HISTOLOGICAL ASSESSMENT OF THE AQUEOUS EXTRACT OF SOYBEAN (*Glycine max*) ON THE FETAL LIVER OF WISTAR RATS

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A PROJECT WORK SUBMITTED TO THE DEPARTMENT OF ANATOMY, UNIVERSITY OF BENIN, BENIN CITY, IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF BACHELOR OF SCIENCE (B.Sc.) DEGREE IN ANATOMY.

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CERTIFICATION

This is to certify that this project work titled "**HISTOLOGICAL ASSESSMENT OF THE AQUEOUS EXTRACT OF SOYBEAN (*Glycine max*) ON THE FETAL LIVER OF WISTAR RATS**" was carried out by AMOS QUEENDALYN UBOGU (BMS1902014) in the Department of Anatomy, School of Basic Medical Sciences, College of Medical Sciences, University of Benin.

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EXTERNAL EXAMINER **DATE**

DEDICATION

This work is dedicated to God Almighty, who has given me grace and strength to be alive and provided all of my needs. Also, to my parents; late Mr Amos Ubogu and Mrs Roseline Ubogu for your ending labour for me. May God richly bless you.

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My sincere and profound gratitude goes to God almighty for Divine Providence and the Holy Spirit for divine guidance and direction during the period of this research.

My heartfelt gratitude goes to my Parents; my dad, late.Mr Amos Ubogu and my mom, Mrs. Roseline Amos Ubogu. I also appreciate my siblings; Dr. Anthony, Engr. Anderson, Mrs Emily, Miss Rachael Ubogu, Mrs Anthonia, Mrs Vivian Chinyere and Engr Godwin Akpan Uko for their continuous financial support, prayers and encouragement. I sincerely appreciate my supervisor; Mrs Ejeguo Akporobo for her immense guidance, supervision, patience, and professional advice throughout the time of this study. I also wish to acknowledge and appreciate the immense support provided by Mr Sam during the Laboratory bench work for this research, and also my senior colleague, Mr John for his immense support.

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ABSTRACT

Soybean is an annual herbaceous legume plant that has diverse applications, serving as animal feed, fodder, and human food and beverage, including flour, starch, oil, spices, and culinary herbs. Soybean have been reported to have a variety of pharmacological properties including hepatoprotective activity. It's effect on the fetus is rather relatively unknown. Thus, this study aimed to investigate the effect of soybean (*Glycine max*) on the histology of fetal liver in Wistar rats. Eighteen (18) adult juvenile Wistar rats with an average weight of 60 g were randomly assigned into three (3) groups (A-C). Group A served as control; Group B was administered 250 mg/Kg body weight of *Glycine max*; Group C was administered 250 mg/Kg body weight of *Glycine max.*The administration lasted for 21 days and was done orally using an orogastric tube. After the duration of administration, the animals were weighed, anaesthetized and sacrificed, with the liver processed for routine hematoxylin and eosin staining for histological evaluation. From this study, results showed a significant decrease ($p<0.05$) in body weight change in the groups administered *Glycine max* when compared to control. There was also a significant decrease ($p<0.05$) in fetal weight and Crown-rump length in the group given 500mg/Kg body weight of *Glycine max* when compared to control. Histological findings showed relatively normal liver architecture in the groups given *Glycine max* when compared to control as evidenced by developing hepatocytes radiating from central vein, and sinusoids. In conclusion, findings from this study suggest that Soybean resulted in significant alterations in maternal and fetal health outcomes (especially at 500 mg/Kg body weight dosage). However, the observed effects may be primarily physiological as there were histological alterations.

CHAPTER ONE

INTRODUCTION

1.1 Background to The Study

Pregnancy is a delicate and transformative period, marked by physiological changes to support the growth and development of the fetus (Murray and Hendley, 2020). This period is characterized by increased nutrient requirements, hormonal fluctuations, and adaptations in various organs to accommodate the growing fetus (Obeagu *et al*., 2023). The placenta acts as a barrier to protect the fetus from harmful substances in the maternal circulation (Murray and Hendley, 2020), but it is not completely impermeable. Substances consumed during pregnancy, including food, drugs, and environmental toxins, can cross the placenta and directly impact the developing fetus (Gómez-Roig *et al*., 2021). During pregnancy, the maternal body undergoes significant changes to meet the nutritional needs of the developing fetus. Maternal diet plays a crucial role in providing essential nutrients for fetal growth and development. However, maternal exposure to harmful substances, such as alcohol, tobacco, and certain medications, can have detrimental effects on fetal health (Obeagu *et al*., 2023). The delicate balance of maternal-fetal health highlights the importance of understanding how substances taken during pregnancy can influence fetal development.

Substances taken during pregnancy can have profound effects on fetal development. Alcohol consumption (Gómez-Roig *et al*., 2021), for example, can lead to fetal alcohol spectrum disorders, characterized by physical, behavioral, and cognitive impairments. Similarly, smoking during pregnancy is associated with increased risks of preterm birth, low birth weight, and developmental delays. Maternal diet, including the consumption of certain foods and dietary supplements, can also impact fetal development (Murray and Hendley, 2020). Soybean is a plant widely consumed for its nutritional benefits (Shea *et al*., 2020). It is rich in proteins, vitamins, minerals, and phytochemicals, including isoflavones (Kim *et al*., 2021). Isoflavones are known for their estrogenic properties (Gómez-Zorita *et al*., 2020) and have been studied for their potential effects on pregnancy outcomes. Some studies suggest that soybean consumption during pregnancy may influence fetal development and pregnancy outcomes (Wang *et al*., 2021; Rizzo *et al*., 2022). However, the existing evidence is inconclusive, and more research is needed to understand the effects of soybeans on pregnancy outcomes.

The liver plays a crucial role in pregnancy by metabolizing nutrients, hormones, and toxins (Yang *et al*., 2023). It is also involved in the production of proteins essential for fetal growth and development (Hou *et al*., 2020; Yang *et al*., 2023). Impairment of liver function during pregnancy can lead to serious complications, including preeclampsia, intrauterine growth restriction, and preterm birth (Joo *et al*., 2021; Terrault and Williamson, 2022). The fetal liver is particularly vulnerable to the effects of maternal factors, as it is still developing and maturing during pregnancy. Understanding how substances like soybean affect the fetal liver can provide valuable insights into the mechanisms underlying these complications and inform strategies for their prevention and management.

1.2 Statement of Research Problem

Despite the delicate nature of pregnancy and the known influence of maternal diet on fetal development, there is a lack of comprehensive understanding regarding the potential impact of soybean consumption on pregnancy outcomes, particularly on fetal liver development. While soybeans are rich in nutrients and bioactive compounds, including isoflavones (Shea *et al*., 2020; Kim *et al*., 2021), which have been studied for their estrogenic properties, their effects on the developing fetus remain unclear. Given the critical role of the liver in pregnancy, where it is involved in metabolism, detoxification, and protein synthesis, any disruption to its development could have significant implications for fetal health and survival. Therefore, there is a need to investigate the histological effects of soybeans on fetal liver development to determine if maternal soybean consumption poses any risks or benefits to fetal health.

1.3 Aim of The Study

The study was aimed at assessing the effect of soybeans on the histology of Fetal liver in Wistar rats. The specific objective of this study was to assess

- Phytochemical composition of *Glycine max*
- Change in body weight and fetal weight of experimental animals
- Crown-rump length in Wistar Rats.
- Histology of the Fetal liver.

1.4 Justification of The Study

Pregnancy is a critical period characterized by unique physiological changes to support fetal development (Murray and Hendley, 2020). Maternal diet during pregnancy plays a crucial role in providing essential nutrients for optimal fetal growth and development. Soybean, a commonly consumed plant, is rich in nutrients and bioactive compounds, such as isoflavones, (Obeagu *et al*., 2023) which have been studied for their potential health benefits. However, the effects of soybean consumption on pregnancy outcomes, particularly on fetal liver development, remain poorly understood.

The liver is a vital organ involved in various metabolic and detoxification processes crucial for fetal survival (Yang *et al*., 2023). Disruption of fetal liver development can lead to serious complications, including metabolic disorders and impaired growth. Therefore, the need to understand how soybean consumption during pregnancy affects fetal liver development is essential for identifying potential risks or benefits associated with maternal soybean intake.

CHAPTER TWO

LITERATURE REVIEW

2.1 Plant of Study: Soybean

2.1.1 Description of Soybean

Soybean is an annual herbaceous legume plant belonging to the pea family Leguminosae and the subfamily Papilionoideae (Grassini *et al*., 2021). It thrives in tropical, subtropical, and temperate environments. The genus name *Glycine* was assigned by Linnaeus in his initial version of Genera Plantarum (Lin *et al*., 2023). The botanical name *Glycine max* was established by Merrill in 1917 and has since been recognized (Grassini *et al*., 2021). Soybean was first introduced to Nigeria in 1908, but initial attempts to cultivate it at Moor Plantation, Ibadan, were unsuccessful. However, it gained successful cultivation in 1928 in the Savanna ecology and later spread to other parts of northern Nigeria, particularly becoming a cash crop in the Tiv division of Benue Province (now Benue State). While most of the crop was exported to Europe as a cash crop, a small amount was used as food in the northern states (Olawale *et al*., 2021; Olatunde *et al*., 2021).

