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VERNONIA CALVOANA (BITTER LEAF) AND *SOLANUM GILO* (SCARLET EGGPLANT) LEAVES DIETARY SUPPLEMENTATION, IT EFFECTS ON OXIDATIVE STRESS MARKERS AND LIVER ENZYMES IN STREPTOZOTOCIN-INDUCED DIABETIC RATS

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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 glutathione concentration in combined group (low dose) compared to diabetic control. Catalase and malondialdehyde concentration reduced significantly ($P<0.05$) in combined group (low dose) while, superoxide dismutase concentrations were significantly reduced in combined group (high dose) compared to groups 4 and 5. Aspartate aminotransferase and alkaline phosphatase activities increased, while alanine aminotransferase activity reduced in all the treated groups significantly ($p<0.05$) in high dose compared to diabetic control Conclusively, dietary supplementation of *Vc* and *Sg* showed significant anti-oxidant effects on streptozotocin-induced diabetic Wistar rats.

Keywords: oxidative stress, diabetic, dietary supplementation, liver enzymes

1.0 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 nonenzymatic 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 done the rate of oxidation of macromolecules (1). 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 (3).

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 (5). 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 (6). This in turn promote 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 (7).

In the cells of the body the antioxidant enzymes consist of three well known classes of enzyme; catalases (CAT), superoxide dismutase (SOD), and glutathione peroxidases (GPX), which play important roles in maintaining homeostasis into cells response to oxidative stress. Reactive oxygen species (ROS), production in the body have been associated with the development of various disease conditions, including diabetes mellitus (DM) and associated complications (1).

Apart from its use as an edible vegetable, studies have reported that *V. calvoana* leaf is traditional used in local medicine as an anti-helmintic, anti-protozoal and anti-hyperglycemic agents according to (8). 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 (9).

Antioxidants such as the flavonoids present in medicinal plants such as the *Vernonia* species like *Vernonia. Calvoana*, 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 (6).

Solanum gilo (Scarlet eggplant) commonly call " Iri" in Gabu in Yala LGA of Cross River State, has be 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 this is according to the report of (10). While in scientific studies *S. gilo* has been shown to be effective in the management of hyperglycemia, obesity and have noticeable positive roles in improving liver function.

2.0 Materials 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 was 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 and *Solanum gilo* 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 (11).

2.6 Induction of diabetes

Diabetes mellitus was induced using the method by (12).

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 based on (11).

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 500mg/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 Collection of blood and preparation of sera samples

Blood was collected via cardiac puncture, allowed to clot and centrifuged for 15 minutes at 2000 rpm. The clear (supernatant) was then collected by aspiration into plain tube carefully corked and refrigerated for biochemical analysis.

2.10 Homogenization of liver tissues

Liver tissue was homogenized according to the method described by[7]. In brief, 0.2g of liver tissue was homogenized in 1000 µl of phosphate buffer of pH 7.4. The homogenate was emptied into a clean tube and allowed to stand for 10 minutes. Then, it was centrifuged for 10 minutes at 3000rpm, after which the supernatant was aspirated into a new clean tube and refrigerated in preparation for biochemical assays.

2.10.1 Determination of glutathione reductase (GSH) activity (mM/l) as described by (13)

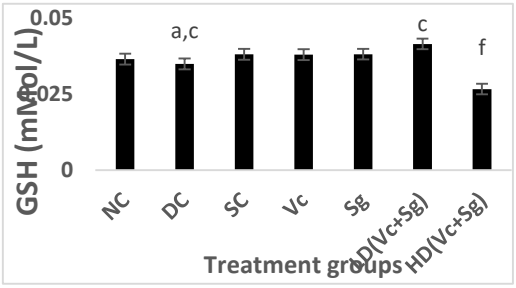

Fig 1: Effect of treatment on glutathione reductase

2.10.2 Determination of catalase (CAT) activity (µmole-protein) as described by (14)

Procedures:

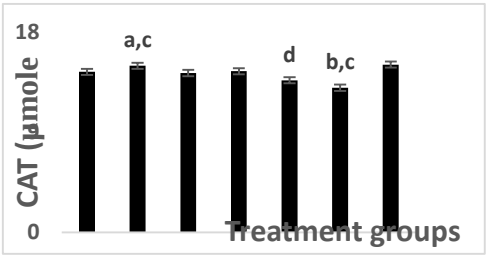

Fig 2:Effect of treatment (GSH) activity (mM/L). n=6,
on catalase (CAT) activity (µmole-protein).

