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POTENTIAL EFFECT OF DIETARY SUPPLEMENT ON THE HISTOLOGY OF THE LIVER AND KIDNEY OF STREPTOZOTOCIN-INDUCED DIABETIC WISTAR RATS USING *VERNONIA CALVOANA* (BITTER LEAF) AND *SOLANUM GILO* (SCARLET EGGPLANT) LEAVES.

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Abstract

This study investigated the effect of dietary supplementation of *Vernonia calvoana* (*Vc*) and *Solanum gilo* (*Sg*) separately and in combined form, on oxidative stress indices in streptozotocin-induced diabetic Wistar rats. A total of 42 male Wistar rats, weighing 130-150g were obtained and divided into groups. All the animals were fasted overnight, body weight and fasting blood glucose measured. Except for group 1, all other groups were administered 50mg/kg body weight streptozotocin intraperitoneally. Group 1 and 2 were normal and diabetic control, group 3 were administered 500mg/kg metformin, groups 4 and 5 were treated with 2% (2g) each of *Vc* and *Sg* separately supplemented diet, respectively. Group 6 was treated with a combination of 2% (1% each of *Vc* and *Sg*) supplemented diet (low doses), while group 7 was similarly treated with a combined 4% (2% each of *Vc* and *Sg*) supplemented diet (high doses). The results showed significant ($p<0.05$) increase in the tissue repairs in groups treated with high dosage of the leaves extract compared to combined group (low dose). Histological result agreed with the biochemical results. Conclusively, dietary supplementation of *Vc* and *Sg* showed significant anti-oxidant effects on streptozotocin-induced diabetic Wistar rats.

Keywords: oxidative stress, diabetic, dietary supplementation, histology

1. Introduction

Oxidative stress results from imbalance between reactive oxygen species (ROSs) generation and their removal, resulting in cellular damage. In humans, oxygen plays very important role as the final acceptor of electrons in the mitochondria through the electron transport chain which is vital for human life (1). Despite the important effect of this process, it has been reported to also generate toxic metabolites referred to as 'reactive oxygen species (ROS) (2). Reactive oxygen species are mainly produced by mitochondria, during both physiological and pathological conditions leading to the formation of O_2 through cellular respiration by lipoxygenase and cyclooxygenase (1).

Basically, the production of ROS depends on both enzymatic and non-enzymatic reactions. The enzymatic reactions which generate ROS are those involved in respiratory chain,

prostaglandin synthesis, phagocytosis, and cytochrome P450 system (3) while NADPH oxidase, xanthine oxidase, and peroxidases are involved in the generation of superoxide radical. Once formed, takes part in several reactions that lead to the formation of hydrogen peroxide, hydroxyl radical (OH), peroxynitrite (ONOO−), and hypochlorous acid (HOCl).

Studies have reported the ability of the ROS to leak across the mitochondria membranes into the cytoplasm where they cause cellular damage through oxidization of a variety of biologically important molecules, including DNA, proteins, lipids, and carbohydrates (4).

To prevent these damages, ROS level in the body have to be regulated by several defense mechanisms, which involve a number of antioxidant and detoxifying enzymes; the antioxidants are molecules with the ability to prevent or slow down the rate of oxidation of macromolecules (5). Their role as antioxidants is to lower or terminate these chain reactions by removing free radicals or inhibiting other oxidation reactions by being oxidized themselves, thus acting as reducing agents (6).

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion and insulin action or both. Chronic hyperglycemia is associated with long-term damage, dysfunction, and failure of normal functioning of various organs, especially the eyes, kidneys, nerves, heart, and blood vessels (7). In diabetes, oxidative stress is as a result of free radicals generated during autoxidation of glucose and increase in the production of glycoxylation products, notably, HbA1c at a level above the recommended plasma value of < 7% as stated by (8). This in turn promotes an increase in intracellular levels of advanced glycation end products. Also, auto-oxidation of glucose generates ROS that accelerate lipid peroxidation with corresponding accumulation of lipoxidation end products and more free radicals (9).

Apart from its use as an edible vegetable, studies have reported that *V. calvoana* leaf is traditionally used in local medicine as an anti-helmintic, anti-protozoal and anti-hyperglycemic agents according to (10). There is also a claim that *V. calvoana* can effectively be used as an antidote for food poisoning and also for curing naval aches and constipation (11).

