Effects of Soya Bean (*Glycine Max*) Extract on the Histology of Livers of Sniper Induced Rats

Johnson Agbai Ukwa [1]., Njoku Oji Ifegwu [2]*., Eke Ugorji Iheanacho [3]., kelechi Uzoma Akatobi [3]., Cosmos Sopuruchi Agim [3]., Azunna Uchenna [3]., Chamberlin Jamike Elem [4]., Justice Ugonna Okoli [1].

[1]Department of Human Anatomy, Faculty of Basic Medical Sciences, Abia State University, Uturu, Abia State

[2]Department of Community Health, School of Health Sciences, Abia State College of Health Sciences and Management Technology Aba, Abia State

[3]Department of Medical Biochemistry, Faculty of Basic Medical Sciences, Abia State University, Uturu, Abia State

[4]Department of Human Kinetics and Sports Sciences, Ignatius Ajuru University of Education, Rumuorlumeni, Port Harcourt, Rivers State

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ABSTRACT

Objective: This research was carried out to investigate the effect of Soya bean (*Glycine max*) extract on the histology of livers of sniper-induced rats.

Methodology: Twenty-five rats weighing 110 g to 150 g were procured and acclimatized for two weeks, thereafter; they were divided into five groups of five rats each, and were housed in cages. The groups were designated as groups A - E. Group A served as the control group and was not induced, while groups B – E were induced. Group A and B received distilled water only, while groups C – E received Vitamin C, 200 mg/kg of body weight and 400 mg/kg of body weight of extracts of Soya bean respectively for 21 days orally through oro-gastric tube. On the 22nd day, the animals were weighed, sacrificed via chloroform inhalation, and livers were harvested for histological study.

Results: Histopathological findings showed normal hepatic architecture with central vain (CV) and active hepatocyte (H) in group A, severe degeneration with severe aggregate intra-hepatic inflammation (IHI) in group B, moderate healing with mild aggregate intra-hepatic inflammation (IHI) with active hepatocyte (H) in group C, mild healing with moderate aggregate intra-hepatic inflammation (IHI) with active hepatocyte (H) in group D, and moderate healing with mild aggregate intra-hepatic inflammation (IHI) in group E.

Conclusion: The extract of Soya bean has ameliorating effect on the histology of livers of sniper-induced rats, and the ameliorating effect improves with increase in the dosages of the extract

Keywords: Sniper; Liver; Soya bean; Ameliorating.

INTRODUCTION

Sniper or Dichlorvos (2,2-dichlorovinyl dimethyl phosphate, commonly abbreviated as an DDVP [1] refers to an organophosphate widely used as an insecticide to control household pests, in public health, and to protect stored products from insects [2]. It is a colourless liquid [3] with aromatic odour, and is soluble in water [6]. Its density is 1.425 g/cm^3 (23.35 g/in^3) at 25 °C (77 °F), melting point below −60 °C (−76 °F) and a boiling point of 140 °C (284 °F) at 27 hPa [4]. Like other organophosphate insecticides, Dichlorvos inhibits acetylcholinesterase, associated with the nervous systems of insects [2], and is claimed to damage DNA of insects [5].

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Dichlorvos enters the air, water, and soil when it is used and manufactured. It can also enter the environment when waste containing dichlorvos is disposed of in landfills [2]. Because it is soluble in water, it dissolves when it enters a body of water, and evaporates into the air easily, but is broken down by water vapor such as humidity [4]. It does not bind to soil, but is broken down slower in soil than in the air. The broken down products are far less harmful than dichlorvos is. It is not stored in plants, animals, or humans [2]. It is effective against mushroom flies, aphids, spider mites, caterpillars, thrips, and whiteflies in greenhouses and in outdoor crops [2]. Also, it is used in the milling and grain handling industries and to treat a variety of parasitic worm infections in animals and humans [2]. It is fed to livestock to control botfly larvae in manure, and acts against insects as both a contact poison and an ingested poison. It is available as an aerosol and soluble concentrate [2]. It is also used in pet flea collars and "no-pest strips" in the form of a pesticide-impregnated plastic; this material has been available to households since 1964 and has been the source of some concern, partly due to misuse [6].

