origin, they originate from the anterolateral portion of the

abdominal aorta. The vessels proceed via the deep inguinal ring, cross the ureter, and join the spermatic cord while traveling through the retroperitoneum. The arteries of the vas deferens and the cremasteric artery provide the testes with additional blood flow (Pandey *et al*., 2016)

--Venous Drainage;
The testes' venous outflow occurs through the pampiniform plexus, which is located in front of the vas deferens. To generate the testicular vein, the veins converge superiorly (Kotian *et al*., 2016). The right testicular vein empties into the vena cava, and the left testicular vein drains into the left renal vein

--Lymphatic Drainage;
The lymphatics from the testes empty into the preaortic lymph nodes, following the same route as the testicular arteries. (Pandey *et al.*, 2016)

2.3.13 INNERVATION OF THE TESTIS

From the same embryonic level as the kidneys, the testes are derived. As a result, they have 90% sympathetic autonomic innervation, originating from the T10-L1 segments, and 90% parasympathetic innervation, originating from the S2-4 segments. (Koopman, Svingen., 2013) Superior spermatic nerves, middle spermatic nerves, and inferior spermatic nerves are the three groups of autonomic nerves that accompany the gonadal arteries and vas deferens to the epididymis and testis.

CHAPTER THREE

3.0 MATERIALS AND METHOD

3.1 IDENTIFICATION OF PLANT

Glycine Max was bought from the New Benin Market, Benin City, Edo State. The plant was extracted at the department of Plant Biology and Biotechnology of the University of Benin. It was given an herbarium number, UBH-G470.

3.2 PREPARATION OF EXTRACT

The Soybean extraction process was essentially followed according to the method described by Aiyegoro and Oko, 2010 with little modifications. Initially, the Soy bean was dried in an oven set at 100^{o}C for almost four hours. Next, the dry soy beans were ground into a powder.

Three extractions of the 1.5kg of powdered soy beans were made in 41.25L of distilled water at room temperature using a shaker for 48 hours. Whatman No.1 filter paper and a Buchner funnel were used to filter the extract. The resulting aqueous extract filtrate was rapidly frozen at -40^{o}C and freeze-dried in a freeze dryer for 48 hours. To achieve the required concentrations for this study, the extracted material was reconstituted using distilled water.

3.3 PHYTOCHEMICAL ANALYSIS

Determination of Total Protein Content

Little changes were made to the methodology utilized by Sudipta *et al*. 2020 in order to determine the extract's total protein content. The calibration curve, which was utilized to determine the unknown protein concentration was prepared using Bovine Albumine serum as the reagent.

The sample extracts were mixed with 4-5ml of reagent 1 (48ml of 2% sodium carbonate in 0.1N sodium hydroxide + 1ml of 1% sodium potassium tartrate + 1ml of 0.5% copper sulphate) and incubated for 15 minutes. Subsequently, each sample was combined with 0.5ml of freshly produced reagent 2 (1-part FolinCiocalteau: 1 part water) and incubated in the dark for 30 minutes. Following that, the absorbance at 660nm was determined and the protein content is given as Ug BSAE/ml of fresh extract.

Determination of Total Phenolic Content

The Folin-Ciocalteu reagent was used to calculate the total phenolic content of the extract, slightly altering the Singleton and Rossi (1965) method and using tannic acid as a standard.

In a nutshell, 1.0ml of the extract solution (250Ug/ml) was added to the test tube. Next, 1.0 milliliter of Folin-Ciocalteu reagent was added, and the flask's contents were well combined. 15.0ml of 20% Na_2CO_3 was added after 5 minutes, and it was left to stand for two hours. Using a UV-Vis spectrophotometer (Jenway 6100, Dunmow, Essex, U.K.), the absorbance was measured at 760nm. Using an equation derived from the common tannic acid calibration graph, the total phenolic content was calculated as Ug of tannic acid equivalent (TAE).

Determination of Alkaloids Content

Using Harborne's (1973) approach, the total alkaloid content was determined. Following the addition of 100ml of 20% acetic acid in ethanol and the weighted addition of 5g of the extract, the beaker was capped and left to stand for two hours. A water bath was used to concentrate the extract to a quarter of its initial volume after it had been filtered. After the extract started to precipitate, concentrated ammonium hydroxide was applied drop by drop until the precipitation was complete. After letting the mixture settle completely, the precipitate was filtered, cleaned with 1% ammonia solution, dried and weighed. All samples were analysed in triplicates.

$$\text{Alkaloids (\%)} = \frac{Weight\ of\ Residue}{Weight\ of\ Sample} \times 100$$

Flavonoid Content determination

Aliquots of the homogeneous cabbage extract (1.5g) were measured in triplicate to evaluate the flavonoid concentration (Ilahy *et al*., 2011). Flavonoid analysis was conducted using thirty-microliter aliquots of the methanolic extract. Following the addition of 90µ of methanol to dilute the samples, 6µ of 10% aluminium chloride ($AlCl_3$), 6µ of 1mol/l sodium acetate (CH_3CO_2Na), and 170µ of methanol were added. After 30 minutes, 415nm was used to measure the absorbance. To calculate the flavonoid content (Ug Qe/g), quercetin was used as the standard.

Estimation of Total Saponins Content

The method reported by Makkar *et al*., which was somewhat modified from the vanillin-sulphuric acid colorimetric reaction, was used to estimate the concentration of total saponins. To 250µL of distilled water, approximately 50µL of plant extract was added. 250µL of vanillin reagent (equivalent to 800mg of vanillin in 10mL of 99.5% ethanol) was added to this. Next, 2.5 millilitres of 72% sulphuric acid were added and thoroughly mixed. This solution was maintained for 10 minutes at 60^{o}C in a water bath. It was chilled in ice cold water for 10 minutes and the absorbance at 570nm was measured. Standard saponin solutions ranging from 0 to 25ppm were made

using the saponin stock solution. The test samples and standard solutions were handled in the same way. PPM was used to express the values.