Antioxidants such as the flavonoids present in medicinal plants such as the *Vernonia* species like *Vernonia. Calvoana*, commonly known as ‘’ekeke” in Yakurr was reported to be effective in the treatment of diabetes, due to their ability to reverse the effect of ROS and oxidative damage in the cells in diabetic conditions (8).

Solanum gilo (Scarlet eggplant) also known as “Iri” in Gabu, in Yala LGA, has been used in traditional medicine for weight reduction, treatment of several ailments including asthma, allergic rhinitis, nasal catarrh, skin infections, rheumatic disease and swollen joint pains, gastro-esophageal reflux disease, constipation, dyspepsia and much more, according to the report of (12). While in scientific studies *S. gilo* has shown to be effective in the management of hyperglycemia, obesity and have noticeable positive roles in improving liver function.

2. Material and methods

2.1 Plant materials

Large quantities of Fresh *Vernonia calvoana* and *Solanum gilo* leaves were gotten from a garden in Ijiman, Yakurr Local Government and Gabu-Yala Local Government Areas of Cross River State. Samples of both plants were taken to the Department of Botany, University of Calabar, for identification and authentication. This was carried out by a Botanist in the Department, Mr. Daniel Offiong. Voucher specimens with identification numbers: Herb/Bot/UCC/0188 and Herb/Bot/UCC/0040, respectively, were deposited in the Herbarium of the Department of Botany, University of Calabar, Calabar, Nigeria.

2.2 Ethical approval

Ethical approval for the treatment and handling of experimental animal and human subjects was obtained from the Faculty Animal Research Ethics Committee on Use and Care of Experimental Animals, Faculty of Basic Medical Sciences, University of Calabar with the approval number; 174PHY2121.

2.3 Processing of plant samples

Vernonia calvoana (bitterleaf and *Solanum gilo* (scarlet eggplant) leaves were plucked from their stems and subjected to thorough washing under running water, after which, they were allowed to drain of water. The leaves were dried under ambient conditions, crushed and blended into powder using a dry moulinex super blender (LM2070-4A, Dubai, United Arab Emirates), then subsequently stored in an air tight container until required.

2.4 Animals and feed

Forty-two (42) male Wistar rats, weighing 130-150grams, were obtained from the Animal House of the College of Medical Sciences, University of Calabar, Nigeria. The feed was purchased from Pfizer Livestock Feed, Abia State, Nigeria.

2.5 Animal experimentation

Forty-two (42) male Wistar rats, weighing 130-150grams, were obtained from the Animal House of the College of Medical Sciences, University of Calabar, Nigeria, and moved to the Animal House of the Department of Biochemistry University of Calabar, Calabar, Nigeria. There were aclimatized for 7 days on rat pallets and water *ad libitum* and maintained under standard housing conditions of adequate ventilation and room temperature ($25^0C \pm 5^0C$) and relative humidity (46% ±5%) with a natural 12hr light-dark cycle as stated by (13).

2.6 Induction of diabetes

2.6.1 Diabetes mellitus was induced using the method by (14).

The rats were fasted over night with free access to water and tested for fasting blood sugar using a lancet which was used to puncture the tip of the tail. Blood glucose was monitored using Accu-Chek glucometer. The STZ was dissolved in 0.9 % normal saline. The rats were induced with STZ at 55 mg/kg via intraperitonael injection. After which the animals were allowed free palletized rat chow for a period of two weeks, before the administration of drug and extract. At the end of the two weeks the blood glucose level rose to ≥ 210 mg/dl which indicated the establishment of diabetes.

2.7 Preparation of extracts into powdered plant samples

The rat feed was prepared in three forms. First, 2% powdered sample of either *V. calvoana* or *S. gilo* was mixed with 98% of rat chow (by measuring 2g of the plant sample and made up to 100g with normal rat chow) separately. Secondly, a combination of 2% of both plant samples measured and mixed with 98% of normal rat chow (by measuring 1g of each of the powdered samples), mixed together and made up to 100g with normal rat chow. Thirdly, a combination of 4% of both plant samples measured and mixed with 96% of normal rat chow (by measuring 2g of each of the powdered samples mixed together and made up to 100g with normal rat chow).

2.8. Treatment of animals with prepared plant samples and normal rat chow

After the one-week acclimatization period, the rats were re-weighed and fed with different quantities of commercial growers Vital Feed, and supplemented rates of inclusion samples of *Vernonia calvoana and Solanum gilo* leaves at different proportions for a period of twenty-eight (28) days. Different dose proportions of the leaves were supplemented into the feed as described by (12).

