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Ameliorating effect of soya bean (*Glycine max*) extract on the histology of the kidneys of sniper-induced rats

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Abstract

Objective: This research study was carried out to investigate the ameliorating effect of Soya bean (*Glycine max*) on the histology of kidneys 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 kidneys were harvested for histological study.

Results: Histopathological findings showed normal renal architecture with glomeruli (G), bowman space (BS), renal tubules (RT) and active tubular cell (TC) in group A, severe hemorrhagic glomeruli (HG) in group B, renal tissue with active glomeruli (G) and tubular cell (TC) in group C, mild regeneration with active tubular cells and moderate glomerular necrosis (GN) in group D, and renal tissue with active glomeruli (G) and tubular cell (TC) in group E.

Conclusion: The extract of 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.

Keywords: Kidney; Sniper; Soya bean; Ameliorating

1. Introduction

The kidneys are bilateral bean-shaped organs that are reddish-brown in colour and located in the posterior abdomen [1]. They are responsible for the filtering and excreting waste products from the blood, and are also for water and electrolyte balance in the body. Metabolic waste and excess electrolytes are excreted by the kidneys to form urine which is transported from the kidneys to the bladder by the ureters and leaves the body via the urethra, which opens out into

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the perineum in the female and passes through the penis in the male. They lie retroperitoneally in the abdomen, and on either side of the vertebral column [1], and typically extend from T12 to L3, although the right kidney is often situated slightly lower due to the presence of the liver. Each kidney is approximately three vertebrae in length. The kidneys are enclosed in complex layers of fascia and fat, and are arranged as follows (deep to superficial): - renal capsule, perirenal fat, renal fascia and pararenal fat. Internally, the kidneys have an intricate and unique structure. The renal parenchyma can be divided into two main areas, these are the outer cortex and inner medulla [1]. The outer cortex extends into the medulla, dividing it into triangular shapes known as renal pyramids. The apex of a renal pyramid is referred to as renal papilla. Each renal papilla is associated with a structure known as the minor calyx, which collects urine from the pyramids. Several minor calices merge to form a major calyx. Urine passes through the major calices into the renal pelvis, a flattened and funnel-shaped structure. From the renal pelvis, urine drains into the ureter, which transports it to the bladder for storage.

The medial margin of each kidney is marked by a deep fissure called the renal hilum which acts as a gateway to the kidney – normally the renal vessels and ureter enter/exit the kidney via this structure. The kidneys are supplied with blood via the renal arteries, which arise directly from the abdominal aorta, immediately distal to the origin of the suprior mescemteric artery. The renal artery enters the kidney via the renal hilum. At the hilum level, the renal artery forms an anterior and a posterior division, which carry 75% and 25% of the blood supply to the kidney, respectively [1]. The kidneys are drained of venous blood by the left and right renal veins which leave the renal hilum anteriorly to the renal arteries, and empty directly into the inferior vena cava [1]. Lymph from the kidney drains into the lateral aortic (or para-aortic) lymph nodes, which are located at the origin of the renal arteries [1]. The nephron is the functional unit of the kidney [2]. Each nephron consists of one renal corpuscles and its associated tubules. The kidney as a whole consists of many nephrons (millions) with their associated blood vessel. The renal corpuscles are the sites where the process of urine formation begins with a filtrate of blood plasma. Each renal corpuscle consists of an epithelial cup called Bowman's capsule enclosing a knot of capillaries called the glomerulus [2].

Sniper or Dichlorvos (2,2-dichlorovinyl dimethyl phosphate, commonly abbreviated as an DDVP [3] refers to an organophosphate widely used as an insecticide to control household pests, in public health, and protecting stored products from insects [4]. It is a colourless liquid [5] 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 [6]. Like other organophosphate insecticides, Dichlorvos inhibits acetylcholinesterase, associated with the nervous systems of insects [4], and is claimed to damage DNA of insects [7].

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 [4]. 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 [4]. It is effective against mushroom flies, aphids, spider mites, caterpillars, thrips, and whiteflies in greenhouses and in outdoor crops [4]. Also, it is used in the milling and grain handling industries and to treat a variety of parasitic worm infections in animals and humans [4]. 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 [4]. 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 [8].