Soybean is a yearly, upright, and hairy herbaceous plant, with a height range of 30 to 183 cm depending on the genotype (Lin *et al*., 2023). It displays two growth habits: determinate and indeterminate. The determinate type is shorter, with fewer leaves but more pods, while the indeterminate types are taller, with more leaves and fewer pods along the stem (Akmalovna *et al*., 2022). The plant's flowers are small, inconspicuous, and self-fertile, appearing in the leaf axils. The stems, leaves, and pods are covered with fine brown or gray hairs. The leaves are trifoliate, oval in shape, and 5-10 cm long, with three to four leaflets per leaf (Lin *et al*., 2023). The fruit is a hairy pod, typically 5-8 cm long, growing in clusters and containing two to four seeds each. Soybean's growth habits vary, with plant height ranging from less than 0.2 to 2.0 meters (Olatunde *et al*., 2021). The pods, stems, and leaves are covered with fine brown or gray hairs. The primary leaves are unifoliate, opposite, and ovate in shape, while secondary leaves are trifoliate, alternate, and compound, with four or more leaflets per leaf (Müller *et al*., 2021; Lin *et al*., 2023). Leaflets measure 6–15 cm in length and 2–7 cm in width. Leaves usually fall before seeds mature (Zilli *et al*., 2021). Soybean's root system consists of a taproot from which a lateral root system emerges. Its flowers are inconspicuous and self-fertile, appearing in leaf axils, and can be white, pink, or purple (Lin *et al*., 2023).

Fig. 2.1: *Image showing Glycine max plant (Zilli et al., 2021)*

The fruit is a hairy pod growing in clusters of three to five, straight or slightly curved, 3-8 cm long, and typically containing two to four seeds measuring 5-11 mm in diameter (Grassini *et al*., 2021). Soybean seeds vary in size and shape, usually oval but ranging from almost spherical to elongated and flattened. Seed coat colors include black, brown, blue, yellow, green, and mottled. Mature bean hulls are hard, water-resistant, and protect the cotyledon (Zilli *et al*., 2021). A visible scar on the seed coat called the hilum, has a micropyle at one end allowing water absorption for sprouting (Akmalovna *et al*., 2022).

2.1.2 Scientific Classification of Soybean

Domain	Eukaryota
Kingdom	Plantae
Phylum	Spermatophyta
Subphylum	Angiospermae
Class	Dicotyledonae
Order	Fabales
Family	Fabaceae
Subfamily	Papilionoidae
Genus	*Glycine*
Species	*Glycine max* (Akmalovna *et al*., 2022)

2.1.3 Uses of Soybean

Soybean has diverse applications, serving as animal feed, fodder, and human food and beverage, including flour, starch, oil, spices, and culinary herbs (Feng *et al*., 2020; Hu *et al*., 2020). Additionally, it has traditional medicinal and pharmaceutical uses. The fermented seeds are used to alleviate colds, fevers, and headaches, and they exhibit estrogen-like activity in the body (Feng *et al*., 2020; Raza *et al*., 2021; Bittencourt *et al*., 2021). Soybean diets are also valued for their ability to treat acidosis (Feng *et al*., 2020). Furthermore, soybean plays an environmental role in soil improvement by fixing nitrogen from the atmosphere (Raza *et al*., 2021). As a legume, soybean is capable of self-nitrogen fixation through a symbiotic relationship with nitrogen-fixing bacteria, specifically *Bradyrhizobium japonicum*, found in its root nodules (Hu *et al*., 2020; Bittencourt *et al*., 2021). Soy oil extracted from soybean is widely utilized in various industries for the production of pharmaceuticals, plastics, papers, inks, paints, varnishes, pesticides, cosmetics, and as a renewable energy source (Hu *et al*., 2020; Raza *et al*., 2021).

2.1.4 Phytochemical Constituents Soybean

Soybeans are rich in phytochemical constituents that contribute to their health-promoting properties. One of the key phytochemicals found in soybeans is isoflavones, including genistein and daidzein, known for their antioxidant and estrogenic effects (Amani-Machiani *et al*., 2021). These compounds are believed to play a role in the potential health benefits associated with soy consumption, such as reducing the risk of certain cancers and heart disease (Modgil *et al*., 2021). Additionally, soybeans contain phytosterols, including beta-sitosterol, which has been shown to help lower cholesterol levels and reduce the risk of heart disease (Yang *et al*., 2021; Modgil *et al*., 2021). Saponins, another class of phytochemicals found in soybeans, have been studied for their potential anticancer and cholesterol-lowering effects (Shahrajabian *et al*., 2021). Phytic acid, present in soybeans, can bind to minerals in the digestive tract and reduce their absorption (Modgil *et al*., 2021). However, some studies suggest that phytic acid may also have antioxidant properties and other health benefits (Amani-Machiani *et al*., 2021). Soybeans also contain protease inhibitors, which can inhibit the activity of enzymes involved in protein digestion and may have anticancer properties (Amani-Machiani *et al*., 2021). Lectins, found in soybeans, are proteins that can bind to carbohydrates and have been studied for their potential anticancer and immunomodulatory effects (Modgil *et al*., 2021). Soybeans also contain flavonoids, such as quercetin and kaempferol, which have antioxidant and anti-inflammatory properties (Yang *et al*., 2021). Phenolic acids, including ferulic acid and caffeic acid, are also found in soybeans and contribute to their antioxidant properties (Yang *et al*., 2021).

2.1.5 Pharmacological Activities of Soybean

2.1.5.1 Anti-cancer Activity

Isoflavones derived from soy protein have been shown to reduce tumor development in a dose-dependent manner. This finding aligns with several in vitro studies indicating that daidzein and genistein, two forms of soybean isoflavones, can inhibit cell growth. Genistein, in particular, has been identified as a potent and selective inhibitor of tyrosine kinases, which regulate cell proliferation. Furthermore, genistein works in conjunction with transforming growth factor-β, a crucial growth factor, to halt the cell cycle and subsequently impede cell growth. Soybean isoflavones also inhibit 5α-reductase, an enzyme that converts testosterone to dihydrotestosterone, thereby potentially preventing prostate cancer associated with elevated dihydrotestosterone levels (Hao *et al*., 2020; Rusdi *et al*., 2021).

Various other phytochemicals present in soybeans, including phytosterols, soya saponins, phenolic acids, and phytates, exhibit anticancer properties. Phytosterols not only inhibit carcinogen synthesis but also suppress cancer cell proliferation, angiogenesis, invasion, and metastasis, while potentially promoting the apoptosis of malignant cells by lowering blood cholesterol levels (Rusdi *et al*., 2021). Soyasaponins have been extensively studied for their anticancer effects on human colon cancer, liver cancer, and breast cancer. Proposed mechanisms include direct cytotoxicity, induction of apoptosis, anti-estrogenic effects, inhibition of tumor cell metastasis, anti-mutagenic effects, bile acid binding action, and normalization of carcinogen-induced cell proliferation. Phytic acid has also garnered attention for its role in cancer prevention, controlling experimental tumor growth, progression, and metastasis. Studies have demonstrated that phytates exhibit anti-neoplastic properties in the breast, colon, liver, leukemia, prostate, sarcomas, and skin cancer (Pabich *et al*., 2021; Rusdi *et al*., 2021; Jan *et al*., 2022).

2.1.5.2 Anti-diabetic Activity

Studies conducted on diabetic patients consuming soybean diets have revealed several potential benefits. Soy-based diets have been found to have a positive impact on hypertension, hypercholesterolemia, atherosclerosis, and obesity, which are common conditions in diabetic patients (Dukariya *et al*., 2020; Das *et al*., 2022). Therefore, incorporating soy into the diet could be a beneficial choice for diabetic individuals. Furthermore, replacing animal protein with soybean protein may help reduce renal hyperfiltration, proteinuria, and renal acid load, thus lowering the risk of renal disease in type 2 diabetes patients (Janani *et al*., 2021; Das *et al*., 2022). It is widely recognized that a high-fiber diet, especially one rich in soluble fiber, can help control plasma glucose levels in diabetics. Both short- and long-term studies have shown improvements in blood glucose levels with the consumption of soybeans, which are high in fiber (Nakai *et al*., 2020). Additionally, an experiment using an aqueous extract of Glycine max seeds demonstrated antihyperglycemic activity (Dukariya *et al*., 2020).