3.4 EXPERIMENTAL ANIMALS

Eighteen (18) juvenile Wistar rats with an average weight of 35g - 69.5g were used for this research work. They were obtained from the animal house of the Department of Anatomy, Faculty of Basic Medical Sciences, University of Benin, Benin City and were housed in standard cages with varying numbers per group which were kept in the animal house of the department. Their mothers were acclimatized in their various cages all through the period of mating, gestation and administration. Throughout the experiment, the animals were housed in conventional conditions with a 12-hour light and 12-hour dark cycle. The babies where birthed in healthy conditions and was isolated with their mothers in new conducive cages from the others. After six (6) weeks of careful observed growth, the offspring from each group were weighed anesthetized and sacrificed. The testis was harvested, weighed and taken for biochemical and historical analysis.

3.5 EXPERIMENTAL MATERIALS

Plastic cages, sawdust, ceramic plates, beaker, orogastric tube, extract of *Glycine max,* refrigerator, running water, distilled water, normal saline, pipette, light microscope, formal saline, frosted slides, cotton wool, methylated spirit, iodine, dissection table, dissecting set, disposable gloves, sample bottles, formalin, oven, water bath, embedding mould, rotatory microtome, slide tray and coverslips.

3.6 EXPERIMENTAL DESIGN AND PROCEDURE

Eighteen (18) animals were allotted to three groups with varying numbers of rats. Animals in group A served as the control group while those in other groups served as the experimental groups administered with *Glycine Max* extract.

Group A (Control Group) animals were fed with vital feed and treated with distilled water.

Group B animals were administered 250mg/kg body weight of *Glycine max* extract.

Group C animals were administered 500mg/kg body weight of *Glycine max* extract.

Administration of the extract was with the aid of an orogastric tube orally for the gestation period before the animals were sacrificed.

3.7 TERMINATION OF EXPERIMENT

On the day the animals were to be sacrificed, we measured and documented the final weight of every animal. Each male rat's testis was individually placed within tissue bottles with the proper labelling for easy identification, the testis

was extracted, weighed and its corresponding diameter measured. Both Normal Saline and neutral formalin were used in the fixation of the testis inside the bottles. Studies on antioxidants also involved the collection of testes.

3.8 ANTIOXIDANT DETERMINATION PROTOCOL

DETERMINATION OF CATALASE (CAT)

Catalase (CAT) activity was estimated by the method described by Cohen et al., (1970).

Reagents

Hydrogen peroxidase (H_2O_2)
Sulphuric acid (6M) H_2SO_4

Preparation of reagents

0.01M $KMnO_4$ was prepared by distilling 0.158g of $KMnO_4$ in 100ml of distilled water. Phosphate buffer (pH 7.4) 0.426g of $NaHPO_4$ and 0.240g of NaH_2PO_4 was weighed and dissolved in 100ml of distilled water. 6M H_2SO_4 and 32.3ml of conc. H_2SO_4 was added to 66.7ml of distilled water.

Procedure

To an unknown volume of plasma (0.5ml), 5.0ml of H_2O_2 was added. This was mixed by inversion and allowed to stand for 30min. The reaction was stopped by adding 1.5ml of 6M H_2SO_4 and 7ml of 0.01M $KMnO_4$. These were mixed by inversion and allowed to stand for 10min. The absorbance was read at 480nm within 30-60 seconds against distilled water. The enzyme blank was run simultaneously with 1.0ml of distilled water instead of hydrogen peroxide. The enzyme activity was expressed as μmoles of H_2O_2 decomposed/min/mg/protein.

Calculation

$$Activity = \frac{OD/Min \times V}{M \times V \times L \times Y}$$

Where OD = Absorbance
L= Light path
V= Total volume of reaction sample
M= Molar coefficient of H_2O_2 (40/m/cm)
V= Volume of sample

Y= mg protein in the sample

ESTIMATION OF SUPEROXIDE DISMUTASE ACTVITY (SOD)

This was determined according to the methods of Masra and Fridorich (1972)

Principle

Adrenaline undergoes auto oxidation rapidly to adrenochrome whose concentration can be determined at 420nm with the aid of a spectrophotometer. The auto oxidation of adrenaline depends on the presence of superanions.

Superoxide dismutase inhibits the auto-oxidation of adrenaline by catalysing the breakdown of superoxide anion. The degree of inhibition reflects the activity of SOD which is determined at 420nm.

Reagent and preparation

Carbonate buffer (0.05M) pH 10.2: This was prepared by dissolving 0.2014g of Na_2CO_3, 0.2604g $NaHCO_3$ and 0.0372g of EDTA in 100ml of distilled water. The pH was adjusted to 10.2 using Sodium hydroxide.

Hydrochloric acid (0.005M): This was prepared by adding 0.044 concentration of HCL to 99.96mls of distilled water.

Adrenaline solution (0.3mM): This was prepared by dissolving 0.01098g of adrenaline in 100mls of 0.005M HCL solution.

Plasma volume of 100ml was mixed with 125ml of carbonate buffer and 150ml of adrenaline solution. 100ml of distilled water was mixed with 1.25ml of carbonate buffer as reference sample. These were mixed and absorbance read at 420nm.

These were mixed and read at 420nm

$$\%\ Inhibition\ =\ \frac{(O.D\ test\ -OD\ ref)}{OD\ test}\ \times 100$$

Enzyme concentration can thus be calculated

$$Unit\Big/mg\ Protein\ =\ \frac{\%\ Inhibition}{50\ \times\ Y}$$

Where Y = mg of protein in the volume of sample used

ESTIMATION OF GLUTHATHIONE PEROXIDASE (GPx)

This was determined according to Nyman (1959)

Principle

This is based on the oxidation of pyrogallol to purpurogallin by peroxidase activity, resulting to a deep brown colour disposition, read at 420nm.

Reagent and preparation

Pyrogallol (20mM): 0.2552g of pyrogallol was dissolved in 100mls of distilled water.

Procedures

To an aliquot of plasma (0.2ml), 2.5ml of phosphate buffer, 2.5ml of H_2O_2, 1.5ml of distilled water and 2.5ml of pyrogallol was added.
The reaction was allowed to stand for 30mins at room temperature. A deep brown colour was formed which was read at 480nm.