Group 1 previously not induced or administered with STZ were fed with commercial grower feed

(Vital Feed Brand) to represent the Normal Control Group. In the STZ administered group were fed with normal rat chow to represent the diabetic (Group 2) and standard (Group 3) control groups. In addition to normal rat chow Group 3 was administered with a calculated dose of Metformin 500 mg/kg body weight orally.

While the last four (4) of the STZ administered groups were fed with the prepared meal of plant plus rat chow as follows. Group 4 was fed with 2% powdered sample of *V. calvoana* (that is 2g in 100g of feed). Group 5 was fed with 2% powdered sample of *S. gilo* (that is 2g in 100g of feed). Group 6 was fed with a combination of 1g each of powdered samples (that is 2g in 100g) of both *V. calvoana and S. gilo* to represent low dose. Group 7 was fed with a combination of 4% powdered samples (that is 4g in 100g) of both *V. calvoana* and *S. gilo* to represent high dose. The rats were fasted 12 hrs before the time of sacrifice. The rats were anaesthetized using ketamine 90 mg/kg and sacrificed.

2.9. Processing of the liver for histopathological examination

This was prepared using the method described by (15).

Fixation: The tissue was fixed in 10 percent neutral buffered formalin.

Dehydration: There was complete removal of water molecules using ascending grade of alcohol (70%, 90%, absolute 1, 2, 3 and 4) for 30 minutes each.

Dealcoholization/Clearing using xylene (1, 2, 3): The tissue was dealcoholize using xylene for 20 minutes.

Impregnation/infiltration: The tissue was impregnated using molten paraffin wax for 20 minutes each – two changes.

Embedding the tissue: Paraffin wax was heated to molten form using an oven and Bunsen burner and kept on. The tissue was put into the molten paraffin wax. The embedding mold was used to scoop a quantity of molten paraffin wax and the tissue embedded in it, then covered with a tissue casset. The embedded tissue was placed in an ice-blocked bath. The removal of the embedded tissue from the block forms a tissue block. The tissue block was then taken and placed in a microtome for trimming. The Mayer's egg albumin was used on the slides to aid adhesion.

2.10. Histopathological study of the liver

Tissue sectioning: A microtome machine was used to cut or section the tissue into tiny sections of 4µ. Staining technique (haematoxylin and eosin dyes): Dewax: The slides were dewaxed in xylene for 30 minutes and hydrated using descending grade of alcohol (absolute, 90%, 70%) then water. The section was stained in heamatoxylin for 10 minutes. The section was rinsed in water. The section was differentiated in 1% acid alcohol briefly. They were rinsed in water. The slides were put in blue scott tap water substitute for 5 minutes. The slides were counter stained in Eosin for 30sec.They were rinsed in water. The slides were dehydrated in ascending grade of alcohol (70%, 90%, absolute). The slides are cleared in xylene and mounted using DPX mountant.

Microscopy: The micrographs were taken using a leica 750 DM high power microscope.

3. Result/Discussion

The normal control group 1 (with magnification X100 and X400), presented a normal histology of the liver. The section of the liver with a preserved architecture showed plate of hepatocytes radiating outward from the central vein area. The hepatocytes are swollen and have abundant granular cytoplasm and a prominent nucleus with coarse chromatin pattern. The separating sinusoidal spaces are dilated, congested and contain mild mononuclear cells. The portal areas show an intact limiting plate hepatocytes and contains mild infiltrate. There is portal inflammation as shown in plate 1 and 2 below.

Group 2, the diabetic control group (plate 3 and 4), showed section of the liver shows a preserved

architecture with plates of hepatocyte radiating outward from the central vein. The hepatocytes are moderately swollen and have abundant cytoplasm with round to oval nuclei. The cells are separated by dilated sinusoidal space containing mild inflammatory infiltrate. The portal areas show an intact limiting plate hepatocytes and contains mild infiltrate. Results suggested a cellular injury on the liver tissues.

Group 3, the metformin control group showed section of the liver with a preserved architecture shows plate of hepatocytes radiating outward from the central vein area. The hepatocytes are swollen and have abundant granular cytoplasm and a prominent nucleus with coarse chromatin pattern. The separating sinusoidal spaces are dilated, congested and contain mild mononuclear cells. The portal areas show an intact limiting plate hepatocytes and contains mild infiltrate. There is mild portal inflammation as shown in plate 5 and 6 below.