2.1.5.3 Cardio Protective Activity

In a study, Basharat *et al*. (2020) assessed the impact of crude extracts from Glycine max seeds on the serum lipid profiles of ad-libitum high-cholesterol-fed male albino Wistar rats. The observed protective effect is attributed to soybean's lipid-lowering and antioxidant properties, which stem from the presence of phytochemicals such as isoflavones, phytic acid, soyasaponins, and phytosterols. Consumption of soybean protein has been linked to a reduced risk of cardiovascular disease, with animal studies indicating that replacing dietary animal protein with soybean protein leads to reductions in total and low-density lipoprotein (LDL) cholesterol levels in the blood.

The role of soybean in preventing cardiovascular disease, particularly its LDL cholesterol-lowering effects, has been extensively studied in controlled clinical trials. According to

Swallah *et al*. (2023), there is a hypothesis suggesting that isoflavones may inhibit the development of atherosclerosis by acting as antioxidants against LDL oxidation, which is a key step in the formation of atherosclerotic plaques. Isoflavones consumed in soy diets have been associated with various cardioprotective effects, including the reduction of LDL cholesterol, inhibition of pro-inflammatory cytokines and cell adhesion proteins, inhibition of platelet aggregation, and improvement in vascular reactivity. The hypocholesterolemic effect of soya saponins, which contribute to their cardiovascular protective properties, has been well-established. Studies have shown that soya saponins reduce cholesterol levels, further supporting their role in cardiovascular health (Swallah *et al*., 2023).

2.1.5.4 Anti-Osteoporosis Activity

Studies have been conducted to assess the potential effects of soybean products on bone density and the risk of osteoporosis. Research by Zhang *et al*. (2024), including Sun *et al*. (2021) and Cai *et al*. (2021), suggests that isoflavones and soybean protein, when consumed as part of a soybean-based diet, may moderately increase bone mass. Genistein, a type of soy isoflavone, has been found to directly inhibit bone resorption, as reported by Zhang *et al*. (2024), and Cai *et al*. (2021). Additionally, daidzein, another soy isoflavone, has been shown to increase bone mass in postmenopausal women, a population prone to osteoporosis (Messina, 1999). Studies using adult rats that had undergone ovariectomy, a procedure that mimics postmenopausal bone loss, also supported the bone-preserving effects of soy isoflavones. These studies found that ovariectomized rats fed a diet rich in soybean protein had greater bone density compared to those fed a diet based on casein.

Randomized controlled studies utilizing isoflavone extracts or pure genistein, as reported by Cai *et al*. (2021), have further supported the beneficial effects of soy isoflavones on maintaining bone mineral density. These studies concluded that soy isoflavones have a mild but significant and independent effect in preserving bone mineral density.

Shabbir *et al*. (2022) conducted a study to investigate the potential effects of soybean products on bone density and the risk of osteoporosis. The study findings revealed that isoflavones and soybean protein, when consumed as part of a soybean-based diet, moderately increased bone mass. Genistein, a type of soy isoflavone, was found to directly inhibit bone resorption. Additionally, daidzein, another soy isoflavone, was shown to increase bone mass in postmenopausal women, a population at high risk for osteoporosis. Studies using adult rats that had undergone ovariectomy, a procedure that mimics postmenopausal bone loss, also supported the bone-preserving effects of soy isoflavones. These studies found that ovariectomized rats fed a diet rich in soybean protein had greater bone density compared to those fed a diet based on casein. Randomized controlled studies utilizing isoflavone extracts or pure genistein further supported the beneficial effects of soy isoflavones on maintaining bone mineral density. These findings suggest that soybean products may have a positive impact on bone health and could potentially help reduce the risk of osteoporosis.

2.1.5.5 Anti-Obesity Activity

Obesity, characterized by an imbalance in energy regulation, is closely linked to hyperinsulinemia, insulin resistance, and lipid metabolism abnormalities. It stands as one of the primary risk factors for developing type II diabetes, cardiovascular disease, atherosclerosis, and certain cancers (Jung *et al*., 2021). The satiating effect of high-protein meals on appetite and food intake in humans is well-documented (Khatun *et al*., 2023). Numerous studies in both animals and humans have demonstrated that soy protein consumption can lead to reductions in body weight and fat mass, as well as lower plasma cholesterol and triglyceride levels. In obese individuals, dietary inclusion of soy protein has been shown to decrease body weight and fat mass while also improving lipid profiles (Khatun *et al.*, 2023). Research suggests that soy proteins may positively impact lipid absorption, insulin sensitivity, fatty acid metabolism, and other hormonal, cellular, or molecular processes associated with obesity (Greaves *et al.*, 2000). These findings highlight the potential of soy protein as a beneficial dietary component in managing obesity and its associated health risks.

2.1.5.6 Anti-Menopausal Activity

Menopause marks a natural stage in every woman's life as she ages. However, thermoregulatory disruptions such as hot flashes (HF), night sweats, mood swings, and reduced energy levels can make this period challenging (Lee *et al.*, 2023). Hot flashes manifest as sudden sensations of heat in the face, neck, and chest (Park *et al.*, 2020). The inclusion of dietary soy has garnered significant interest due to reports of reduced menopausal discomfort and lower morbidity rates from hormone-dependent diseases in soy-consuming Asian populations compared to non-soy-consuming Western populations. Epidemiological studies in Japanese women have indicated that soy product consumption may offer a protective effect against menopausal symptoms (Lee *et al.*, 2024).

Research has shown that daily supplementation of isolated soybean protein-containing natural isoflavones in the diet of postmenopausal women can reduce the frequency of hot flashes (Park *et al.*, 2020; Lee *et al.*, 2023; Lee *et al.*, 2024). It is hypothesized that in postmenopausal women, isoflavones bind to free estrogen receptors, providing a weak estrogenic effect. This potential benefit suggests a dietary alternative to postmenopausal hormone replacement therapy (Natarelli *et al.*, 2023). Numerous human studies, comprising peri-menopausal and postmenopausal women experiencing menopausal symptoms, have investigated the hypothesis that soy products alleviate these symptoms. Results from these studies, where women consumed soy proteins for at least 4 weeks, have shown beneficial effects on menopausal symptoms (Natarelli *et al*., 2023; Lee *et al*., 2024).

2.1.5.7 Oestrogenic Activity

Theoretically, soy isoflavones can be useful as a source of oestrogen from outside the body (Jargin, 2020). Oestrogen-like effects have been proposed as one of the major mechanisms of action of isoflavones related to their pharmacological effects (Anisah *et al*., 2021). Based on the oestrogenic effect of isoflavones, they can be used for the prevention and treatment of hormone-dependent incidences of osteoporosis, breast cancer and menopausal symptoms in women (Jargin, 2020). Prevailing hypothesis is that isoflavones may act like anti-oestrogen when they are present in concentration of high oestrogen, and like oestrogen when they are in a low-oestrogen environment (Megha *et al*., 2023).

2.1.5.8 Hepatoprotective Activity

Research carried out in animals suggests that soy protein ingestion exerts its lipid-lowering effect by lowering the absorption of cholesterol in the intestine and increasing faecal bile acid excretion, thereby reducing hepatic cholesterol content and enhancing the removal of low-density lipoproteins LDL (Ren *et al*., 2021). Hepatic cholesterol metabolism has been observed to be directly affected by soy protein diet (Vishnupriya and Kowsalya, 2022).

Also, an *in vivo* study indicated that soya saponins inhibit the elevation of liver transaminases when administered orally to rats with peroxidized corn oil. Damage to the liver caused by peroxidized salad oil is inhibited by the addition of soya saponin during peroxidation. Structure-activity relationship suggests that the sugar moiety linked at the C-3 (third carbon) may play an important role in the hepatoprotective actions of soyasaponins present in soybeans (Vishnupriya and Kowsalya, 2022).

2.1.5.9 Neuroprotective Activity

In 2018, a study was conducted to find out the effect of soy isoflavones on scopolamine-induced memory impairment in mice. The results revealed that isoflavones found in soy exhibit neuroprotective effects which may be partially due to the enhancement of cholinergic system function, suppression of oxidative stress, and activation of the ERK/CREB/BDNF signal pathway. Soy isoflavones have been reported to improve cognitive function in adults, without severe adverse effects and also have the potential to reduce the risk of dementia and decline in cognition (Irnidayanti *et al*., 2021).

In another study, soybean oligopeptides (SOPs) were isolated from soy protein to evaluate its neuroprotective effect. The findings suggest that SOPs can protect PC12 cells (pheochromocytoma differentiated from neural crest cells) against Hydrogen peroxide-induced lipid peroxidation and cell death. These neuronally differentiated cells directly model sympathetic neurons which are usually affected by neurodegenerative diseases and also share important properties with CNS dopaminergic neurons (Khosravi and Razavi, 2021).