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 [9]. 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 [9]. 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 [9]. Its misuse poses significant risks to human health, manifesting in both short-term and long-term consequences [10]. Long-term exposure may lead to severe health implications, including developmental abnormalities in offspring, memory loss, reduced fertility, and potential carcinogenic effects [10]. These adverse effects highlight the importance of adhering to safety guidelines to mitigate the risks associated with dichlorvos exposure [10]. Short-term inhalation exposure to high concentrations of dichlorvos (DDVP) could contribute to moderate toxicological effects of the heart [11]. Acute and prolonged exposure may lead to death, genotoxic, neurological, reproductive, carcinogenic, immunological, hepatic, renal, respiratory, metabolic, dermal and other systemic effects [12]. Its toxicity is due to the ability of the compound to inhibit acetyl cholinesterase at cholinergic junction of the nervous system [12].

Soya bean or soy bean or soybean (*Glycine max*) [13] 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 [14]. 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 [15]. Their fruits are pods that contain 2 to 4 seeds each, which are the soya beans [15]. 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 [15].

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.

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 [16]. 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 [15].

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 [17]. Research has shown that its consumption has a hypouricaemic effect, and is harmless the kidney [18].

Therefore, this research work aims at educating the public on the ameliorating effect of soya bean extract on the histology of kidney 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, stockfish, and crayfish by traders and farmers as is use is dangerous to health.

2. Material and methods

2.1. 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.

2.2. 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/067. The were washed under running water to remove impurities, and were dried thoroughly under the sun for 48 hours and later ground 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 were dried to yield the crude soya bean extract which was stored in an airtight containers 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.

2.3. 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 [20].

2.4. Experimental protocol

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.

2.5. 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.

3. Results

3.1. 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 kidney (x400) (H/E) of the animals of group A (GPA) control section showing normal renal architecture with glomeruli (G), bowman space (BS), renal tubules (RT) and active tubular cell (TC).
- Micrograph 2 is the result of the histology of the kidney (x400) (H/E) of the animals in group B (GPB) induced with sniper only and without treatment showing severe hemorrhagic glomeruli (HG).
- Micrograph 3 is a photomicrograph of group C (GPC) section of kidney (x400) (H/E) induced with sniper and treated with vitamin C showing renal tissue with active glomeruli (G) and tubular cell (TC).
- Micrograph 4 is a photomicrograph of group D (GPD) section of kidney (x400) (H/E) induced with sniperl and treated with 200 mg/kg extract showing mild regeneration with active tubular cells and moderate glomerular necrosis (GN)
- Micrograph 5 is a photomicrograph of group E (GPE) section of kidney (x400) (H/E) induced with sniperl and treated with 400 mg/kg extract showing renal tissue with active glomeruli (G) and tubular cell (TC).