Calculations

$$Activity = \frac{OD/Min \times vt \times Df}{E \times Vs \times Y}$$

OD = Absorbance of test
Vt = Total volume of reaction mixture
Df = Dilution factor = 1
E = Molar extinction co-efficient (12/m/cm)
Vs = Volume of sample
Y = mg of protein used

DETERMINATION OF MALONDIALDEHYDE (MDA)

Malonaldehyde was determined using the thiobarbituric acid assay (Buege and Aust, 1978)

Principle

Malonaldehyde which is a product of lipid peroxidation react with thiobabituric acid (TBA) to give a red species.

Procedure

A volume of plasma (1.0ml) was added to 2.0ml of TCA-TBA-HCL and mixed thoroughly. The solution was heated for 15mins in a boiling water bath. After cooling, the flocculent precipitate was

removed by centrifuged at 1000g for 10min. The absorbance was determined using the formula;

$$MDA\ (mol/mg\ protein) = \frac{A \times V \times 100}{M \times V \times Y}$$

A= Absorbance
V= Total volume of reaction mixture
M= Molar extinction coefficient
V= volume of the sample
Y= mg protein

3.9 HISTOLOGICAL ANALYSIS

3.9.1 PARAFFIN TISSUE PROCESSING

Subsequent to the tissue (placentas) harvesting and fixation in 10% formalin, the tissues underwent the following processing after being left to stand for roughly 72 hours in order to obtain adequate tissue penetration:
Tissues were dehydrated in progressively higher alcohol grades (ranging from 70% to 90% and absolute alcohol), with ethanol being the most preferred alcohol.
Alcohol was cleared with the use of xylene as a clearing agent. In order to eliminate alcohol from tissue samples, tissues were put through two modifications.
The tissues were infiltrated three times in a heated oven between 65 and 70 degrees Celsius using a solution of melted paraffin wax. The last change lasts roughly thirty minutes, although the first two only lasted fifteen minutes each.
The embedding technique involved utilizing an embedding mould, into which melted paraffin wax was poured, and the infiltrated tissues were arranged longitudinally to create a longitudinal section.
Tissue blocks were formed when the molten paraffin wax was allowed to cool and solidify.
Using a rotary microtome, tissue was sectioned into 5 micron-thick sections to resemble thin sheets of material.

3.9.2 HEMATOXYLIN AND EOSIN STAINING METHOD

- A 20% alcohol solution was used to disseminate the ribbon-like portions of excellent tissue, which were then sliced and floated in a water bath set at 30 degrees Celsius.

- After being sectioned, the tissues were placed on glass slides and let to air dry.

- The tissue slices underwent a 15-minute xylene treatment to eliminate surplus paraffin wax. They were then hydrated by passing them through progressively higher alcohol concentrations (100%, 90% and 70%) and finally submerging them in water, each for around 5 minutes.

- H&E dyes were utilized to stain the tissue. Hematoxylin was used to stain the tissues for 10 minutes.

- Blueing or washing tissues under running water was done.

- Sections were counterstained for 5-10 minutes using 1% eosin.

- After that, the tissues were then rinsed in water.

- The tissues underwent a fast dehydration process using increasing alcohol grades (ranging from 70% to 90%) and absolute alcohol for a duration of 5 minutes.

- After 5 minutes of xylene clearing, the slides were mounted with a glass coverslip using an appropriate mountant, a combination of Xylene and Distrene Plasticizer (DPX).

3.10 DATA ANALYSIS

The IBM Statistical Package for Social Science (SPSS) was used to analyse the data. After cleansing the data, the Kolmogorov-Smirnov test was used to determine if it was normal. One-way analysis of variance (ANOVA) and post-hoc LSD were used to examine the animal body weights, relative organ weights and serum hormone levels. The results were presented using tables and graphs, with values deemed significant at $p<0.05$. Every animal research group was regarded as a single experimental unit, and all of the data produced for each animal were incorporated in the analysis.

CHAPTER 4

4.0 RESULTS

4.1 PHYTOCHEMICAL ANALYSIS

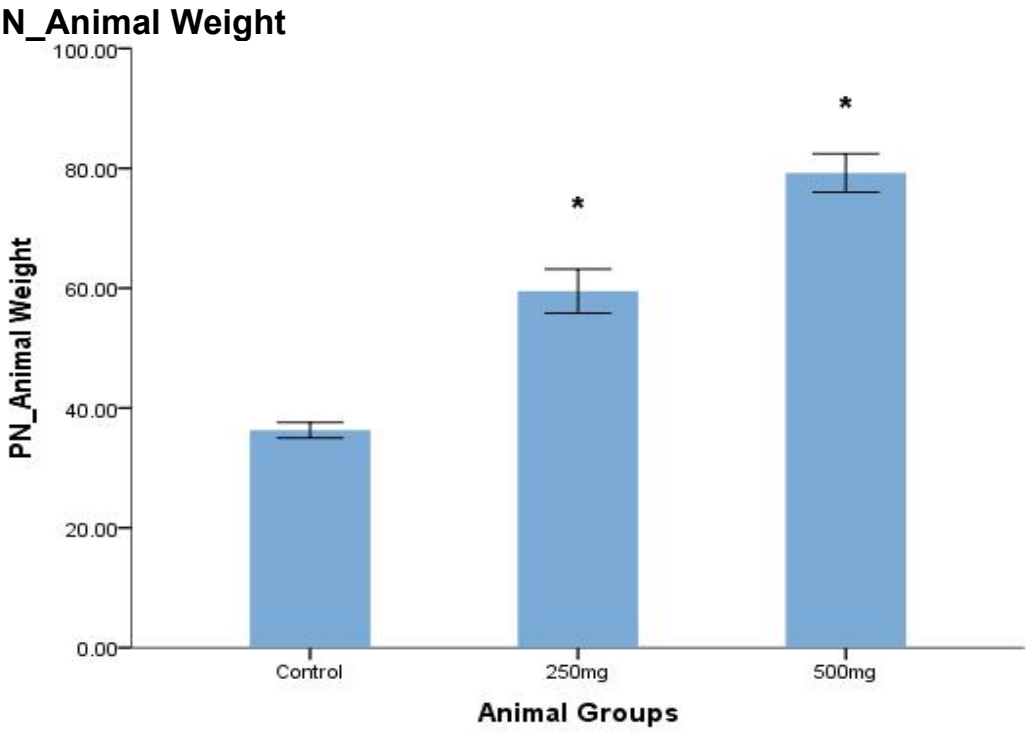
Table 4.1: Showing the qualitative analysis of *Glycine max* extract