The findings of the comparative study on the neuroprotective effects of soybean and *tempeh* extracts suggested soybean as a beneficial food for anti-inflammation within the brain. In another study to evaluate the effect of soybean on amnesic mice induced with alprazolam, it was observed that soybean administration significantly reversed amnesia in a dose-dependent manner. This positive effect was attributed to the oestrogenic effect of soy isoflavones. Soy isoflavones tend to imitate the activity of oestrogen in the brain, thereby improving cognitive functions. Soy isoflavones have also been reported to increase cholinergic transmission by indirectly facilitating acetylcholine in the brain through activation of choline acetyltransferase (Qiao *et al*., 2022)

2.2 Organ of Study: The Liver

The liver is an accessory organ of the digestive system present in vertebrates and some other animals. It has a wide range of functions, including detoxification, protein synthesis, and the production of biochemicals necessary for digestion. The liver is necessary for survival; there is currently no way to compensate for the absence of liver function in the long term, although new liver dialysis techniques can be used in the short term (Singh, 2011). This organ plays a major role in metabolism and has several functions in the body, including glycogen storage, decomposition of red blood cells, plasma protein synthesis, hormone production, and detoxification. The liver's highly specialized tissues regulate a wide variety of high-volume biochemical reactions, including the synthesis and breakdown of small and complex molecules, many of which are necessary for normal vital functions (Maton *et al*., 2003).

2.2.1 Gross Anatomy of the Liver

The liver is a reddish-brown organ with four lobes of unequal size and shape. A human liver normally weighs 1.44–1.66 kg (3.2–3.7 lb), (Cotran *et al*., 2005) and is a soft, pinkish-brown, triangular organ. It is both the largest internal organ (the skin being the largest organ overall) and the largest gland in the human body. It is located in the right upper quadrant of the abdominal cavity, resting just below the diaphragm. The liver lies to the right of the stomach and overlies the gallbladder. It is connected to two large blood vessels, one called the hepatic artery and the other called the portal vein (Shneider and Sherman, 2008). The hepatic artery carries blood from the aorta, whereas the portal vein carries blood containing digested nutrients from the entire gastrointestinal tract and also from the spleen and pancreas. These blood vessels subdivide into capillaries, which then lead to a lobule. Each lobule is made up of millions of hepatic cells which are the basic metabolic cells. Lobules are the functional units of the liver. Two major types of cells populate the liver lobes; parenchymal and non-parenchymal cells (Cotran *et al*., 2005). 80 percent of the liver volume is occupied by parenchymal cells commonly referred to as hepatocytes. Non-parenchymal cells constitute 40 percent of the total number of liver cells but only 6.5 percent of its volume. Sinusoidal hepatic endothelial cells, Kupffer cells, and hepatic stellate cells are some of the non-parenchymal cells that line the liver sinusoid (Kmiecz, 2001).

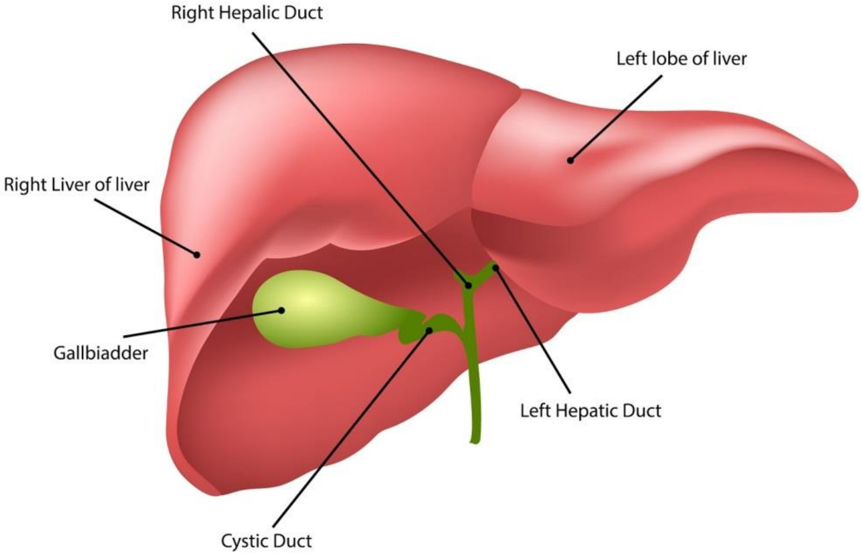


Fig 2.3 Liver (Nelson and Elevitch, 2006)

2.2.2 Blood Supply of the Liver

The liver gets a dual blood supply from the hepatic portal vein and hepatic arteries. Supplying approximately 75 percent of the liver's blood supply, the hepatic portal vein carries venous blood drained from the spleen, gastrointestinal tract, and its associated organs (Kmiecz, 2001). The hepatic arteries supply arterial blood to the liver, accounting for the remainder of its blood flow. Oxygen is provided from both sources; approximately half of the liver's oxygen demand is met by the hepatic portal vein, and half is met by the hepatic arteries (Shneider and Sherman, 2008). Blood flows through the liver sinusoids and empties into the central vein of each lobule. The central veins coalesce into hepatic veins, which leave the liver.

2.2.3 Nerve Supply

The nerves of the liver are derived from the hepatic plexus, the largest derivative of the celiac plexus. The hepatic plexus accompanies the branches of the hepatic artery and portal vein to the liver (Kmiecz, 2001). It consists of sympathetic fibers from the celiac plexus and parasympathetic fibers from the anterior and posterior vagal trunks. Nerve fibers accompany the vessels and biliary ducts of the portal triad. Other than vasoconstriction, their function is unclear (Moore and Dalley, 2006).

2.2.4 Lymphatic Drainage

The liver is a major lymph-producing organ. Between one-quarter and one-half of the lymph received by the thoracic duct comes from the liver. The lymphatic vessels of the liver occur as superficial lymphatics in the subperitoneal fibrous capsule of the liver (Glisson capsule), which forms its outer surface, and as deep lymphatics in the connective tissue, which accompany the ramifications of the portal triad and hepatic veins (Moore and Dalley, 2006). Most of the lymph is formed in the perisinusoidal spaces (of Disse) and drains to the deep lymphatics in the surrounding intralobular portal triads. Superficial lymphatics from the anterior aspects of the diaphragmatic and visceral surfaces and the deep lymphatic vessels accompanying the portal triads converge toward the porta hepatis. They drain to the hepatic lymph nodes scattered along the hepatic vessels and ducts in the lesser omentum (Leslie and James, 2007). Efferent lymphatic vessels from the hepatic nodes drain into celiac lymph nodes, which in turn drain into the chyle cistern, a dilated sac at the inferior end of the thoracic duct. Superficial lymphatics from the posterior aspects of the diaphragmatic and visceral surfaces of the liver drain toward the bare area of the liver (Moore and Dalley, 2006). Here they drain into phrenic lymph nodes or join deep lymphatics that have accompanied the hepatic veins converging on the IVC and pass with this large vein through the diaphragm to drain into the posterior mediastinal lymph nodes. Efferent vessels from these nodes join the right lymphatic and thoracic ducts (Moore and Dalley, 2006).

2.2.5 Embryology of the liver

The origin of the liver lies in both the ventral portion of the foregut endoderm (endoderm being one of the 3 embryonic germ cell layers) and the constituents of the adjacent septum transversum mesenchyme. In the human embryo, the hepatic diverticulum is the tube of endoderm that extends out from the foregut into the surrounding mesenchyme (Maton *et al*., 2003). The mesenchyme of the septum transversum induces this endoderm to proliferate, branch, and form the glandular epithelium of the liver. A portion of the hepatic diverticulum (that region closest to the digestive tube) continues to function as the drainage duct of the liver, and a branch from this duct produces the gallbladder (Gilbert, 2000). Besides signals from the septum transversum mesenchyme, fibroblast growth factor from the developing heart also contributes to hepatic competence, along with retinoic acid emanating from the lateral plate mesoderm.

The hepatic endodermal cells undergo a morphological transition from columnar to pseudostratified resulting in thickening into the early liver bud. Their expansion forms a population of bipotential hepatoblasts (Lade and Monga, 2011). Hepatic stellate cells are derived from mesenchyme (Berg *et al*., 2010). After the migration of hepatoblasts into the septum transversum mesenchyme, the hepatic architecture begins to be established, with liver sinusoids and bile canaliculi appearing. The liver bud separates into the lobes. The left umbilical vein becomes the ductus venosus and the right vitelline vein becomes the portal vein. The expanding liver bud is colonized by hematopoietic cells. The bipotential hepatoblasts begin differentiating into biliary epithelial cells and hepatocytes (Lade and Monga, 2011). The biliary epithelial cells differentiate from hepatoblasts around portal veins, first producing a monolayer, and then a bilayer of cuboidal cells. In the ductal plate, focal dilations emerge at points in the bilayer, become surrounded by portal mesenchyme, and undergo tubulogenesis into intrahepatic bile ducts (Gilbert, 2000).