DDVP is a conceivably cytotoxic substance that can induce skin irritation after prolonged exposure because of the presence of dichlorovenyl side chain that effectively associates with cell proteins and elicits cell degradation [7]. It produces a genotoxic structural alert, a thiophosphate functional group (trimethyl phosphate) that is responsible for the in vitro mutagenesis. It induces a weak DNA methylation that can repress gene transcription on promoter site, yet, this might not be effective in human exposure because of the rapid metabolism in an in vivo assays [7]. It can be potentially mutagenic if higher quantity is inhaled, applied topically, and ingested through food as it has been used to preserve farm products [7]. Its misuse poses significant risks to human health, manifesting in both short-term and long-term consequences [8]. Long-term exposure may lead to severe health implications, including developmental abnormalities in offspring, memory loss, reduced fertility, and potential carcinogenic effects [8]. These adverse effects highlight the importance of adhering to safety guidelines to mitigate the risks associated with dichlorvos exposure [8]. Short-term inhalation exposure to high concentrations of dichlorvos (DDVP) could contribute to moderate toxicological effects of the heart [9]. Acute and prolonged exposure may lead to death, genotoxic, neurological, reproductive, carcinogenic, immunological, hepatic, renal, respiratory, metabolic, dermal and other systemic effects [10]. Its toxicity is due to the ability of the compound to inhibit acetyl cholinesterase at cholinergic junction of the nervous system [10].

The liver is a peritoneal organ positioned in the right upper quadrant of the abdomen [11]. It is the largest visceral structure in the abdominal cavity, and the largest gland in the human body that is predominantly located in the right hypochondrium and epigastric areas, and extends into the left hypochondrium of the abdomen. As an accessory digestion gland, it performs a wide range of functions, such as synthesis of bile, glycogen storage and clotting factor production [11]. The important functions of the liver are bile synthesis, which helps carry away waste and break down fats in the small intestine during digestion; synthesis of certain proteins for blood plasma, production of cholesterol and special proteins to help carry fats through the body; stores and releases glucose as needed, processes hemoglobin to use its iron content (the liver stores iron); changes harmful ammonia to urea; clears the blood of medicines and other harmful substances; regulates blood clotting; fights infections by making immune factors and removing bacteria from the bloodstream; and clears bilirubin. [12]. When the liver has broken down harmful substances, they are excreted into the bile or blood. Bile by-products enter the intestine and ultimately leave the body in bowel movements [12]. Blood by-products are filtered out by the kidneys and leave the body in the form of urine.

It has two surfaces – the diaphragmatic and viscera. The diaphragmatic surface forms the anterosuperior surface of the liver [11]. It is smooth and convex, fitting snugly beneath the curvature of the diaphragm. Its posterior aspect is not covered by visceral peritoneum and is in direct contact with the diaphragm is known as the “bare area” of the liver. The visceral surface is on the posteroinferior surface of the liver [11]. With the exception of the fossa of the gallbladder and porta hepatis, it is covered with peritoneum [11]. It is moulded by the shape of the surrounding organs, making it irregular and flat. It lies in contact with the right kidney, right adrenal gland, right colic flexure, transverse colon, first part of the duodenum, gallbladder, oesophagus and the stomach is also has a number of ligaments that attaches it to the surrounding structures. The ligaments are formed by a double layer of peritoneum and include falciform ligament, coronary ligament (anterior and posterior folds), Triangular ligaments (left and right), and lesser omentum In addition to these supporting

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ligaments, the posterior surface of the liver is secured to the inferior vena cava by hepatic veins and fibrous tissue.it has hepatic recesses which are anatomical spaces between the liver and surrounding structures. The hepatic recesses are of clinical importance as infection may collect in them, forming an abscess. They include subphrenic spaces, subhepatic space and Morison's pouch [11].