Group 4, the *Vc* group showed section of the liver with a preserved architecture shows plate of hepatocytes radiating outward from the central vein area. The hepatocytes are swollen and have abundant granular cytoplasm and a prominent nucleus with coarse chromatin pattern. The separating sinusoidal spaces are dilated, congested and contain mild mononuclear cells. The portal areas showed an intact limited plate hepatocytes and contains mild infiltrate. There is mild portal inflammation shown in plate 7 and 8 below.

Group 5, the *Sg* group showed section of the liver with a preserved architecture shows plate of hepatocytes radiating outward from the central vein area. The hepatocytes have abundant granular cytoplasm and a prominent nucleus with coarse chromatin pattern. The separating sinusoidal spaces are dilated, congested and contain mild mononuclear cells. The portal area contains the portal vein, hepatic artery and bile duct with an intact limiting plate hepatocyte. There is mild portal inflammation as shown in plate 9 and 10 below.

Group 6, the low dose (*Vc+Sg*) group showed section of the liver shows a preserved architecture with plates of hepatocyte radiating outward from the central vein. The hepatocytes have abundant cytoplasm with round to oval nuclei. The cells were separated by dilated sinusoidal space containing mild inflammatory infiltrate. The portal area is moderately expanded and contains sparse mononuclear inflammatory infiltrates as shown in plate 11 and 12.

Group 7, the high dose (*Vc+sg*) group showed section of the liver shows a preserved architecture with plates of hepatocyte radiating outward from the central vein. The hepatocytes have abundant cytoplasm with round to oval nuclei. The cells are separated by dilated sinusoidal space containing mild inflammatory infiltrate. The portal area contains hepatocyte, portal vein and hepatic artery with intact limiting hepatocytes. Mononuclear inflammatory infiltrate as seen plate 13 and 14 below.

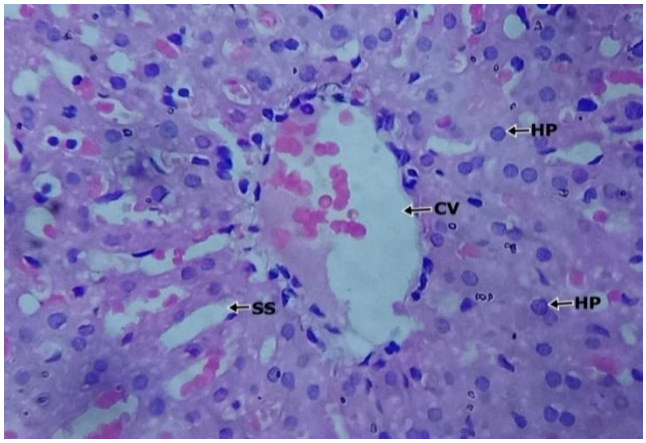

Plate 1: Photomicrograph of section of the liver of Normal control group fed with rat chow 100% + water *ad Libitum* stained with Hematoxylin & Eosin magnification X100.

KEY: HP – Hepatocyte, **CV** – Central Vein, **SS** – Sinusoidal Space

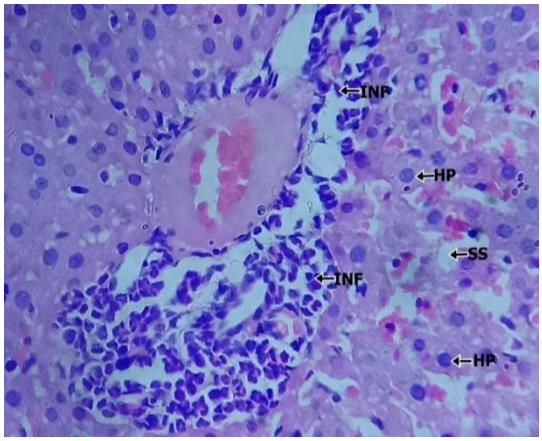

Plate 2: Photomicrograph of section of the liver of Normal control group fed with rat chow 100% + water *ad Libitum* stained with Hematoxylin & Eosin magnification X400.