2.10.3 Determination of malonaldehyde (MDA) activity (nmol MDA/mg protein) as described by (15)

2.10.4 Determination of superoxide dismutase (SOD) activity (U/ml) as described by (16)

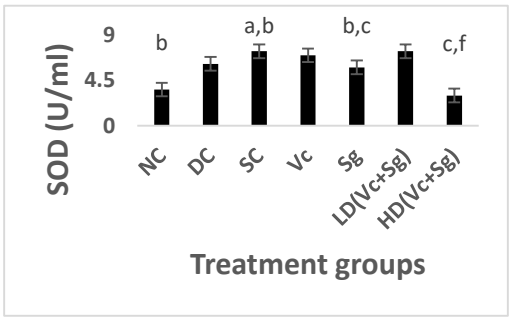

Fig 3: Effect of he treatment on SOD (umol/l)

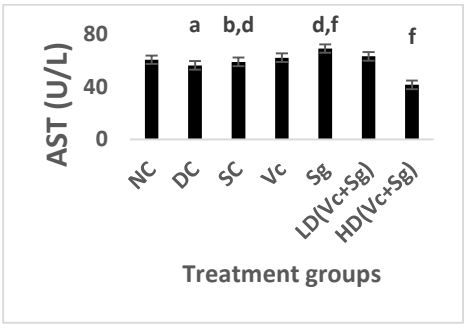

Fig. 4: Effect of treatment on malonaldehyde

2.10.5. Estimation of serum activity of aspartate aminotransferase (AST) (U/L) as described by[24]

2.10.6. Estimation of serum activity of alanine aminotransferase (ALT) (U/L) as described by (17)

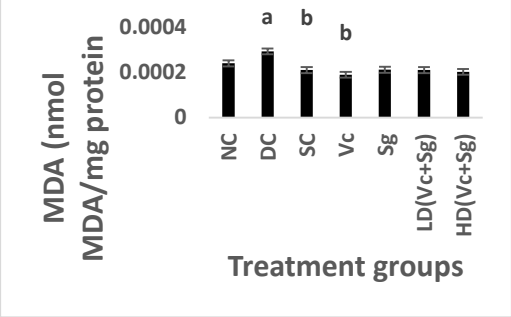

Fig. 5: Effect of treatment on serum activity of aspartate aminotransferase) activity of alanine aminotransferase (ALT).

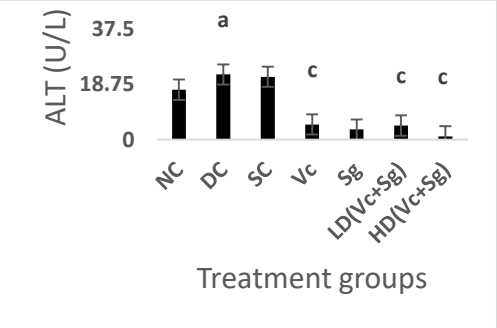

Fig 6: Effect of treatment on serum (MDA) activity (nmol MDA/mg protein) (U/L). (AST) (U/L

2.10.7 Estimation of serum activity of alkaline phosphatase (ALP) (U/L) as described by (18)

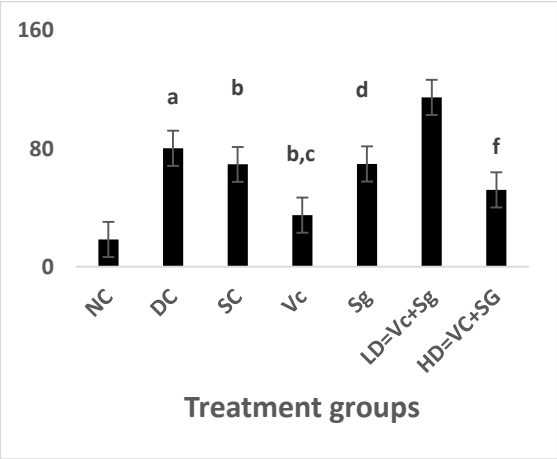

Fig 7: Effect of treatment on serum activity of alkaline phosphatase (ALP) (U/L).

NC= normal control, DC= diabetic control, SC= standard control, *Vc= Vernonia calvoana*, *Sg= Solanum gilo*. LD(*Vc+Sg*)= Low dose(*Vernonia calvoana+Solanum gilo*). HD(*Vc+Sg*)=High dose(*Vernonia calvoana+Solanum gilo*).

3.0 Statistical analysis

The raw data obtained were analyzed using statistical package for social science (SPSS version 20). One-way ANOVA and post-hoc comparison (Least square difference, LSD) were applied to determine any significant difference at $p<0.05$ (or otherwise) among the experimental groups.