The liver is covered by a fibrous layer, known as Glisson's capsule, and is comprised of a large right lobe and smaller left lobe. There are two further "accessory" lobes that arise from the right lobe, which are located on the visceral surface of liver - caudate lobe which is located on the upper aspect of the visceral surface [11]. It lies between the inferior vena cava and a fossa produced by the ligamentum venosum (a remnant of the foetal ductus venosus), and quadrate lobe which is located on the lower aspect of the visceral surface. It lies between the gallbladder and a fossa produced by the ligamentum teres (a remnant of the fetal umbilical vein). Separating the caudate and quadrate lobes is a deep, transverse fissure – known as the porta hepatis. It transmits all the vessels, nerves and ducts entering or leaving the liver with the exception of the hepatic veins [11].

Microscopically, the cells of the liver (known as hepatocytes) are arranged into lobules [11]. These are the structural units of the liver. Each anatomical lobule is hexagonal-shaped and is drained by a central vein. At the periphery of the hexagon are three structures collectively known as the portal triad - arteriole which is a branch of the hepatic artery entering the liver; venule which is a branch of the hepatic portal vein entering the liver; and bile duct which is branch of the bile duct leaving the live [11]. The portal triad also contains lymphatic vessels and vagus nerve (parasympathetic) fibres. Thus, the liver histology reveals a highly organized structure designed for detoxification and metabolism, centered around hexagonal hepatic lobules. Its key features include hepatocytes arranged in plates, radiating from a central vein toward peripheral portal tracts, which contain a bile duct, portal vein, and hepatic artery. Sinusoids, lined with Kupffer cells, allow blood to percolate and bathe hepatocytes [13].

It has a unique dual blood supply: Hepatic artery proper (25%) which supplies the non-parenchymal structures of the liver with arterial blood. It is derived from the coeliac trunkm while the hepatic portal vein (75%) supplies the liver with partially deoxygenated blood, carrying nutrients absorbed from the small intestine, and is the dominant blood supply to the liver parenchyma and allows the liver to perform its gut-related functions (such as detoxification) [11]. The venous drainage of the liver is achieved through hepatic veins. The central veins of the hepatic lobule form collecting veins which then combine to form multiple hepatic veins. These hepatic veins then open into the inferior vena cava. The parenchyma of the liver is innervated by the hepatic plexus, which contains sympathetic (coeliac plexus) and parasympathetic (vagus nerve) nerve fibres [11]. These fibres enter the liver at the porta hepatis and follow the course of branches of the hepatic artery and portal vein. Glisson's capsule, the fibrous covering of the liver, is innervated by branches of the lower intercostal nerves. Distension of the capsule results in a sharp, well localized pain [11]. The lymphatic vessels of the anterior aspect of the liver drain into the hepatic lymph nodes. These lie along the hepatic vessels and ducts in the lesser omentum; and empty in the coeliac lymph nodes which in turn, drain into the cisterna chili [11]. Lymphatics from the posterior aspect of the liver drain into phrenic and posterior mediastinal nodes, which join the right lymphatic and thoracic ducts [11].

Soya bean or soy bean or soybean (*Glycine max*) [14] is a species of legume native to East Asia that is widely grown for its edible bean. It is a staple crop, the world's most grown legume, and an important animal feed [15]. Its plants are annual legumes that can reach the heights of 1 to 1.5 meters (3 to 5 feet), with compound leaves composed of three leaflets, and flowers that are small and typically white or purple [16]. Their fruits are pods that contain 2 to 4 seeds each, which are the soya beans [16]. The seeds are the most commonly used part of the soya bean plant. They can be processed into various products, including oil, meal, tofu, tempeh, and soy milk [16].

They are versatile source of plant-based protein and are used in various forms in human diets, such as tofu, soy milk, soy sauce, and edamame. Its meal is a significant component of livestock and poultry feed due to its high protein content. Its oil is used in cooking, as an ingredient in processed foods, and in industrial applications like biodiesel production, and the compounds are used in the production of medicines and dietary supplements.