KEY: INP – Interlobular bile duct, **INF –** Intercellular follicle, **HP –** Hepatocyte, **SS –** Sinusoidal Space

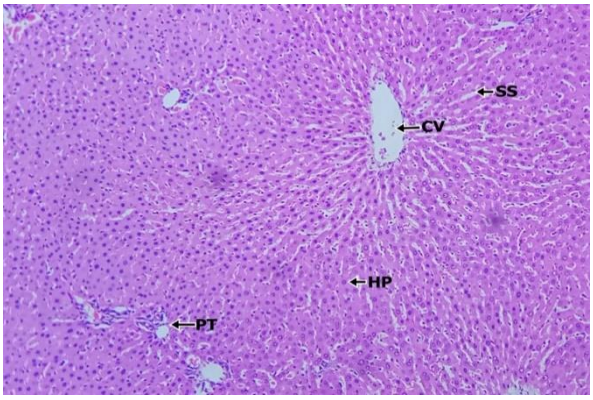

Plate 3: Photomicrograph of section of the live of DC group fed with rat chow 100% + water *ad Libitum* stained with Hematoxylin & Eosin magnification X100.

KEY: SS – Sinusoidal Space, **CV** – Central Vein, **HP** – Hepatocyte, **PT** – Portal Triad

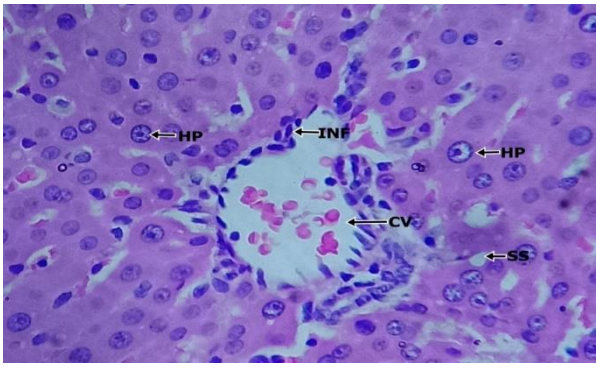

Plate 4: Photomicrograph of section of the liver of DC group fed with rat chow 100% + water *ad Libitum* stained with Hematoxylin & Eosin magnification X400

KEY: HP – Hepatocyte, **INF –** Intercellular follicle, **CV –** Central Vein, **SS –** Sinusoidal space

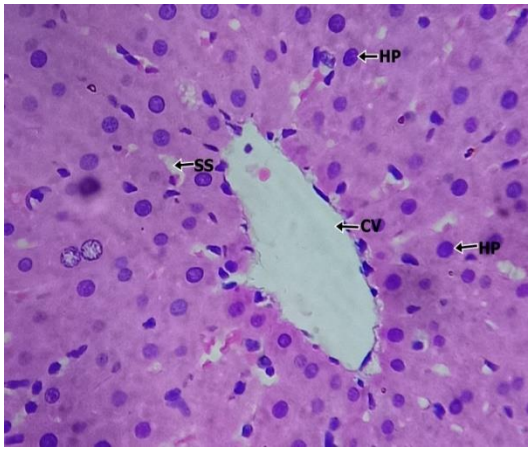

Plate 5: Photomicrograph of section of the liver of SC group treated with Metformin 500mg/body weight + rat chow stained with Hematoxylin & Eosin magnification X100.

KEY: HP – Hepatocyte, **SS –** Sinusoidal Space, **CV –** Central Vein

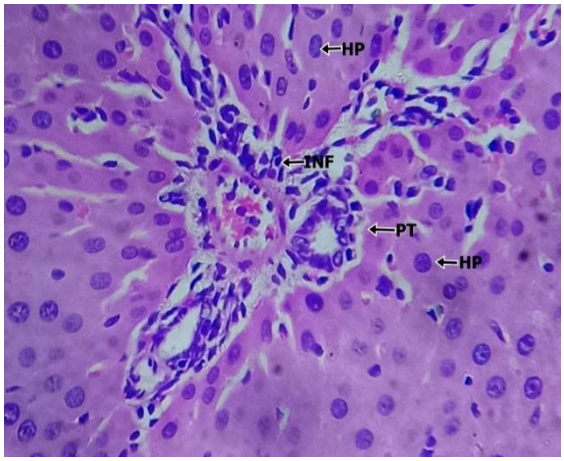

Plate 6: Photomicrograph of section of the liver of SC group treated with Metformin 500mg/body weight + rat chow stained With hematoxylin & Eosin magnification X400.