4.0 Results and discussion

The presence on bioactive ingredients found in leaves of *Vernonia calvoana* and *Solanum gilo* has justified their uses in traditional medicine for the treatment of various clinical conditions such as inflammation, in the management of diabetes (19). Individually these plants have also been considered for their antioxidant properties. The study by (19) reported a reduction following an increase in oxidative stress parameters including malondialdehyde (MDA) while catalase (CAT) and GPX gave reversed results in streptozotocin-induced diabetic rats. In their study they concluded that *V. calvoana* possessed ameliorative effects that aided in mopping up free radicals preventing oxidative stress in the process.

Result obtained for glutathione (GSH) showed that induction of diabetes caused a significant ($p<0.05$) reduction in GSH concentration as indicated in diabetic control (DC) group compared to Normal control (NC) group (Fig 2). Following 28 days treatment, the result further indicated a reverse in the reduction in the low dose of *Vernonia calvoana* and *Solanum gilo* LD (*Vc* + *Sg*) group.

In the treated groups GSH concentration increased significantly in LD (*Vc+Sg*) group compared to DC and SC groups, respectively, followed by SC, *Vc* and Sg groups which did not indicated an effect among them though further increased compared to high dose, HD(*Vc+Sg*) group with the least effect compared to the treated and control groups, respectively. In this current study, results obtained showed that diabetic induction caused a reduction in glutathione (GSH) concentration. This was in line with previous reports that stated that diabetes

induces alteration in activity of the enzyme glutathione peroxidase as well as reductase found in the cell that metabolize peroxide to water and convert glutathione disulfide back into glutathione. The study further stated that an alteration in activities of the enzyme make the cells prone to oxidative stress resulting in cellular injury (20).

In addition, reports have shown that GSH plays a vital role in minimizing lipid peroxidation in cellular membranes as well as other known event that is reported to occur in oxidative stress (21). While other studies noted the ability of diabetes Miletus promote lipid profile disturbances in the cell, making the body more prone to lipid peroxidation. In this current study, treatment with both plants (*Vernonia calvoana* and *Solanum gilo*) were able to reversed the effect of diabetes recorded in DC group, but more significant in a combined form at low dose. This result is in agreement with reports from previous studies that indicated anti-oxidant (22) and anti-diabetic potency of the plants. Thus, in the prevention of cellular damage that leads to oxidative stress in experimental animals (10).

From the result obtained for the activities of catalase (CAT), induction of diabetes caused a significant ($p<0.05$) increase in DC group compared to NC group (Fig 3). This increase were reversed in the treated groups after 28 day of treatment except in the HD (*Vc +Sg*) treated group.

In the treated groups the result further showed that CAT concentration were decreased significantly in LD (*Vc+Sg*) group compared to DC and HD (*Vc+Sg*) groups respectively, followed by Sg group, while SC and *Vc* groups did not indicated an effect among them but reduced compared to DC and HD (*Vc+Sg*) groups respectively.

Unlike the result of GSH a combined treatment of the plant either as low or high gave a result that agreed with the report by (21) which suggested that for the body to function properly the activities of CAT should be maintained avoiding its excess production seen in HD (*Vc+Sg*) group that may cause serious damage to lipids, RAN and DNA while in the case of its deficiency or low activities seen in LD (*Vc+Sg*) leads to event where beta cell of pancreas undergoes oxidative stress by producing elevated levels of ROS resulting in beta cells dysfunction and diabetes.

Result of malonaldehyde (MDA) activities showed that induction of diabetes using streptozotocin STZ) significantly ($p<0.05$) increased its concentration as indicated in DC group compared to NC group (Fig 4). Though following 28 days of treatment with prepared meal of plant samples and standard drug a reverse in the result was obtained.

Furthermore, in the treated groups the result showed that MDA concentration was decreased significantly in *Vc* group compared to other treated groups with no significant ($p>0.05$) effect among them but also decreased compared to DC groups.

Significant ($p<0.05$) increase in superoxide dismutase (SOD) activities were obtained following streptozotocin administration as shown by the increase in DC group compared to NC group (Fig 5), which were reversed only the HD (*Vc+Sg*) group compared to the DC as well as other treated groups after 28 days of treatment with prepared meal of plant samples and standard, respectively.

Also, from the treated groups the result showed that SOD activities were decreased significantly only in the HD(*Vc+Sg*) group compared to DC and standard control (SC) control groups, followed by Sg group which reduced compared to SC significantly but slightly compared to DC groups.