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According to research studies, some of the impressive health benefits of soya beans are that they contain natural polyphenols, which are powerful antioxidants that help to neutralize the effect of free radical molecules cause by oxidative stress that often characterizes the onset of chronic diseases and ageing, help to prevent breast cancer in premenopausal women, have a low glycemic index and can be beneficial for individuals with diabetes [17]. Other benefits include good source of calcium and magnesium, essential minerals for maintaining strong and healthy bones, contain phytoestrogen compounds which serve as estrogen supplements, thus help to regulate hormonal imbalance and neutralize the accompanying symptoms, contain isoflavone compounds that help to promote healthy blood vessel functions, thereby reducing the risk of cardiovascular diseases, and are rich source of protein boosting growth and tissue repair [16].

Soya bean protein may improve alcohol-induced lipid accumulation, oxidative stress and inflammation by decreasing proinflammatory cytokines and CYP2E1 protein expression and by increasing PPARα and CYP4A protein expressions and fecal lipid excretion, thereby producing beneficial effects on ALD during ethanol withdrawal [18]. Research has shown that its consumption has a hypouricaemic effect, and is harmless to the kidney [19]. Lastly, it has also been shown that soya bean has ameliorating effect on the histology of kidneys of sniper-induced rats, and the ameliorating effect improves with increase in the dosages of the extract [20].

Thus, this research work aims at educating the public on the ameliorating effect of soya bean extract on the histology of liver of sniper-induced rats thereby encouraging its consumption especially to reduce the effect of sniper which is being used to preserve food items like beans, stock-fish, and crayfish by traders and farmers as is use is dangerous to health.

MATERIAL AND METHODS

Animal procurement, care and treatment

Twenty-five (25) female wistar rats weighing between 110 g to 150 g were procured and housed at the Animal house of Anatomy Department, Abia State University; Uturu with wire gauze cages in a well-ventilated area, were maintained under standard laboratory conditions of temperature (22+2 ℃), relative humidity (55-65 %) and 12 hours light/dark cycle. They were fed with standard commercial pellet diet and water ad libitum and were also acclimatized for two weeks before the experiment. Their health statuses were closely monitored before and during the experiment. All procedures were carried out in strict accordance with the Institutional guidelines on the care and use of experimental animals.

Collection, identification and preparation of plant material

Soya bean seeds (*Glycine max*) were purchased from a local market in Aba, Abia State, Nigeria, and were authenticated at Herbarium unit, Botany Department, Abia State University, Uturu, Abia State with the Herbarium number ABSU/REC/BHA/068. They were washed under running water to remove impurities, and were dried thoroughly under the sun for 48 hours and later grinded into powder. The dried seeds were grounded into a fine powder using a laboratory blender to obtain the powder. Later the powdered seeds were extracted using ethanol in a soxhlet apparatus. The extract was concentrated with a rotary evaporator, and was dried to yield the crude soya bean extract which was stored in an airtight container at 4 ^{0}C until further use. At the time of use, the filtrate extracts were filtered into a stainless basin with a white cloth and placed in a water bath so as to dry up the water. 250 mg of these extracts /kg body weights were dissolved in 10 mls of distilled water and were administered to the animals.

Induction of Sniper

Sniper was purchased at pharmaceutical shop at Ariaria Market Aba, Abia State, Nigeria. The rats were exposed to 20 mg/kg of Sniper for 4 hours at 3 days interval in an inhalation chamber for 21 days. The lethal-dose (LD_{50}) of sniper in rats has been determined to be 833 (mg/kg) using Spearman-Karber's arithmetic method [21].

Experimental protocol

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The animals were grouped into five (5) groups of five (5) rats each. Different doses of the extracts of soya bean were administered via oral route with the aid of oral gastric tube as shown below:

Group A: The control group + distilled water.

Group B: 20 mg/kg of Sniper only.

Group C: 20 mg/kg of Sniper + Vitamin C.

Group D: 20 mg/kg of Sniper + 200 mg/kg of body weight of extracts of soya bean.

Group E: 20 mg/kg of Sniper + 400 mg/kg of body weight of extracts of soya bean.