KEY: HP – Hepatocyte, **INF** – Intercellular follicle, **PT** – Portal Triad

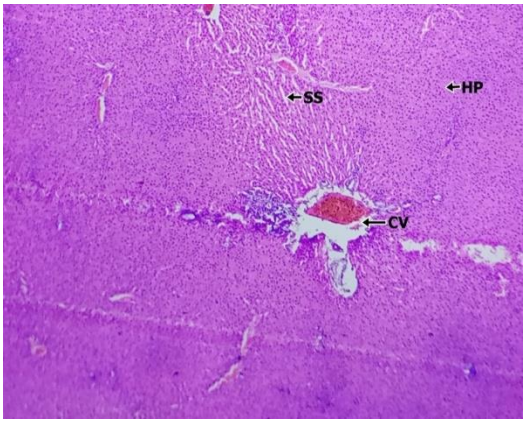

Plate 7: Photomicrograph of section of the liver of *Vc* group treated with 2% *Vernonia calvoana* stained with Hematoxylin & Eosin magnification X100.

KEY: SS – Sinusoidal Space, **HP** – Hepatocyte, **CV** – Central Vein

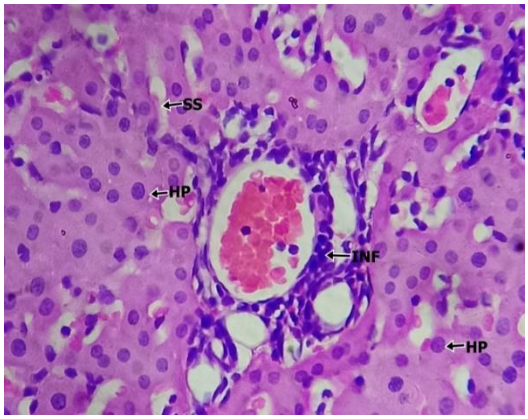

Plate 8: Photomicrograph of section of the liver of *Vc* group treated with 2% *Vernonia calvoana* Libitum stained with Hematoxylin & Eosin magnification X400.

KEY: SS – Sinusoidal Space, **HP** –, Hepatocyte **INF** – Intercellular follicle

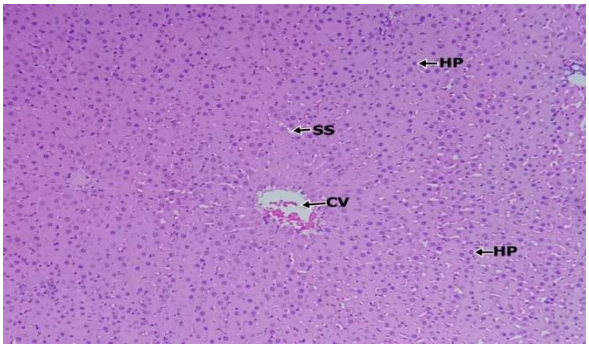

Plate 9: Photomicrograph of section of the liver of *Sg* group treated with2% *Solanum gilo* stained with Hematoxylin & Eosin magnification X100.

KEY: HP – Hepatocyte, **SS** – Sinusoidal Space, **CV** – Central Vein

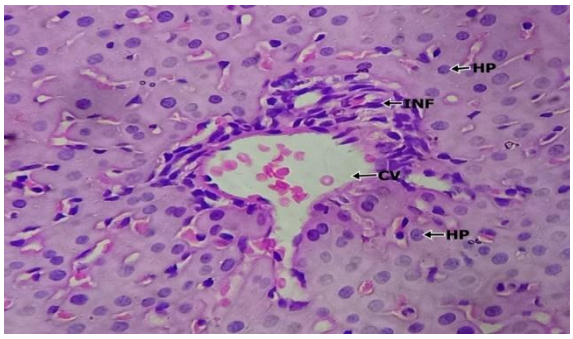

Plate 10: Photomicrograph of section of the liver of *Sg* group treated with 2% *Solanum gilo* stained with Hematoxylin & Eosin magnification X400.