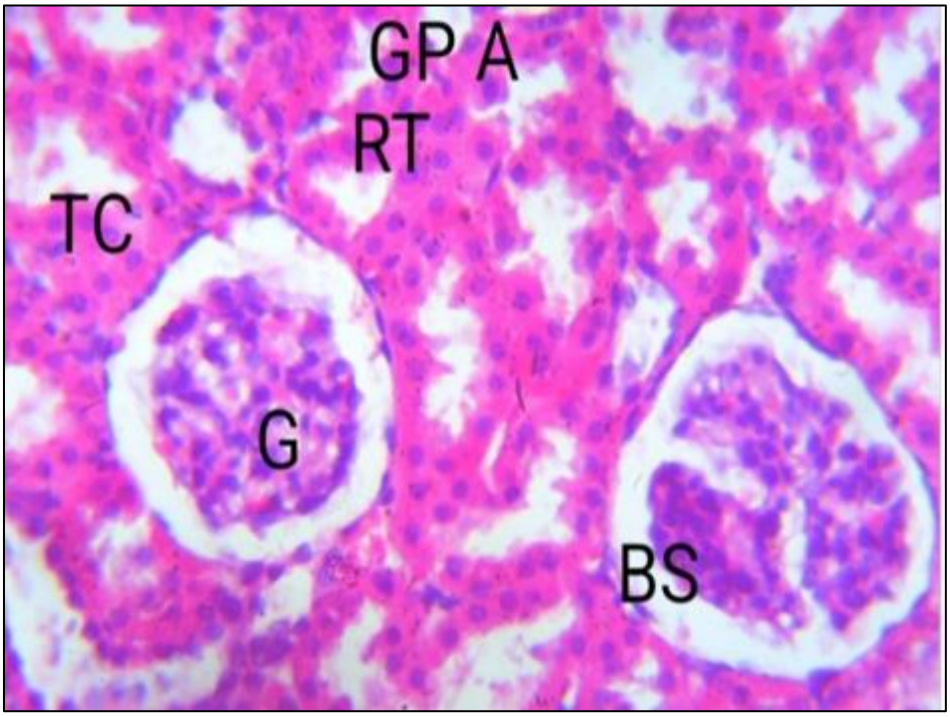

Figure 1 Micrograph 1 (x400) (H/E) is showing normal renal architecture with glomeruli (G), bowman space (BS), renal tubules (RT) and active tubular cell (TC)

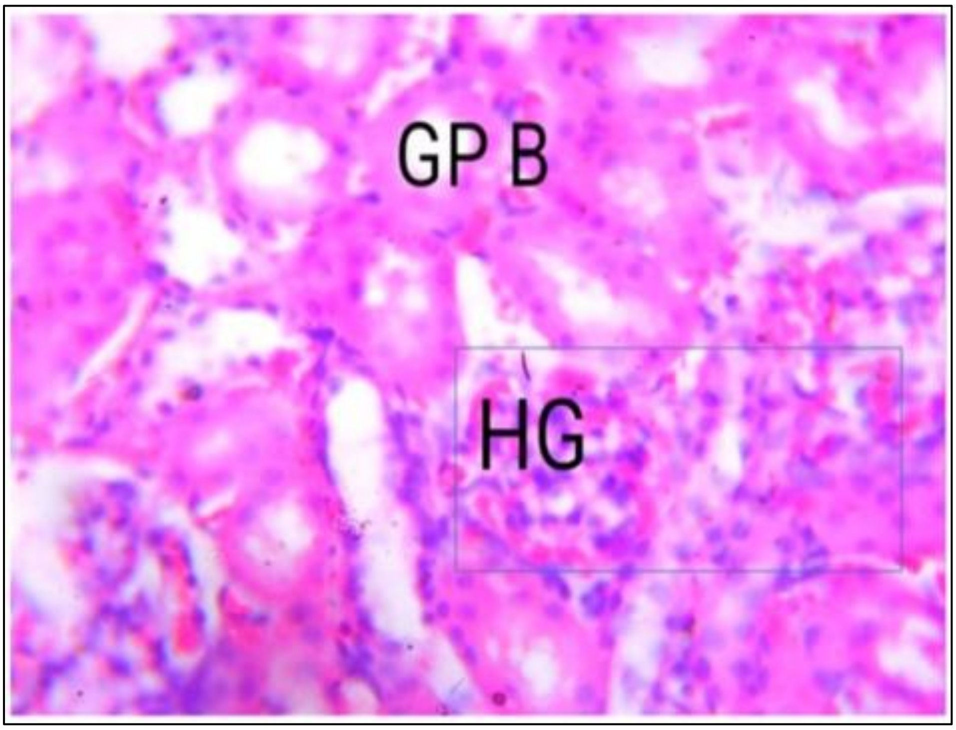

Figure 2 Micrograph 1 (x400) (H/E) is showing severe hemorrhagic glomeruli (HG).