Sample collection and analysis

The extracts were administered for twenty one (21) days. On the 22nd day, the animals were sacrificed by anaestethizing under chloroform vapour and dissected. Livers were harvested from the rats, weighed, and fixed in Bouin's fluid for histological analyses.

RESULTS

Histopathological findings

The histopathological findings of this research work reveals as follows: -

- Micrograph 1 is the result of is the result of the histology of the liver (x400) (H/E) of the animals of group A (GPA) control section showing normal liver architecture with central vein (CV) and active hepatocyte (H).
- Micrograph 2 is the result of the histology of the liver (x400) (H/E) of the animals in group B (GPB) induced with sniper only and without treatment showing severe degeneration with severe aggregate intra hepatic inflammation (IHI).
- Micrograph 3 is a photomicrograph of group C (GPC) section of liver (x400) (H/E) induced with sniper and treated with vitamin C showing moderate healing with mild aggregate intra hepatic inflammation (IHI) with active hepatocyte (H).
- Micrograph 4 is a photomicrograph of group D (GPD) section of liver (x400) (H/E) induced with sniper and treated with 200 mg/kg extract showing mild healing with moderate aggregate intra hepatic inflammation (IHI) with active hepatocyte (H).
- Micrograph 5 is a photomicrograph of group E (GPE) section of kidney (x400) (H/E) induced with sniper and treated with 400 mg/kg extract showing moderate healing with mild aggregate intra hepatic inflammation (IHI).

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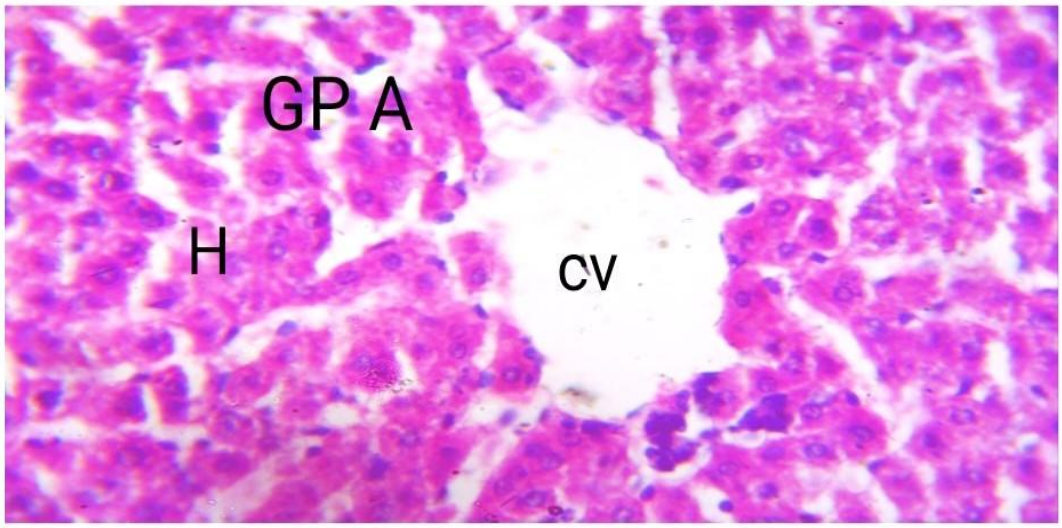

Figure 1 Micrograph 1 (x400) (H/E) is showing normal liver architecture with central vein (CV) and active hepatocyte (H).