++: PRESNT
+: SLIGHTLY PRESENT
-: ABSENT

S/N	PHYTOCHEMICAL	OBSERVATION
1	REDUCING SUGAR	+
2	SAPONINS	++
3	FLAVONOIDS	+
4	PHENOLICS	+
5	TANNINS	-
6	EUGENOLS	+
7	TERPENOIDS	+
8	STEROIDS	-
9	ALKALOIDS	+
10	PROTEIN	++

4.2 STATISTICAL RESULTS

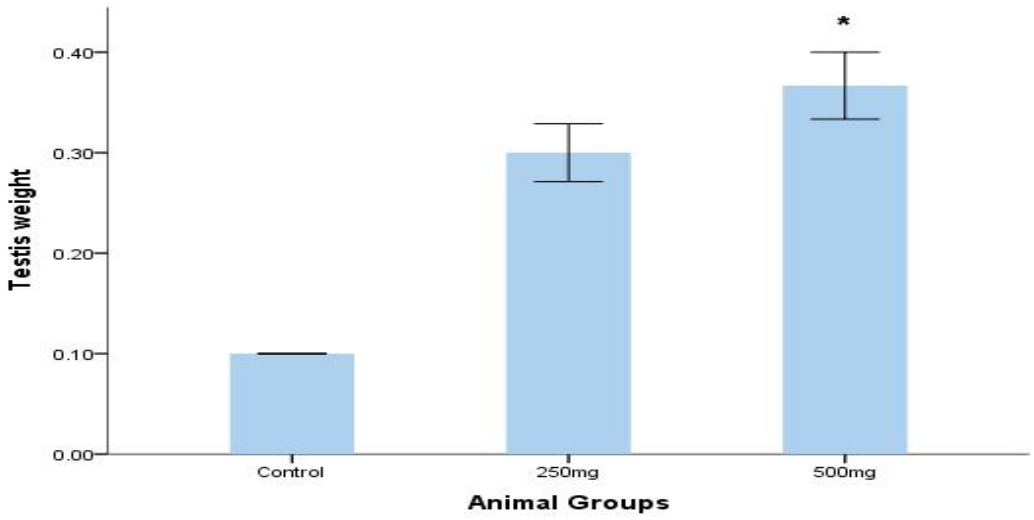
Chart 4.1; Showing the body weight of animals



PN - post natal
* - indicates $p<0.05$

There was statistically significant difference among the body weight of animals from groups B and C who were administered 250mg and 500mg of extract respectively.

Chart 4.2; Showing the testis weight of animals

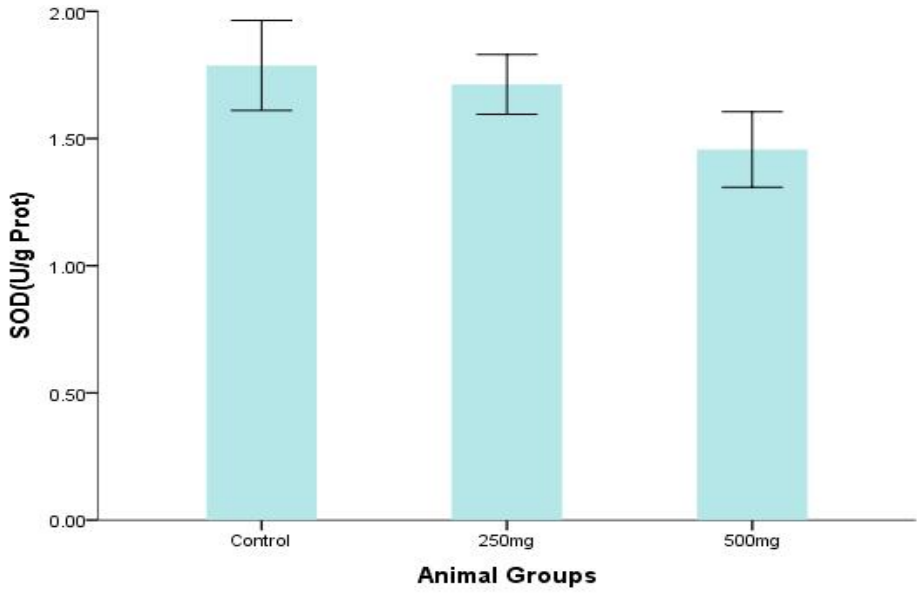


* - indicates p<0.05

There was a major statistical increase in the testicular weight group C.

4.3. OXIDATIVE STRESS

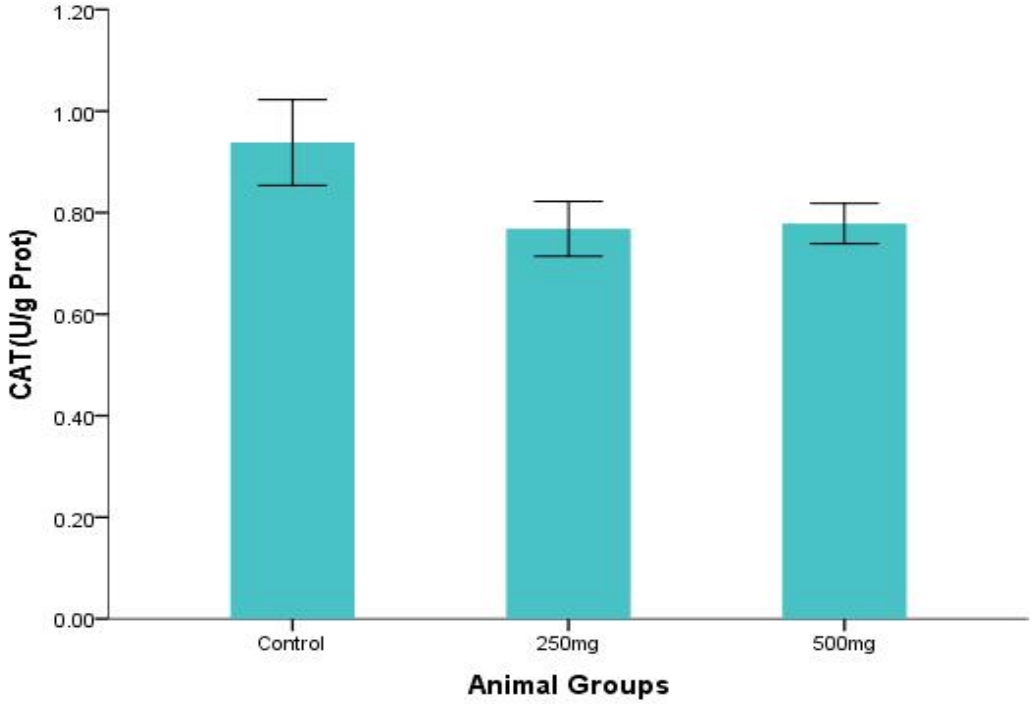
Chart 4.3: Showing test for the superoxide dismutase