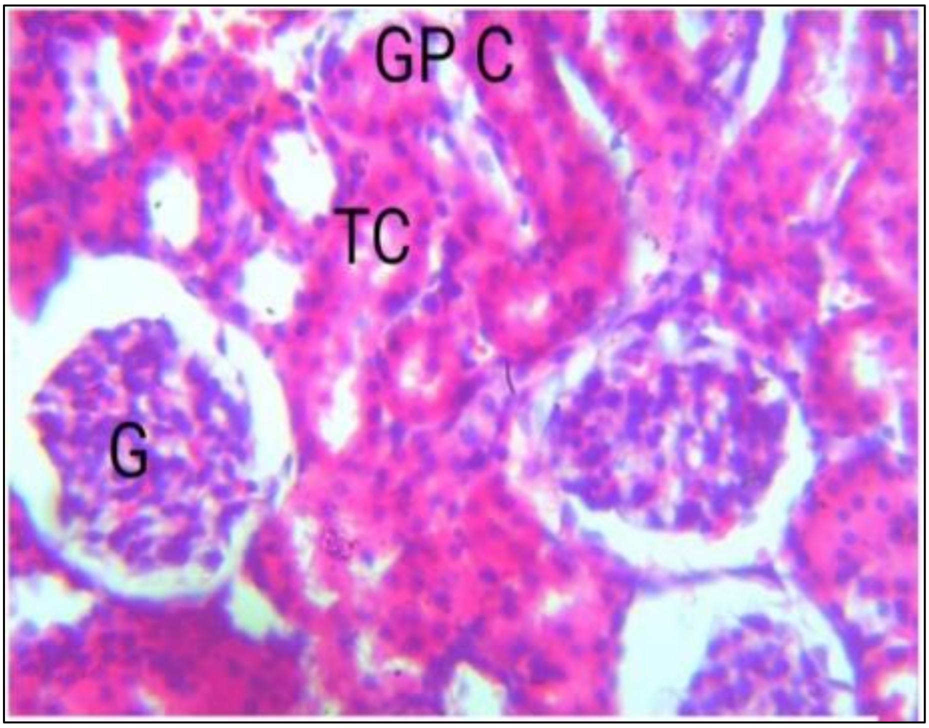

Figure 3 Micrograph 1 (x400) (H/E) is showing renal tissue with active glomeruli (G) and tubular cell (TC)

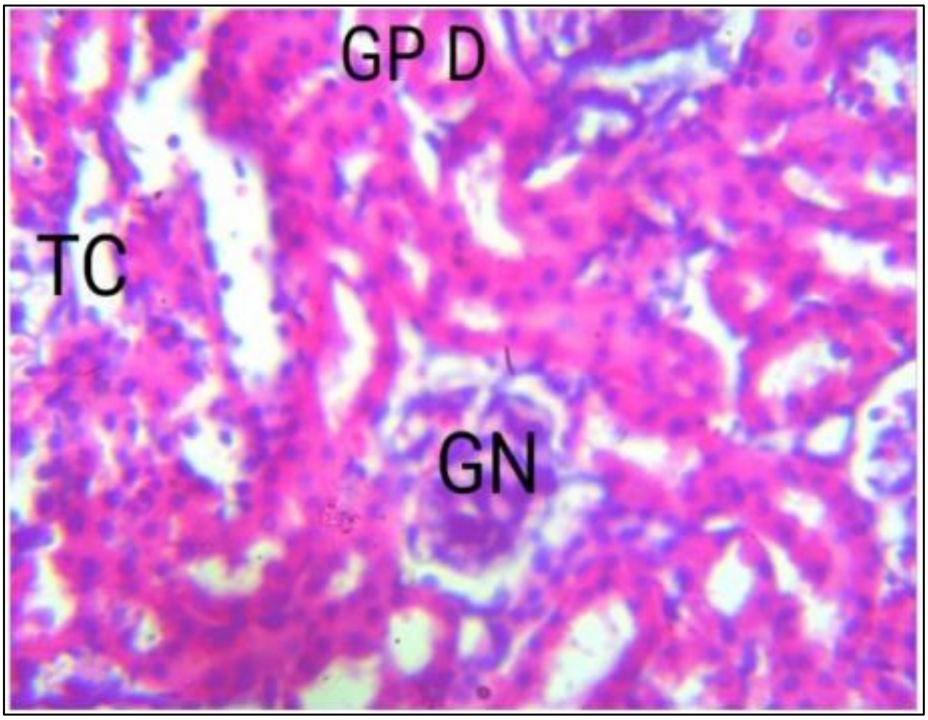

Figure 4 Micrograph 1 (x400) (H/E) is showing mild regeneration with active tubular cells and moderate glomerular necrosis (GN)