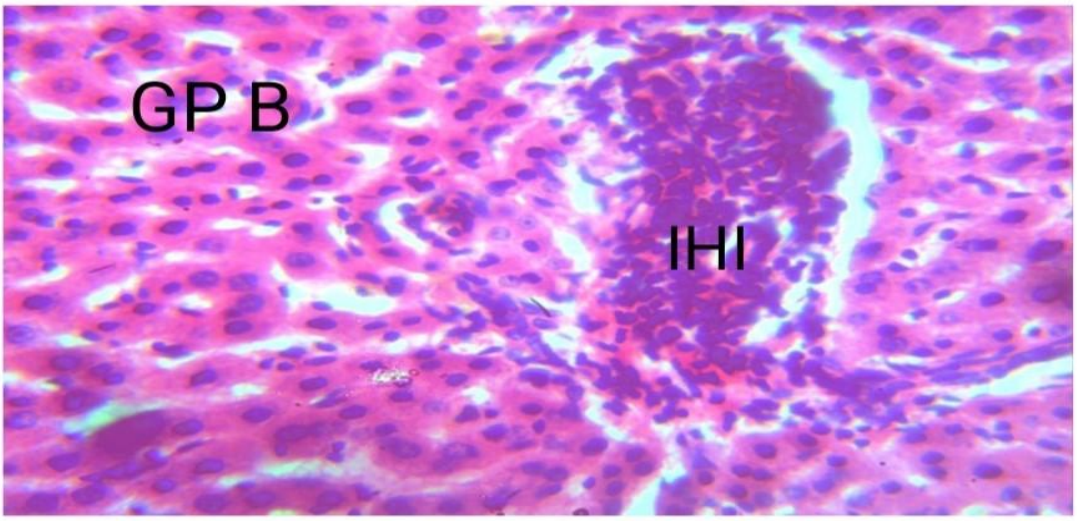

Figure 2 Micrograph 2 (x400) (H/E) is showing severe degeneration with severe aggregate intra hepatic inflammation (IHI).

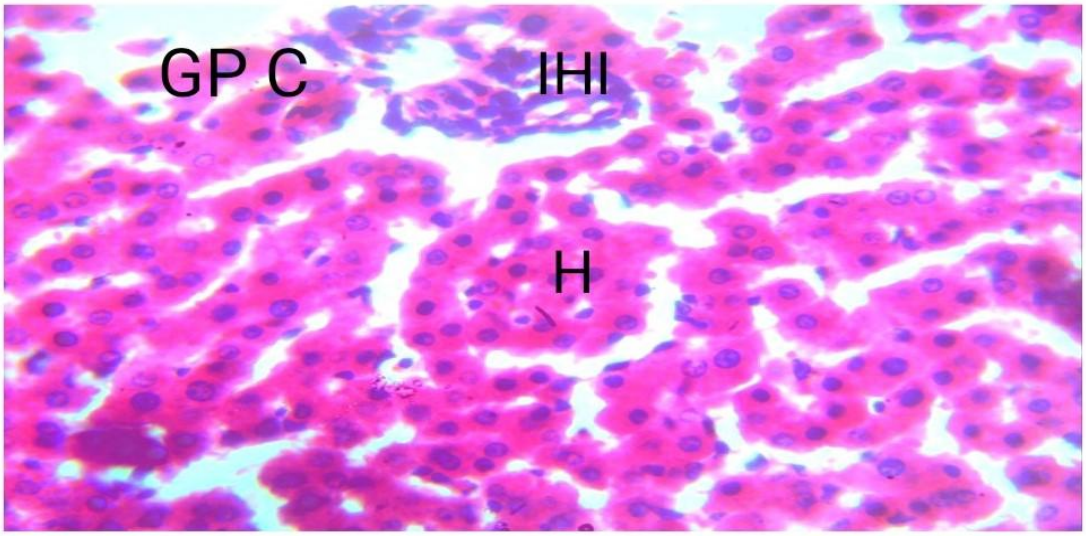

Figure 3 Micrograph 3 (x400) (H/E) is showing moderate healing with mild aggregate intra hepatic inflammation (IHI) with active hepatocyte (H).