Hepatoblasts not adjacent to portal veins instead differentiate into hepatocytes and arrange into cords lined by sinusoidal epithelial cells and bile canaliculi. Once hepatoblasts are specified into hepatocytes and undergo further expansion, they begin acquiring the functions of a mature hepatocyte, and eventually mature hepatocytes appear as highly polarized epithelial cells with abundant glycogen accumulation. In the adult liver, hepatocytes are not equivalent, with position along the portocentrovenular axis within a liver lobule dictating expression of metabolic genes involved in drug metabolism, carbohydrate metabolism, ammonia detoxification, and bile production and secretion (Lade and Monga, 2011).

2.2.6 Histology of the Liver

The liver consists of hexagonal lobules (classical liver lobules) made up of anastomosing cords of hepatocytes radiating away from the central vein, a radicle of the hepatic vein. At the periphery of the lobules in the corners of the hexagon are portal triads/tracts (Chaurasia *et al*., 2013). Each triad contains three structures viz. radicle of the bile duct (hepatic ductal), hepatic artery (hepatic arteriole), and portal vein (portal venule). The plates/cords of hepatocytes are separated by vascular spaces called sinusoids which connect the portal vein of the triad to the central vein of the lobule. The blood flow in the sinusoids is from the periphery of the lobule toward the central vein (Vishram, 2014).

The blood flowing in these wide vessels is prevented from coming in contact with the hepatocytes by the presence of an endothelial lining composed of sinusoidal lining cells. Often, the cells of this endothelial lining do not contact each other, leaving gaps of up to 0.5 μm between them (Singh, 2011). The sinusoidal lining cells also have fenestrae that are present in clusters, known as sieve plates. Thus, particulate matter less than 0.5 μm in diameter may leave the lumen of the sinusoid with relative ease (Leslie and James, 2007).

2.2.7 Functions of the Liver

The liver is a vital organ of the digestive system present in vertebrates and some other animals. It has a wide range of functions, including detoxification, protein synthesis, and the production of biochemicals necessary for digestion (Singh, 2011). The liver is necessary for survival; there is currently no way to compensate for the absence of liver function in the long term, although new liver dialysis techniques can be used in the short term.

This gland plays a major role in metabolism and has a number of functions in the body, including glycogen storage, decomposition of red blood cells, plasma protein synthesis, hormone production, and detoxification. It lies below the diaphragm in the abdominal-pelvic region of the abdomen. It produces bile, an alkaline compound that aids in digestion via the emulsification of lipids (Leslie and James, 2007). The liver's highly specialized tissues regulate a wide variety of high-volume biochemical reactions, including the synthesis and breakdown of small and complex molecules, many of which are necessary for normal vital functions (Maton *et al.,* 1993).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Collection and Identification of Plant

Soybean (*Glycine max*) was purchased at New Benin market, Oredo Local government area, Benin City, Edo State, Nigeria. The Soybean was taken to the Department of Plant Biology and Biotechnology, University of Benin, for identification and authentication. It was identified and assigned herbarium number, **UBH- G470,** in the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Benin City.

3.2 Extraction of Plant Material

The extraction of the *Glycine max* was carried out according to the method described by Aiyegoro and Oko 2010. The *Glycine max* was first dried in an oven at a temperature of 100 ^{o}C for about 4 hours. The dried *Glycine max* was then pulverized to powder form. The powdered *Glycine max* (1.5kg) was extracted thrice in distilled water (41.25 L) at room temperature on a shaker for 48 hours. The extract was filtered using a Buchner funnel and Whatman No.1 filter paper. The filtrate of aqueous extract obtained was quickly frozen at -40°C and dried for 48 h using a freeze dryer. The resulting extract was reconstituted with distilled water to give desired concentrations used in this study. This method of extraction used is known as maceration.

3.3 Phytochemical Screening

Qualitative screening of Cucumis sativus fruit was carried out in the Pharmacognosy Department, University of Benin. Flavonoids, tannins, phenols, saponins, steroids, alkaloids, and terpenoids were screened for their presence in the plant material.

3.3.1 Determination of Total Protein Content

The method used by Sudipta *et al.,* 2020 was adopted with little modification in the determination of Total Protein Content of the extract. Bovine Albumine Serum was used as standard reagent for preparing the Calibration curve against which the unknown concentration of proteins was estimated. 4.5 ml of reagent 1 (48 ml of 2% sodium carbonate in 0.1N sodium hydroxide + 1ml of 1% sodium potassium tartrate + 1ml of 0.5% copper sulphate) was added to the sample extracts and incubated for 15 min. After this, 0.5 ml of freshly prepared reagent 2 (1-part FolinCiocalteau: 1part water) was mixed with each sample and left for 30 min of dark incubation. After that the absorbance was measured at 660 nm and the amount of protein is expressed as Ug BSAE/ ml of fresh extract.

3.3.2 Determination of total phenolic contents

The amount of total phenolics in extract was determined with Folin–Ciocalteu reagent according to the method of Singleton and Rossi (1965) with slight modification using tannic acid as a standard. Briefly, 1.0ml of extract solution (250 Ug/ml) was added in a test tube. Then, 1.0 ml of Folin–Ciocalteu reagent was added and the content of the flask was mixed thoroughly. After 5 min, 15.0 ml Na2CO3 (20 %) was added and allowed to stand for 2 hours. The absorbance was measured at 760 nm using a UV-Vis spectrophotometer (Jenway 6100, Dunmow, Essex, U.K). The total phenolic content was determined as Ug of tannic acid equivalent (TAE) using an equation obtained from the standard tannic acid calibration graph.

3.3.3 Determination of Alkaloid Content

The total alkaloid content was measured using the method described by Harborne (1973). 5g of the extract was weighed into a 250 mL beaker and 100 mL of 20% acetic acid in ethanol was added and covered to stand for 2 hours. This was filtered and the extract was concentrated using a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added dropwise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate was collected by filtration, washed with 1% ammonia solution, dried and weighed. All samples were analyzed in triplicates.

Alkaloid (%) = Weight of residue / Weight of sample x 100

3.3.4 Flavonoid content determination

The flavonoid content was determined on triplicate aliquots of the homogenous cabbage extract (1.5 g) (Ilahy *et al.,* 2011). Thirty-microliter aliquots of the methanolic extract were used for flavonoid determination. Samples were diluted with 90 µl methanol, 6 µl of 10% Aluminum chloride (AlCl3), 6µl of 1mol/l Sodium acetate (CH3CO2Na) were added and finally 170 µL of methanol was added. The absorbance was read at 415 nm after 30 min. Quercetin was used as a standard for calculating the flavonoid content (Ug Qe/g).

3.3.5 Estimation of total saponins content

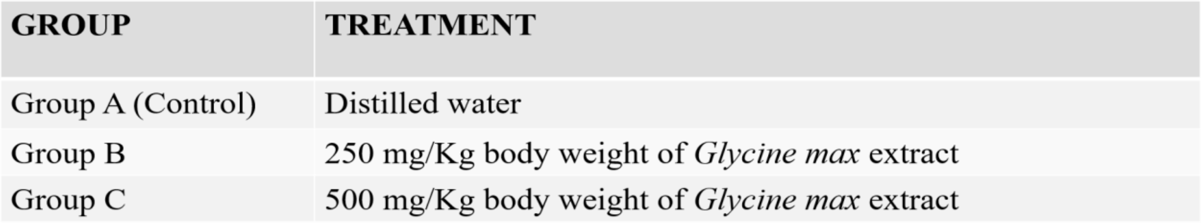
Estimation of total saponins content was determined by the method described by Makkar *et al*. based on vanillin-sulphuric acid colorimetric reaction with some modifications. About 50 µL of plant extract was added with 250 µL of distilled water. To this, about 250 µL of vanillin reagent (800mg of vanillin in 10mLof 99.5%(ethanol)was added. Then 2.5 mLof 72%sulphuric acid was added and it was mixed well. This solution was kept in a water bath at 60°C for 10min. After 10min, it was cooled in ice cold water and the absorbance was read at 570nm. 0- 25ppm standard saponin solutions were prepared from saponin stock solution. The standard solutions were treated similarly to the test samples. The values were expressed as PPM.

3.4 Management of Experiment Animals

Twenty-four (24) adult Wistar rats weighing between 165g and 200g were procured and bred in the Animal house, Department of Anatomy, University of Benin city, Benin City. The animals were kept in polypropylene cages at normal room temperature. The animals had access to standard rat chow (Top feed grower mash) and clean water *ad libitum*. The animals were weighed weekly before commencement and throughout the duration of the experiment using a digital weighing scale calibrated in grams and recorded to the nearest whole number. Protocols for this experiment were in accordance with the guide for the care and use of laboratory animals (National Research Council of the National Academics, 2011).

3.5 Experimental Design

Twenty-four (24) adult Wistar rats were randomly assigned into three (3) groups; Groups A-C comprising of eight (8) rats per group.