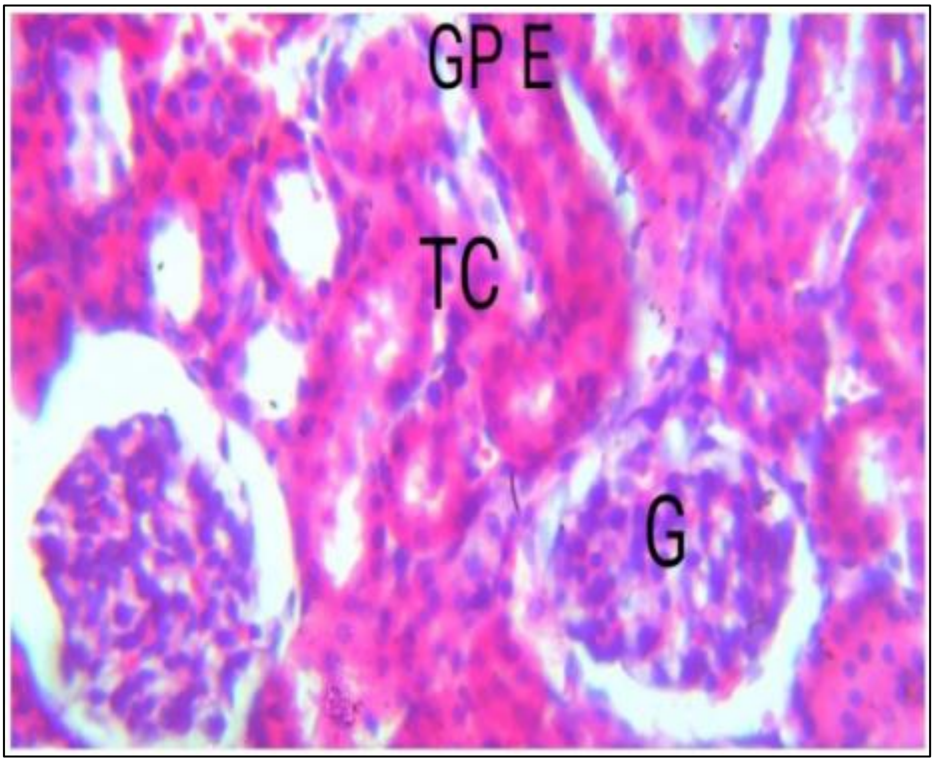

Figure 5 Micrograph 1 (x400) (H/E) is showing renal tissue with active glomeruli (G) and tubular cell (TC)

4. Discussion

The use of dangerous chemicals like sniper to preserve food items like beans, stockfish, and crayfish by traders and farmers is dangerous to health. Thus, the National Agency for Food and Drug Administration and Control (NAFDAC) has issued a stern warning to Nigerians regarding the hazardous practice of using such dangerous chemicals to preserve food items [10]. The agency specifically emphasized the dangers associated with dichlorvos, a chemical commonly utilized by traders to safeguard food from spoilage as its misuse poses significant risks to human health, manifesting in both short-term and long-term consequences. Long-term exposure can result in severe health implications, including developmental abnormalities in offspring, memory loss, reduced fertility, and potential carcinogenic effects [10]. These adverse effects highlight the importance of adhering to safety guidelines to mitigate the risks associated its exposure [10]. Thus, the aim of this research study is to investigate the effect of soya bean extract on the histology of kidney of sipper-induced rats.

The finding of this present study of the kidneys of the animals in group A (GPA) (x400) (H/E) of Micrograph 1 (figure 1) showed normal renal architecture with glomeruli (G), bowman space (BS), renal tubules (RT) and active tubular cell (TC). This is in line with the normal structure of the histology of kindey which according to research study is made up of nephrons, with each nephron consisting of one renal corpuscle and its associated tubules [2]. The kidney as a whole consists of many nephrons (millions) with their associated blood vessel, each renal corpuscle consists of an epithelial cup called the Bowman's Corpuscle enclosing a knot of capillaries called the glomerulus [2],

While, the histopathological result of the histology of the kidneys of the animals in group B (GPB) induced with sniper only (x400) (H/E) of Micrograph 2 (figure 2) showed severe hemorrhagic glomeruli (HG). 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 [12]. Its toxicity is due to the ability of the compound to inhibit acetyl cholinesterase at cholinergic junction of the nervous system [12].

The result of the histology of the kidneys of the animals in group C (GPC) induced with sniper (x400) (H/E) and treated with vitamin C of micrograph 3 (figure 3) showed renal tissue with active glomeruli (G) and tubular cell (TC); in micrograph 4 (figure 4) the result of the histology of the kidneys 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 regeneration with active tubular cells and moderate glomerular necrosis (GN); 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 renal tissue with active glomeruli (G) and tubular cell (TC). 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.

5. Conclusion

The extracts of soya bean have ameliorating effect on the histology of kidneys 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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