KEY: HP – Hepatocyte**, INF** – Intercellular follicle, **CV** – Central Vein

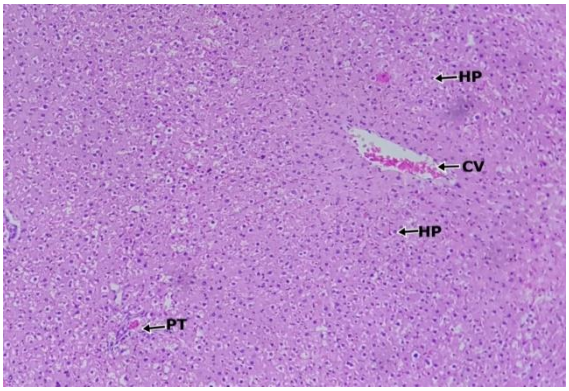

Plate 11: Photomicrograph of section of the liver of LD(*Vc*+*Sg*) group treated with low dose of 1% *Vernonia calvoana* + 1% *Solanum gilo* stained with Hematoxylin & Eosin magnification X100.

KEY: HP – Hepatocyte**, CV** – Central Vein, **PT** – Portal Triad

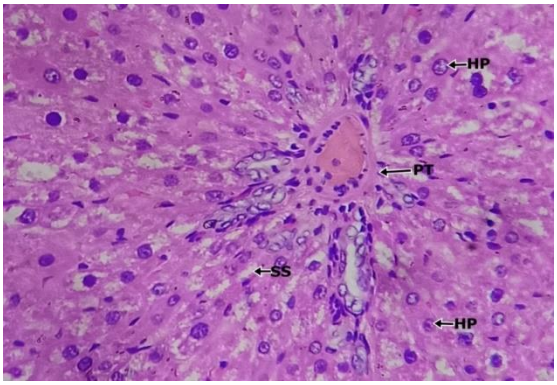

Plate 12: Photomicrograph of section of the liver of LD(*Vc*+*Sg*) group treated with low dose of 1% *Vernonia calvoana* + 1% *Solanum gilo* stained with Hematoxylin & Eosin magnification X400.

KEY: HP – Hepatocyte, **PT** – Portal Triad, **SS** – Sinusoidal Space

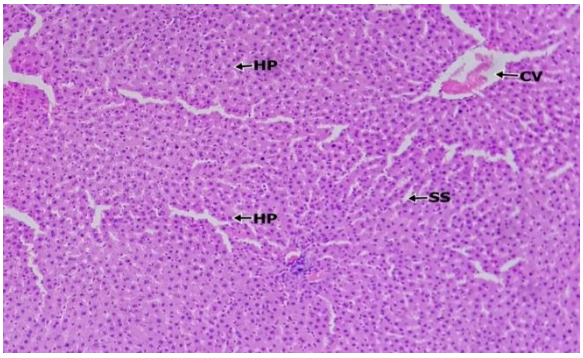

Plate 13: Photomicrograph of section of the HD(*Vc+Sg*) group treated with high dose of 2% *Vernonia calvoana* + 2% *Solanum gilo* stained with Hematoxylin & Eosin magnification X100.

KEY: HP – Hepatocyte, **CV** – Central Vein, **SS** – Sinusoidal Space

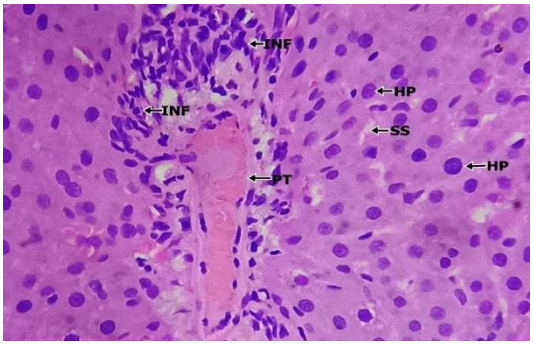

Plate 14: Photomicrograph of section of liver of the liver of HD(*Vc+Sg*) group treated with high dose of 2% *Vernonia calvoana* + 2% *Solanum gilo* stained with Hematoxylin & Eosin magnification X400

KEY: INF – Intercellular follicle, **HP –** Hepatocyte, **SS** – Sinusoidal Space, **PT** – Portal Triad

4. Conclusion

Vernonia calvoana(bitter leaf) and *Solanum gilo* (scarlet eggplant) have many bioactive constituents which are believed to have contributed to the positive effects on the histology of the liver affected by streptozotocin-induced Wistar rats complication. Therefore, *venonia calvoana and solannum gilo* at combined and separate forms were capable of improving liver structure and function in Wistar rats and could be used as a remedy for the management of liver tissues affected by diabetes.

Conflict of interest

There is no conflict of interest.

Acknowledgement

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Authors' declaration

The authors hereby declare that the work presented in this article are original and have not been published elsewhere. Hence, we take any liability for claims related to the content of this article.

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