There was no significant statistical difference among all groups.

Chart 4.4: Showing test for catalase

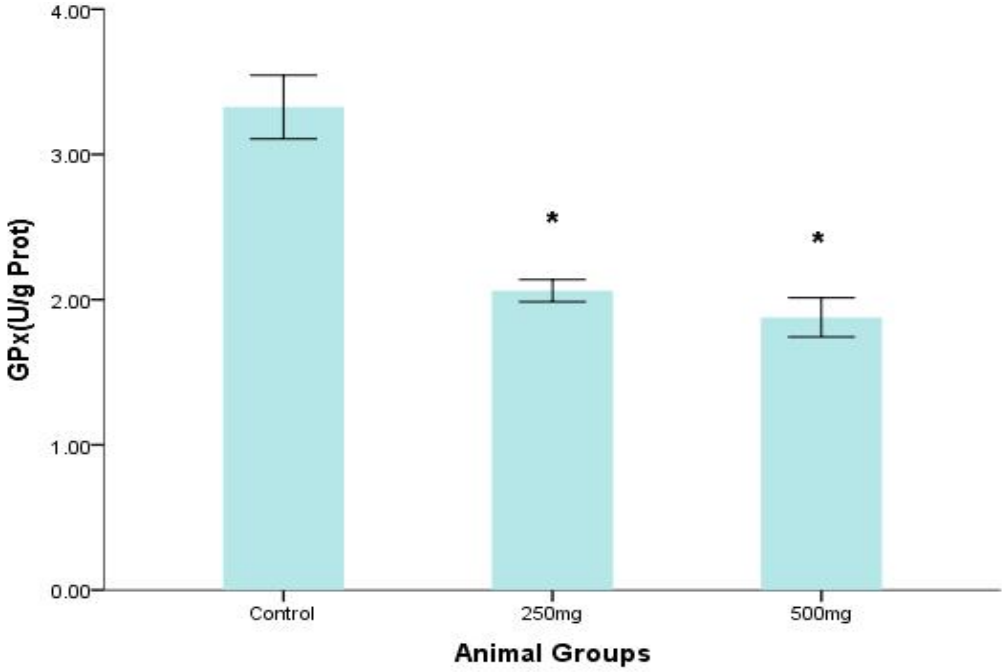
CAT (U/g Prot)



There was no significant statistical difference among all groups.

Chart 4.5: Showing test for glutathione peroxidase

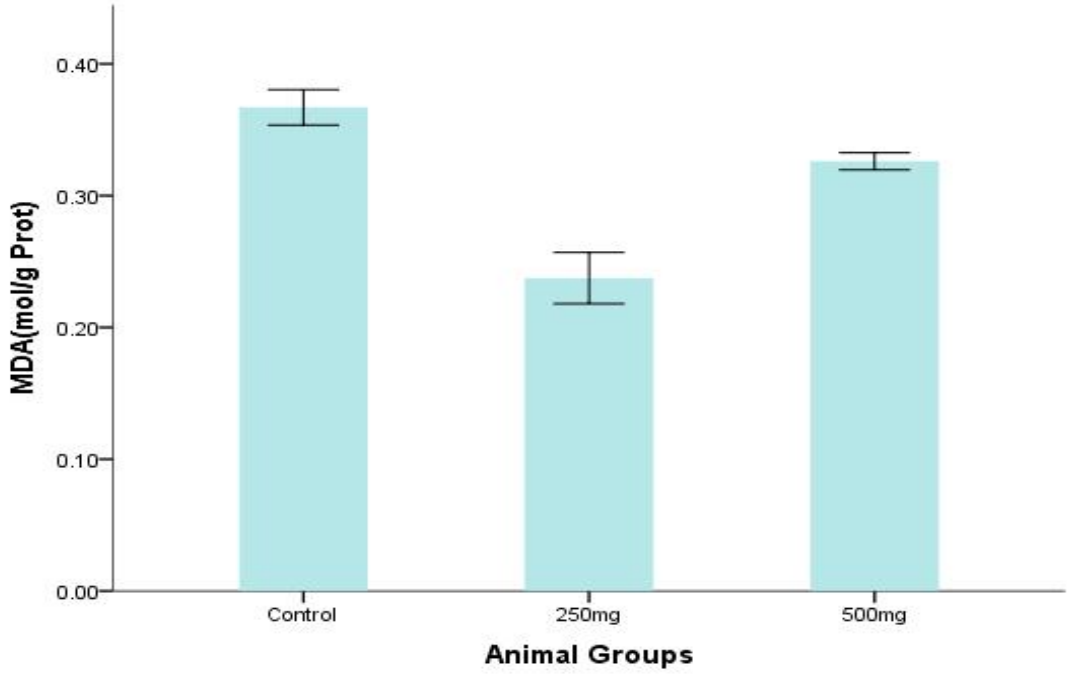
GPx (U/g Prot)



* - indicates p<0.05

There was statistically significant decrease among the results of animals in group B and C who was administered 250mg and 500mg of extract respectively.