GROUP	TREATMENT
Group A (Control)	Distilled water
Group B	250 mg/Kg body weight of *Glycine max* extract
Group C	500 mg/Kg body weight of *Glycine max* extract

Administration lasted for 21 days and all administration was done via oral route using an orogastric tube.

3.6 Method of Sample Collection

At the end of the treatment duration, the rats were weighed and then sacrificed under chloroform anesthesia. Maternal weight, fetal weight and the crown-rump length of the pups was. The liver of the pups was harvested, fixed and then prepared for histological assessments. The tissues were processed for routine haematoxylin and eosin (H&E) staining.

3.7 Histological Procedure

3.7.1 Paraffin Tissue Processing

Following the fixation of the harvested tissues 10% formal saline, Tissues were processed as follows;

- Dehydration of tissues in increasing gradient of 70% to 90% alcohol and absolute alcohol using ethanol as the choice of alcohol.

- Clearance of alcohol was done using xylene as a clearing agent. Tissues were allowed to pass through two changes for total removal of alcohol.

- The tissues were infiltrated in three changes of molten paraffin wax in an oven at a temperature of 65-70C. The changes were done for 15 minutes each, and the last changes of paraffin wax for 30 minutes.

- Embedding was carried out using an embedding mould, into which the molten paraffin wax was poured and the infiltrated tissues were placed in it in a longitudinal orientation to produce longitudinal sections.

- The molten paraffin wax was allowed to cool resulting in solidification to form tissue blocks.

- After trimming, sectioning of the tissue blocks was done using the rotatory microtome to cut tissues blocks.

- After trimming, sectioning of the tissue blocks was done using the rotary microtome to cut tissues into ribbon like sections of thickness of 5 microns.

3.7.2 Hematoxylin and Eosin Staining Method

- Satisfactory and good tissue sections which came out as ribbons were selected and placed in 20% alcohol for spreading of the paraffin sections which are then cut and floated in a water bath at a temperature of 30C.

- The sectioned tissues were picked with slides and allowed to dry.

- The tissue sections were placed in xylene for fifteen (15) minutes to remove excess paraffin wax from the tissues and were then subjected to hydration by passing them through descending grades of alcohol (100%, 90%, 70%) and then into water, all of which lasted for 5 minutes each.

- Staining of the tissues was done using H&E dyes. The tissues were stained in hematoxylin for 10 minutes.
- Tissues were washed in running tap water (a process called blueing).
- Sections were counter-stained with 1% Eosin for 5-10 minutes.
- Tissues were rinsed in water.
- Tissues were dehydrated rapidly through 70% graded alcohol to absolute alcohol for 5 minutes.
- Tissues were finally cleared using xylene for 5 minutes and the slides mounted with glass cover slip using a suitable mount, Distrene plasticzer and xylene (DPX).

3.8 Photomicrography

The sections of the liver were obtained and examined under Leica DM750 research microscope with a digital camera (LeicaCC50) attached. Digital photomicrographs of the tissues sections were taken at x40 and x100 objective magnifications.

3.9 Statistical Analysis

Data were subjected to statistical analysis using the IBM SPSS statistic software (statistical package for social science) (version 21) and relevant statistical values were obtained. One-way analysis of variance (ANOVA) was carried out and presented as mean SEM. LSD post-hoc test was used. Value of $p< 0.05$ were converted into graphical representations in form of bar charts.

CHAPTER FOUR

RESULTS

4.1 Phytochemical Screening

Table 4.1 shows the qualitative phytochemical constituents in Soybean. Results showed the presence of reducing sugars, saponins, flavonoids, phenolics, eugenols, terpenoids, alkaloids, and Protein.

Table 4.1: Qualitative screening of phytochemical constituents of Soybean

S/N	**PHYTOCHEMICAL**	**OBSERVATION**
1	Reducing Sugars	+
2	Saponins	+
3	Flavonoids	+
4	Phenolics	+
5	Tannins	-
6	Eugenols	+
7	Terpenoids	+
8	Steroids	-
9	Alkaloids	+
10	Protein	+

- Absent + present

4.2 Effect of Treatment on Weights

Figure 4.1 shows the change in body weight across the experimental groups. There was a significant decrease ($p<0.05$) in the body weight change of rats treated with 250mg/Kg and 500mg/Kg of Soybean when compared to the control.

Figure 4.2 shows the Fetal weight across the experimental groups. There was a significant decrease ($p<0.05$) in the fetal weight of rats treated with 500mg/Kg of Soybean when compared to the control. However, there was no significant difference ($p>0.05$) in the fetal weight of rats treated with 250mg/Kg of Soybean when compared to the control.

Figure 4.3 shows the crown-rump length across the experimental groups. There was a significant decrease ($p<0.05$) in the crown rump length of rats treated with 500mg/Kg of Soybean when compared to the control. However, there was no significant difference ($p>0.05$) in the crown-rump length of rats treated with 250mg/Kg of Soybean when compared to the control.

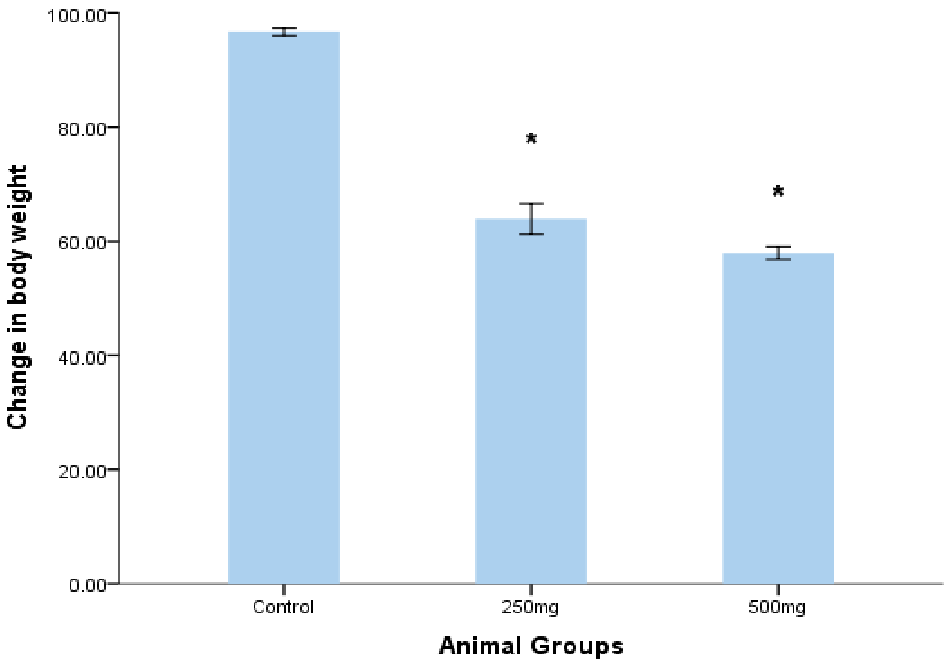


Fig. 4.1: Change in body weight of experimental animals across the groups. Values are given as mean ±SEM. * $p<0.05$ compared to the control.