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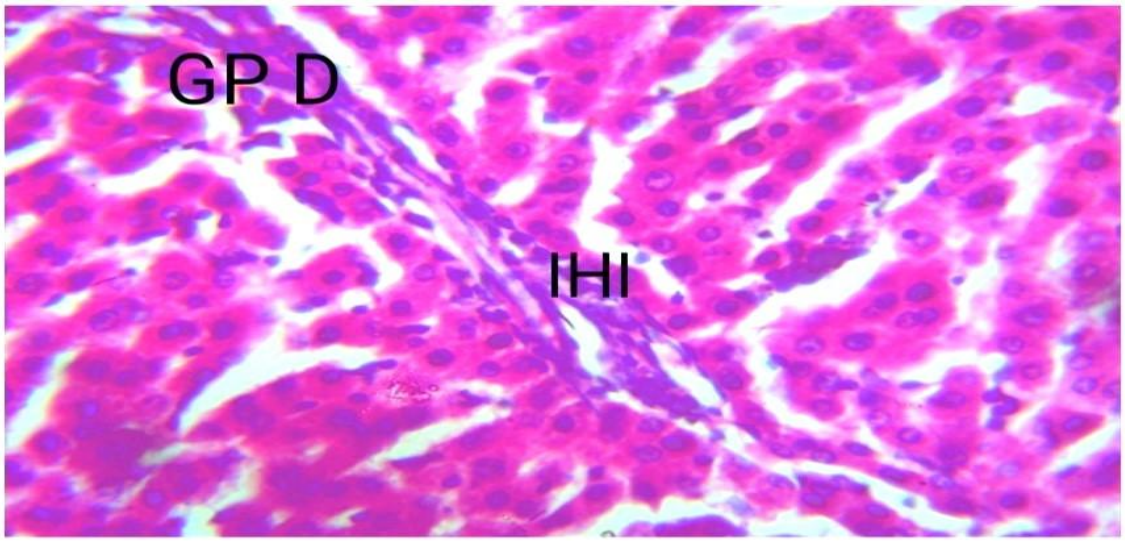

Figure 4 Micrograph 4 (x400) (H/E) is showing mild healing with moderate aggregate intra hepatic inflammation (IHI) with active hepatocyte (H).

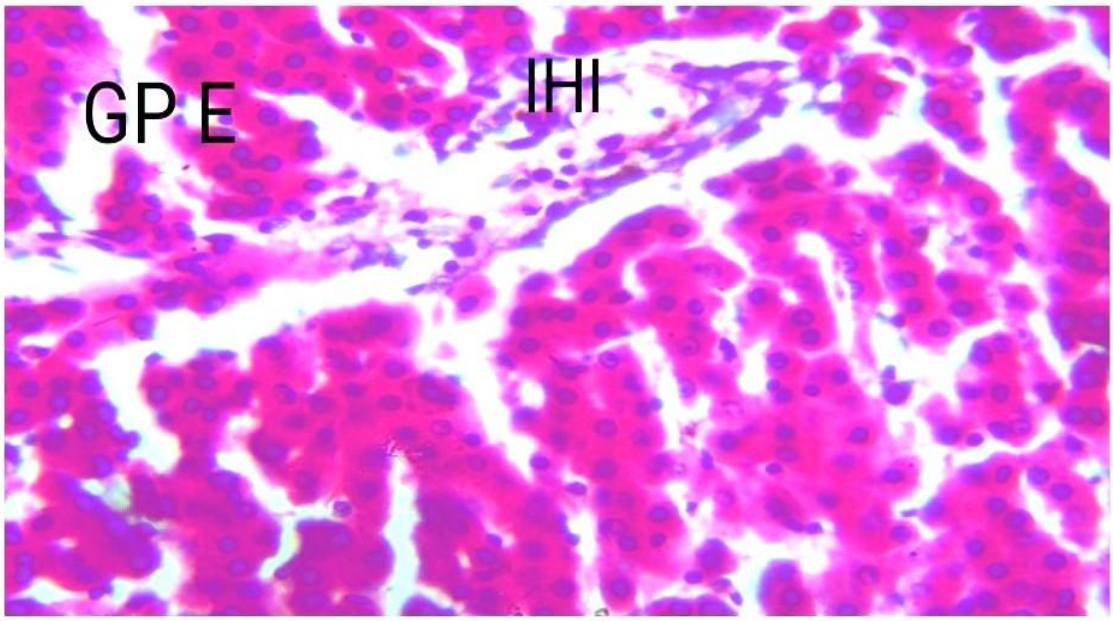

Figure 5 Micrograph 5 (x400) (H/E) is showing moderate healing with mild aggregate intra hepatic inflammation (IHI).