Chart 4.6: Showing test for the malondialdehyde in serum of post-natal rats



There was no significant statistical difference among all groups.

4.4. HISTOLOGY OF THE TESTIS

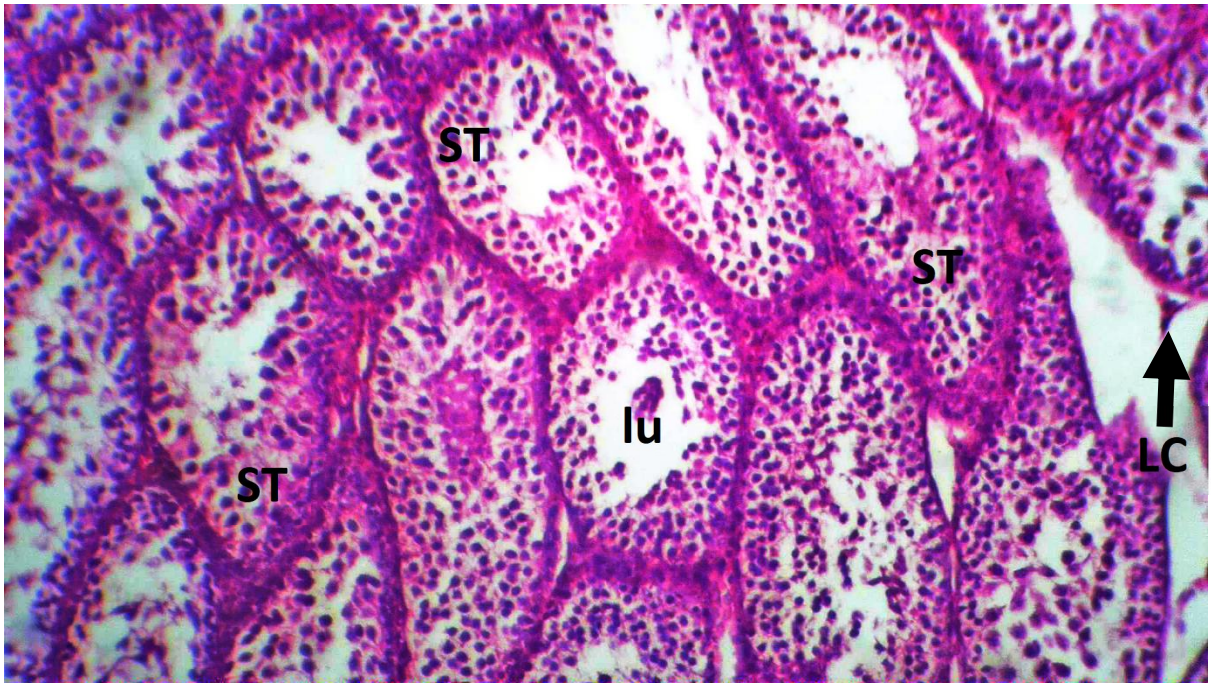


Plate 4.1a: CONTROL (H&E; 100x)

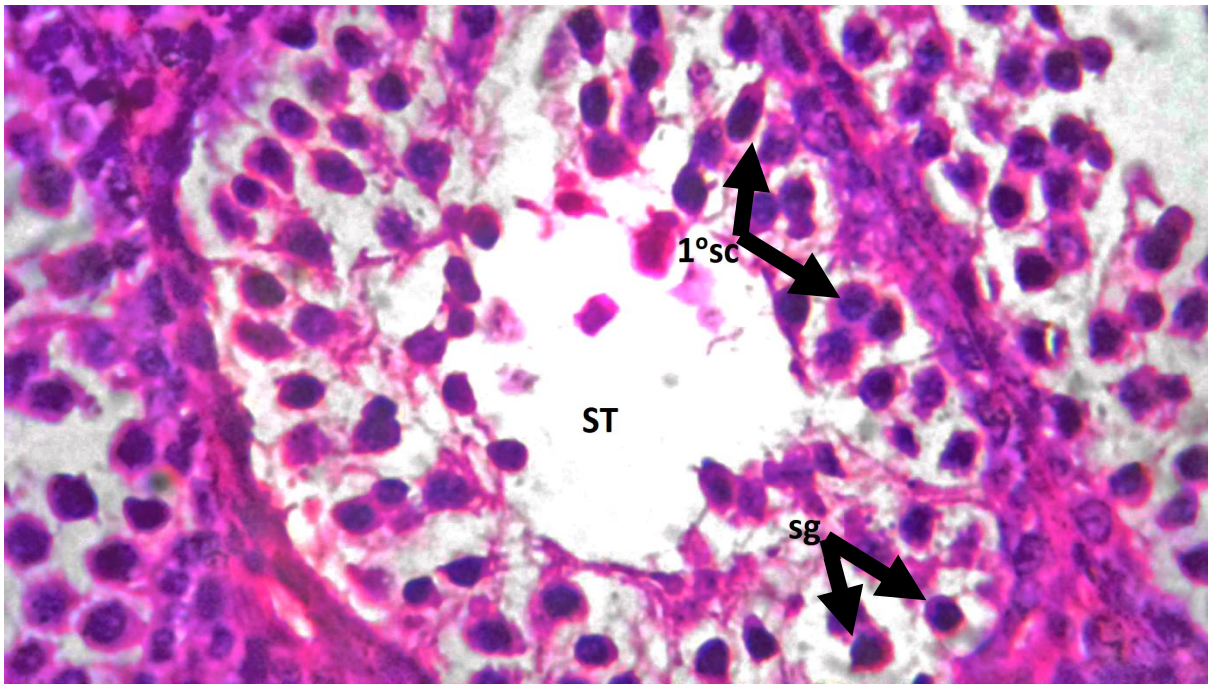


Plate 4.1b: Representative photomicrograph of juvenile (PND42) testis of control group showing normal histological features of juvenile testis: Seminiferous tubules (ST) acquiring lumen (lu); spermatogonia (sg), Sertoli cells linning the ST, Leydig cells (LC) in the interstitial space, primary spermatocytes 1°sc (H&E; 400x):