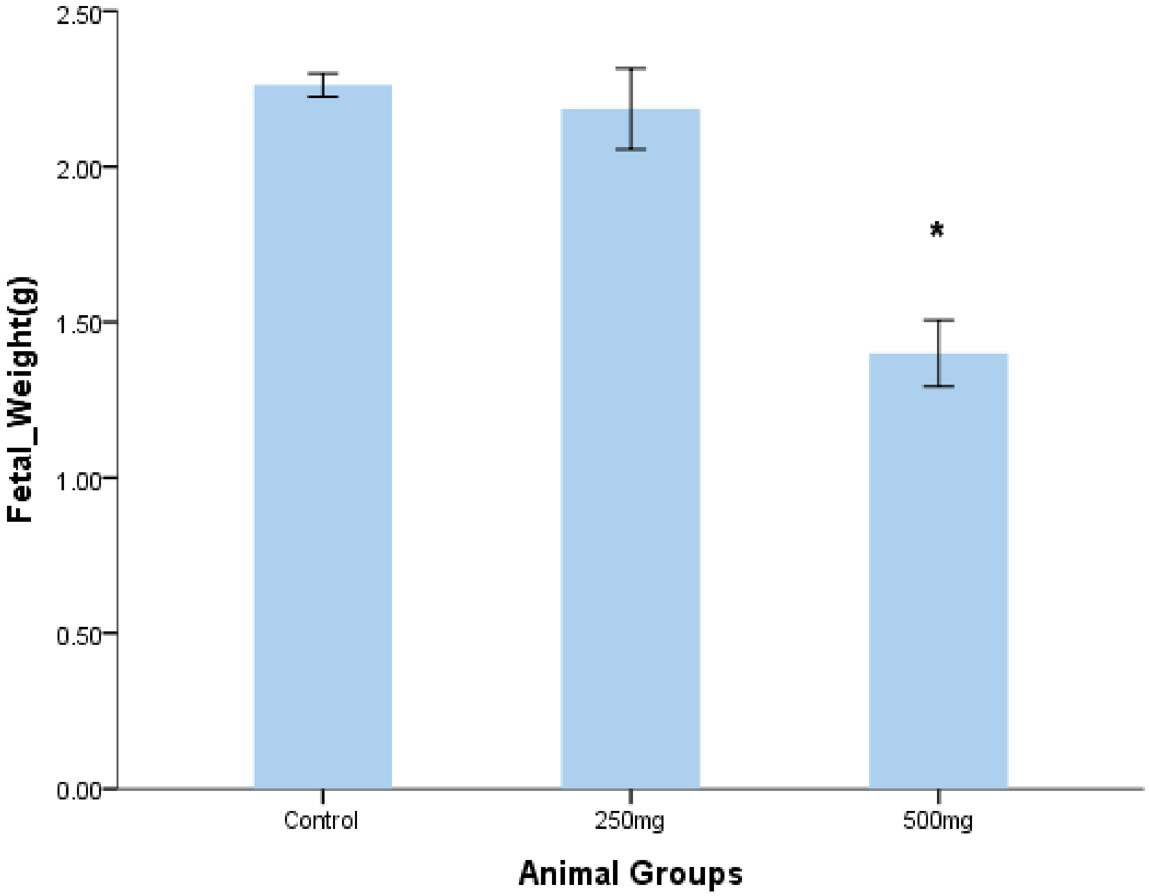


Fig. 4.2: Fetal weight of experimental animals across the groups. Values are given as mean ±SEM. * $p<0.05$ compared to the control.

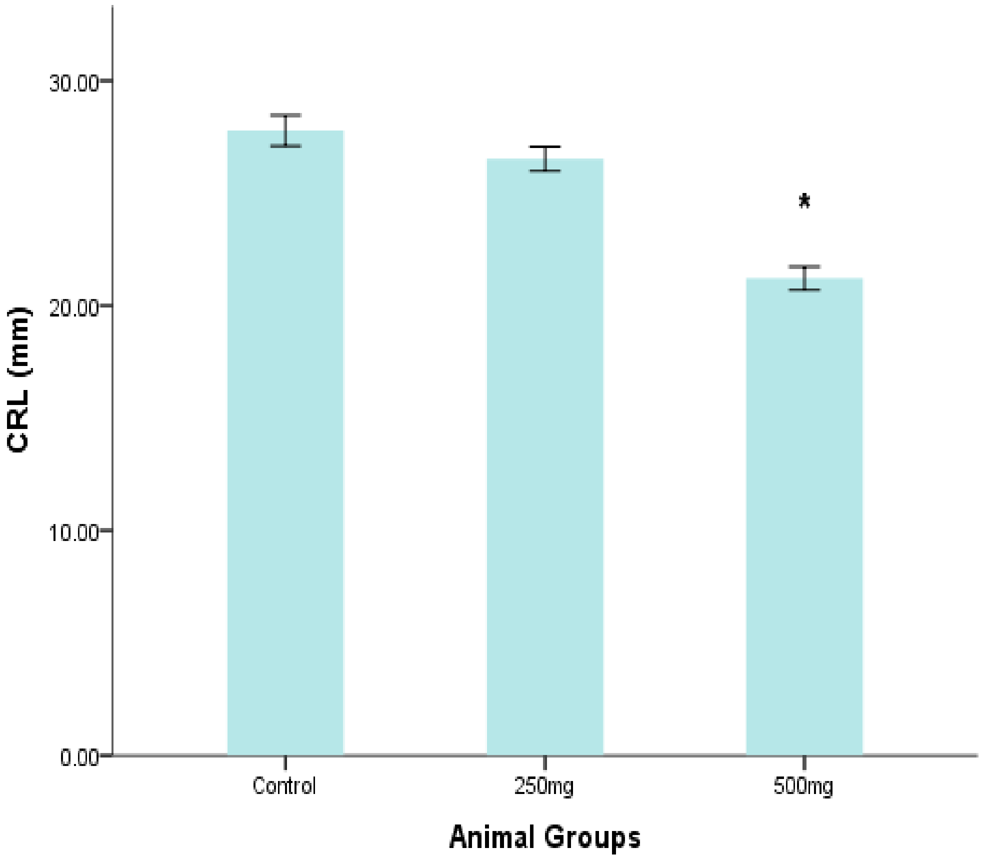


Fig. 4.3: Crown Rump Length of experimental animals across the groups. Values are given as mean ±SEM. * $p<0.05$ compared to the control.

4.3 Effect of Treatment on Histology

Plate 4.1 (A and B) showed the histology of the fetal liver in the control group with developing hepatocytes, radiating from the central vein, and sinusoids. Plate 4.2 (A and B) showed the histology of the fetal liver in the group administered 250mg/Kg of Soybean having histology similar to control with developing hepatocytes, radiating from the central vein, and sinusoids.

Plate 4.3 (A and B) showed the histology of the fetal liver in the group administered 250mg/Kg of Soybean having histology similar to control with developing hepatocytes, radiating from the central vein, and sinusoids.

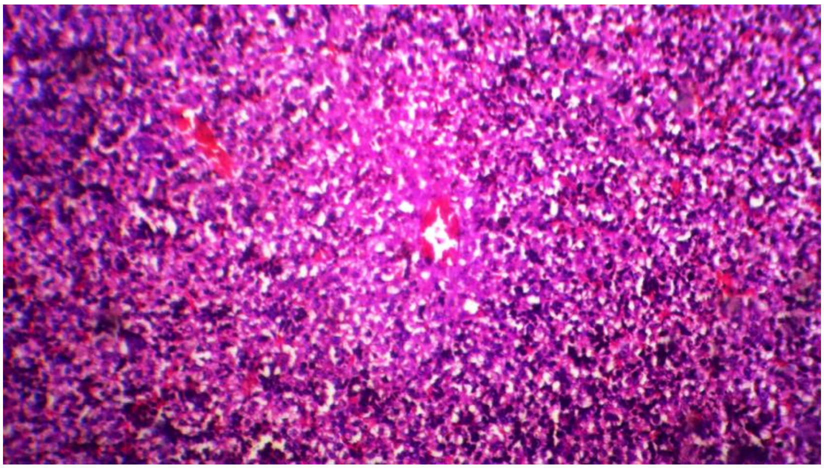


Plate 4.1A showing fetal liver of Control: developing hepatocytes (H) radiating from central vein (CV), sinusoids (S) (H&E; 100×).

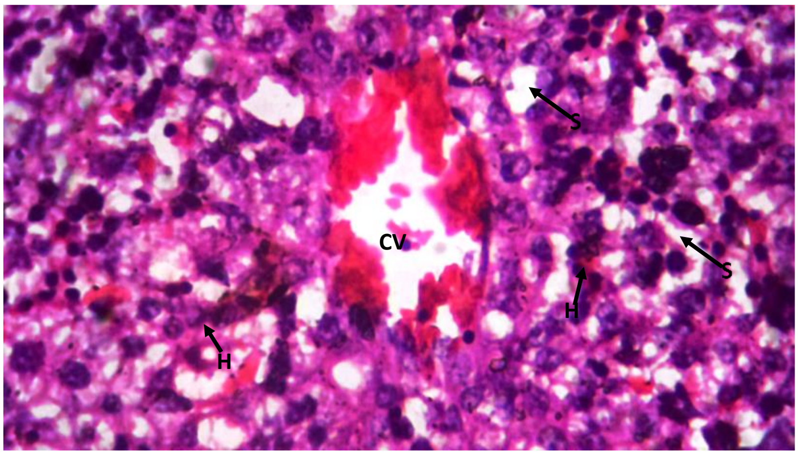


Plate 4.1B showing fetal liver of Control: developing hepatocytes (H) radiating from central vein (CV), sinusoids (S) (H&E; 400×).