DISCUSSION

Dangerous chemicals like sniper that are dangerous to health have been alleged to be used by farmers and traders to preserve food items like beans, stock-fish, and crayfish. This made the National Agency for Food and Drug Administration and Control (NAFDAC) to issue a stern warning to Nigerians regarding the hazardous practice of using such dangerous chemicals to preserve food items [8]. The agency specifically emphasized that the dangers associated with dichlorvos one of the chemicals commonly utilized by traders to safeguard food from spoilage poses significant risks to human health, with its misuse manifesting in both short-term and long-term consequences. Its long-term exposure can result in severe health implications, including developmental abnormalities in offspring, memory loss, reduced fertility, and potential carcinogenic effects [8]. These adverse effects highlight the importance of adhering to safety guidelines to reduce the risks associated with its exposure [8]. Thus, the aim of this research study is to find out the effect of soya bean extract on the histology of the liver of sipper-induced rats.

The finding of this present study of the livers of the animals in group A (GPA) (x400) (H/E) of Micrograph 1 (figure 1) showed normal liver tissue architecture with central vein (CV) and active hepatocyte (H). This is in line with the normal structure of the histology of liver which according to research study is made up of hepatocytes which are arranged into lobules, with each anatomical lobule being hexagonal-shaped and is being drained by a central vein [11]. Also, research has shown that the histology of liver reveals a highly organized structure designed for detoxification and metabolism, centered on hexagonal hepatic lobules with its key features including hepatocytes arranged in plates, radiating from a central vein toward peripheral portal tracts containing a bile duct, portal vein, and hepatic artery [13].

While, the histopathological result of the histology of the livers of the animals in group B (GPB) induced with sniper only (x400) (H/E) of Micrograph 2 (figure 2) showed severe degeneration with severe aggregate intra hepatic inflammation (IHI). This could be due its oxidative effect on the renal tissues as research has shown that acute and prolonged exposure of sniper may lead to death, genotoxic, neurological, reproductive, carcinogenic, immunological, hepatic, renal, respiratory, metabolic, dermal and other systemic effects [10]. Its toxicity is due to the ability of the compound to inhibit acetyl cholinesterase at cholinergic junction of the nervous system [10].

The result of the histology of the livers of the animals in group C (GPC) induced with sniper (x400) (H/E) and treated with vitamin C of micrograph 3 (figure 3) showed moderate healing with mild aggregate intra hepatic inflammation (IHI) with active hepatocyte (H); in micrograph 4 (figure 4) the result of the histology of the livers of the animals in group D (GPD) induced with sniper (x400) (H/E) and treated with 200 mg/kg of body weight of extracts of soya bean showed mild healing with moderate aggregate intra hepatic inflammation (IHI) with active hepatocyte (H); while that of micrograph 5 (figure 5) induced with sniper (x400) (H/E) and treated with 400 mg/kg of body weight of extracts of soya bean showed moderate healing with mild aggregate intra hepatic inflammation (IHI). These positive results could be due to the ameliorating effect of soya bean which increases with the increase in the extract dosages. This could be due to the ability of the extract of soya bean to detoxify the toxins present in sniper thereby reducing markers of oxidative stress that could have caused the damages in micrograph 2 (figure 2) that received not treatment of the extract of the soya bean at all.

CONCLUSION

The extracts of soya bean have ameliorating effect on the histology of livers of sniper-induced rats, and the ameliorating effect is dose-dependent, and improves better with increase in dosages of the extract.

Compliance with ethical standards

ACKNOWLEDGMENTS

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Disclosure of conflict of interest

The authors have no conflict of interest to declare.

Statement of ethical approval

This research work was approved by the Ethical Approval Committee, Faculty of Basic Medical Sciences, Abia State University, Uturu, Abia State, Nigeria.

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