(H&E; 100x)

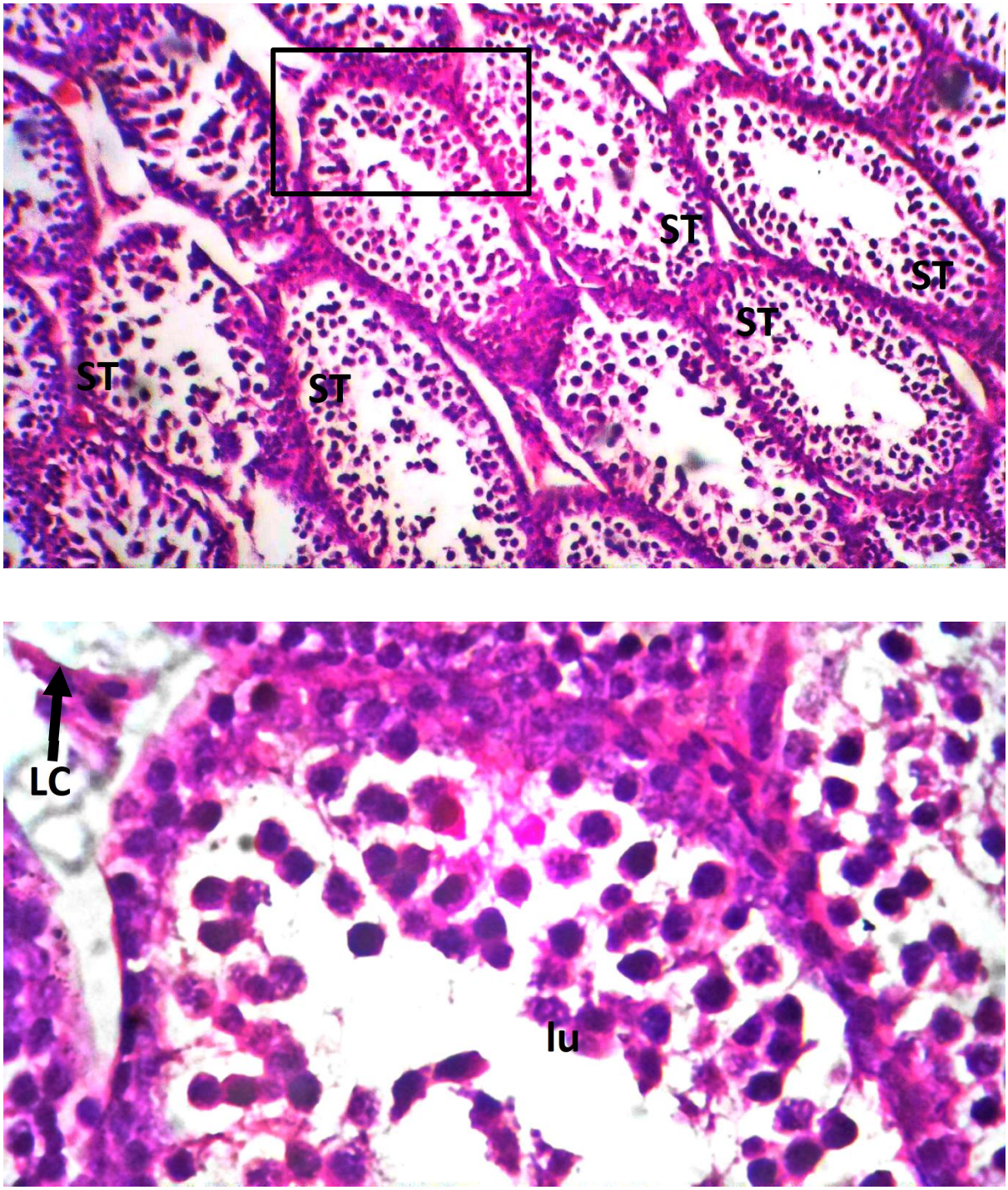


Plate 4.2 a (above) and b (below) of 250mg/kg: similar to control; Seminiferous tubules (ST) acquiring lumen (lu); spermatogonia (sg), Sertoli cells linning the ST, Leydig cells (LC) in the interstitial space, primary spermatocytes 1^{o}sc (H&E; 400x):

(H&E; 100x)