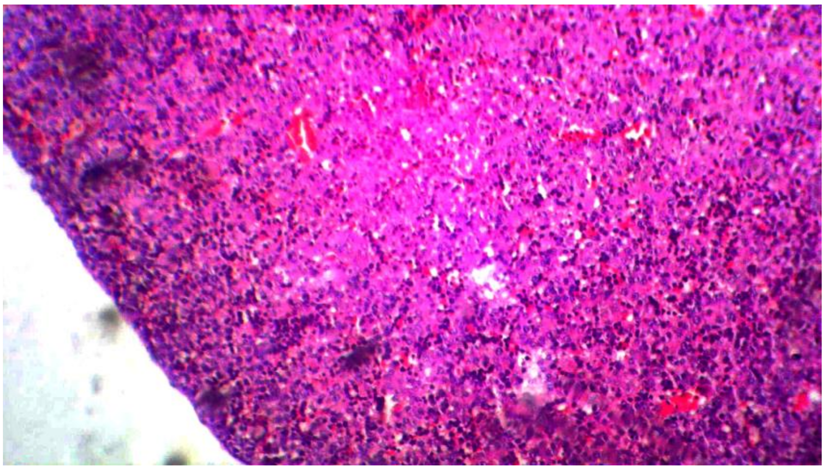


Plate 4.2A showing Fetal liver of rat administered 250mg/Kg of Soybean: similar to the control; developing hepatocytes (h) radiating from central vein, sinusoids (S) (H&E; 100×)

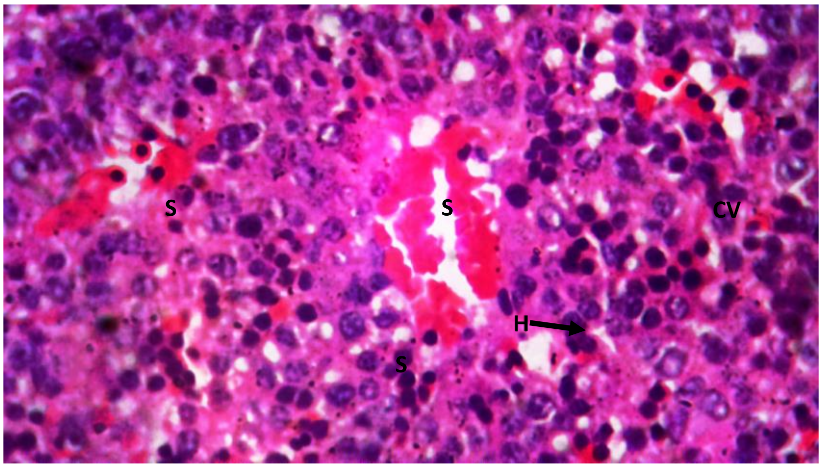


Plate 4.2B showing Fetal liver of rat administered 250mg/Kg of Soybean: similar to the control; developing hepatocytes (h) radiating from central vein, sinusoids (S) (H&E; 400×)

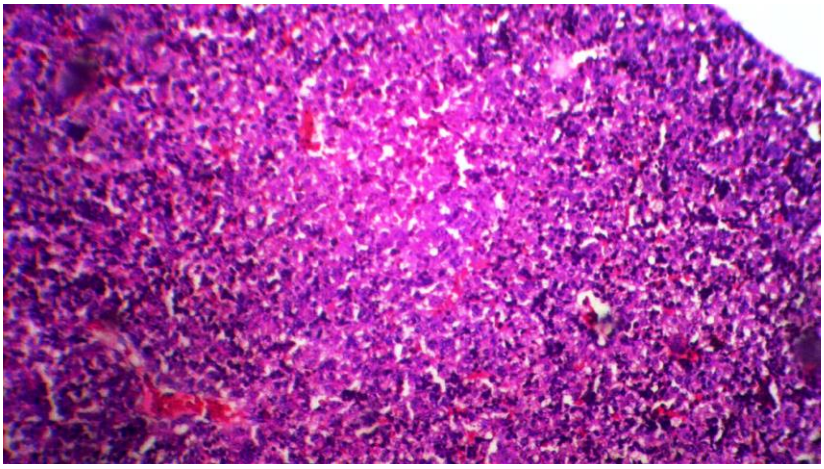


Plate 4.3A showing the Fetal liver of rat administered 500mg/Kg: similar to the control; developing hepatocytes (h) radiating from central vein, sinusoids (S) (H&E; 100×)

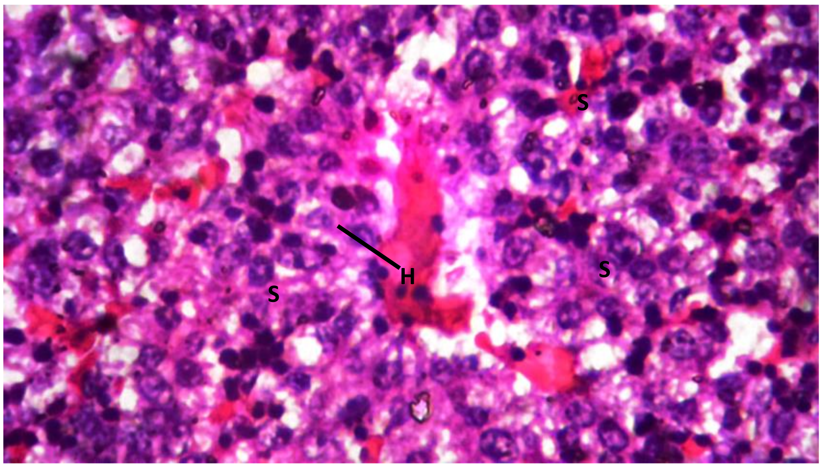


Plate 4.3B showing the Fetal liver of rat administered 500mg/Kg: similar to the control; developing hepatocytes (h) radiating from central vein, sinusoids (S) (H&E; 400×)

CHAPTER FIVE

DISCUSSION, CONCLUSION AND RECOMMENDATION

5.1 DISCUSSION

Soybean is a remarkable botanical resource, containing a rich array of vital minerals. Findings from this study showed the presence of reducing sugars, saponins, flavonoids, phenolics, eugenols, terpenoids, alkaloids, and proteins in soybean. This aligns with previous studies (Chakraborty et al., 2014; Kumar *et al.*, 2022) on the phytochemical composition of this legume. These compounds have been extensively studied for their potential health-promoting properties, including antioxidant, anti-inflammatory, and antimicrobial activities. Reducing sugars serves as a readily available energy source (Du et al., 2020). Saponins, flavonoids, and phenolics are known for their antioxidant properties (Lee et al., 2011; Rahimi and Bagheri, 2020), which may help protect against oxidative stress and associated diseases. Eugenols, terpenoids, and alkaloids have been linked to various health benefits, including anti-inflammatory and antimicrobial effects (Cox-Georgian et al., 2019). The absence of tannins in soybean is consistent with previous studies by (Li et al., 2012), as soybeans are not typically rich in tannins. Tannins are known to interfere with protein digestion and may contribute to astringency in foods (Soares et al., 2020), so their absence in soybean is advantageous. The absence of steroids in soybean is also consistent with previous studies by Wang *et al.* (2011), as steroids are not commonly reported in soybean. Steroids play important roles in biological processes but are not typically abundant in plant-based foods like soybean.

The assessment of body weight is a crucial aspect of evaluating the effects of various substances, including potential therapeutic agents or dietary components, particularly during pregnancy (Kuiper et al., 2001). Changes in body weight can indicate alterations in metabolism, nutrient utilization, or overall health status (Brent, 2004). In this study, there was a significant decrease in body weight change in rats treated with both 250mg/Kg and 500mg/Kg of Soybean compared to the control group. This suggests that soybean administration at these dosages may lead to weight loss or altered weight gain in pregnant rats. This finding is consistent with a study by Zang et al. (2006) who reported weight changes in response to soybean consumption. Also, findings also revealed a significant decrease in fetal weight in rats treated with 500mg/Kg of Soybean compared to the control group. However, no significant difference in fetal weight was observed in rats treated with 250mg/Kg of Soybean compared to the control group, indicating a dosage-dependent effect of soybean on fetal weight. There was a significant decrease in crown-rump length in rats treated with 500mg/Kg of Soybean when compared to the control group. Crown-rump length is an important indicator of fetal growth and development, and alterations in this measurement can have implications for fetal health. Again, no significant difference in crown-rump length was observed in rats treated with 250mg/Kg of Soybean compared to the control group. These findings suggest that high dosages of soybean may have adverse effects on fetal growth and development, as indicated by the decreased fetal weight and crown-rump length. However, the effects appear to be dosage-dependent, with no significant effects observed at the lower dosage of 250mg/Kg. These results are consistent with previous studies (Zang et al., 2006) that have reported adverse effects of high dosages of soybean on fetal development.

Histological findings showed that the fetal liver exposed to 250mg/Kg of Soybean had histological features closely resembling those of the control group. This suggests that soybean administration at this dosage did not induce any significant histological alterations in the fetal liver. Similarly, the liver histology of rats treated with 500mg/Kg also showed no observable histological alterations in the fetal liver when compared with the control. Studies investigating the impact of soybean on liver health have yielded varying results depending on the dosage and duration of exposure. A study by Amer (2012) reported similar findings, showing that soybean administration did not lead to significant histological changes in the liver. However, in contrast, a study by Kosif et al. (2010) found that higher dosages of soybean extract resulted in liver toxicity in animal models.

5.2 CONCLUSION AND RECOMMENDATION

In conclusion, findings from this study show that Soybean do not affect the normal development of the Liver. However, further studies are necessary to assess the long-term effects of soybean consumption on liver development.

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