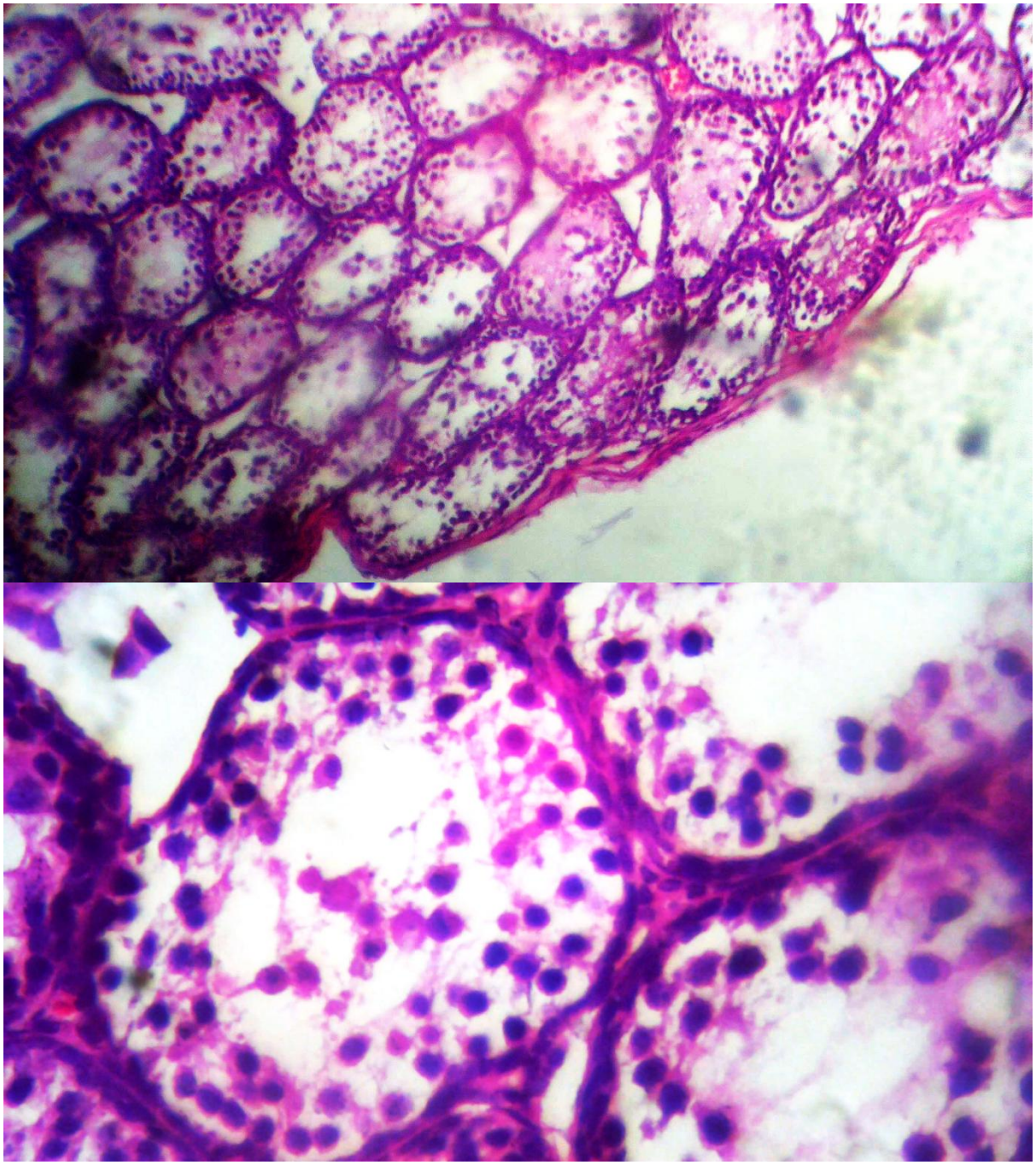


Plate 4.3 a (above) and b (below) of 500mg/kg: similar to control: Seminiferous tubules (ST) acquiring lumen (lu); spermatogonia (sg), Sertoli cells linning the ST, Leydig cells (LC) in the interstitial space, primary spermatocytes 1^{o}sc (H&E; 400x):

CHAPTER FIVE

5.0. DISCUSSION

The development of sexuality is an important bio-psycho-social development (Philibert *et al*., 2013), which takes an adult shape during this transition phase where juvenile testicles develop and attains maturity where by spermatogenesis is completed and matured sperm cell are produced, making male fertility a major concern in reproductive sciences, given the reason why numerous botanicals are currently being screened to determine their effect on the development of the male sex organ and its fertility.

In respect to statistical analysis, there was significant increase in the body weight between the control group and the groups that were administered with the soybean extract after 42 days of careful observation which is in alignment with the research done by Aryani *et al*., (2019) who worked on the effects of G. max on rats testicular steroid hormones, her research reported a significant increase in body weight of animals treated with *G. max* extract but in contrast with Odiase *et al*., (2023) who worked on the effect of *Glycine max* on testis and epididymis, his research shows that aqueous seed extract of *Glycine max* had no effect on the body weight of rats and Mona *et al* (2022) who worked on the dietary soybean sauce impact on testicular tissue of rats in dose and duration dependent, her research shows a decrease in body weight of rats treated with *G. max* extract. This study also shows that the aqeous seed extract of *G. max* also had significant statistical increase in testicular weights of animals which was in contrast with Odiase *et al*., (2023), whose research shows that there was also no significant difference in the testicular weight when treated with *G. max* extract and Aryani *et al*., (2019), who reported decrease in testicular weights of rats treated with *Glycine max.* These results are different because Odiase *et al* (2023) and Aryani *et al* (2019) administered their extract directly on adult male rats while administration was done prenatally. Spermatogenic cell count has an impact on testicular weight (Kianifard *et al*., 2013), possibly due to a increase in germ cells.

An oxidative analysis stress was also carried out and the results shows no significant statistical difference in the super oxide dismutase, catalase as well as the malondialdehyde tests which signifies a positive result, showing the insignificant effect of soybean extract in the developmental juvenile testes, although a slight decrease was observed in the statistical results of the glutathione peroxidase levels on the testis in the group administarted with 250 and 500mg/kg, it however didn't reflect in the histology as the normal histology

was retained, this is in contrast with Mona *et al* (2022), whose research shows an increase in Glutathione levels on treatment with both low and high dose of *G. max.*

Histologically, group A (control group) shows a normal structure of a juvenile developing testis where the tunica albuginia, the seminiferous tubules which contain the sustenticular (Sertoli) cells which serve as the supportive cells on the outer border of the seminiferous tubules and the spermatogenic cells which which proliferate into the primary spermatocytes and then into matured sperm cells and also the connective tissues which contain blood vessels and the Leydig cells are visible in their various stages, this result is in alignment with that of Odiase *et al* (2023), whose result in his control group shows normal testicular cells and Mona et al (2022) whose control group also shows normal histological features. Group B and C who's dams were administered 250mg/kg and 500mg/kg of aqueous *Glycine max* extract respectively also shows normal and progressive development of the juvenile testis without any form of alterations from pathology as those from the control group (group A) also in their various stages, this result is in contrast to those of Odiase *et al* (2023) whose result shows abnormalities in spermatogenesis and spermatogenesis arrest to the animal he admitted with 400mg/kg of *Glycine max* extract and Mona *et al* (2022) whose results also shows spermatogenesis arrest on treatment with low dose *G. max* and had hight restoration of normal histological features on high dose of *G. max*. These results are different possibly because he administered the extract on adult male rats while mine was done prenatally.

5.1. CONCLUSION

This research study shows that the aqueous extract of *Glycine max* (soybean) has no detrimental effect on the development of juvenile testis of post natal male Wistar rats. It is therefore safe to take soybean (*Glycine max*) during pregnancy.

5.2. RECOMMENDATION

It is therefore recommended that further research be carried out on other mechanism of action and effects of the *Glycine max.*

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