Superoxide dismutase (SOD), according to the report by (21)0 acts as the first line of body's defense mechanism against cellular injury caused

by excess production of ROS through catalyzing the sharing of superoxide to other less toxic forms. While malonaldehyde (MDA) is one of the final products of polyunsaturated fatty acids peroxidation in cells have been reported to increase as a result of overproduction of free radicals to indicate cell membrane injury. In this current study both SOD (fig 5) and MDA (fig 4) were increased following administration of streptozotocin (STZ) to induce diabetes. But unlike SOD which only reversed in a combined treatment group low dose of *Vernonia calvoana* and *Solanum gilo* HD (*Vc+Sg*), MDA level were reduced in all the treated groups significantly in *Vc* group compared to the untreated diabetic group (DC group).

A significant ($p<0.05$) decrease in aspartate aminotransferase (AST) activity was observed following diabetic induction with STZ as indicated in DC group compared to NC group. But, following treatment for 28 days the result further showed a reversed AST activity in Sg group compared to the DC as well as other treated groups

Furthermore, in the treated groups the result showed an increase in AST activity compared to DC group except in the HD (*Vc+Sg*) group which further reduced compared to DC as well as other treated groups. The increase in *Sg* group were followed by LD(*Vc+Sg*) and *Vc* groups that increased compared to Sc control group which also showed an increased activity compared to DC and HD(*Vc+Sg*) groups, respectively as in figure 6 below.

Result obtained for alanine aminotransferase (ALT) activity showed that STZ administration caused a significant ($p<0.05$) increase indicated in DC group compared to NC group. The result further showed that following 28 days of treatment a reduction in ALT activity in all the treated groups except in the SC group. While, in the treated groups the result showed that ALT activity were significantly ($P<0.05$) reduced compared to SC group which did not show any effect compared to DC group figure 7 below.

Alkaline phosphatase (ALP) activity result showed a significant ($p<0.05$) increase following diabetic induction with STZ as indicated in DC group compared to NC group. But, following treatment for 28 days the result further showed a reversal in AST activity in all the treated groups except in LD (*Vc+Sg*) which further increased significantly compared to DC and SC control as in figure 8 below.

Furthermore, in the treated groups the result showed a more significant ($p<0.05$) reduction in *Vc* group followed by HD (*Vc+Sg*) group which reduced compared to Sg group and SC control group. The SC and *Sg* groups did not show any change between them but were reduced compared to DC control and LD (*Vc+Sg*) groups, respectively.

Liver function tests carried out in this study to examine the structure and function of the liver following diabetic induction indicated a rise in the activities of aspartate aminotransferase (AST), alkaline phosphatase (ALP), alanine aminotransferase (ALT) in serum. Studies have shown that these enzymes increase in blood following damage which occurs as result of exposure to toxic agents (23), chemicals or drugs as well as clinical event that result in cellular damage such as cirrhosis and oxidative damage. Studies have shown that clinical events like oxidative stress do not only damage the liver structure but induce hepatic damage by causing irretrievable alteration of lipid, protein and DNA contents (24).

On the other hand, the result indicated a reduction in AST activity following administration of STZ. Low activity of AST is reported to normally occur in blood, when body tissues or an organ such as the heart or liver is diseased or damaged and the activities of AST recorded in blood (or serum) is directly related to the extent of the damage to the tissue or organ.

In this study AST activities were significantly increased in the group treated with *Solanum gilo* (*S.g*) group followed by the group treated with a combined meal of both plants at low dose LD(*Vc+Sg*)), then followed by *Vernonia calvoana* (*V.c*) and the standard control group. On the other hand, ALP activities were significantly decreased in Vc group followed by high dose of combined meal of both plant HD (*Vc+Sg*) while *Sg* group though showed a decreased activity compared to the SC group. In ALT, both plants showed significant activities but more in a combined form at high dose HD (*Vc+Sg*) followed by *Sg* group. These results showed that individually the plants were potent agents with the ability to improve liver function in diabetic Wistar rats as well as in a combined form at deferent doses. This report is in agreement with the study by (9) which reported a decrease in AST activity, with an increase in ALP and ALT activities in Wistar rats treated with dietary meal of *Vernonia calvoana* leaves. Similarly, (9) reported the ability of *Solanum* species to improve liver function in hyperglycemic rats.

This result, maybe attributed to the presence of phytochemical components including saponin and flavonoids present in the plants (8, 25, 26) which have also been reported to possess antioxidant properties, and prevent oxidative stress that occur in diabetes resulting in liver injury according to the study by (27).

Furthermore, on *Solanum gilo*, though not much information exists on its potency in the management of different clinical conditions. A report by (28) showed that the plant contained phytochemicals that are potent hypoglycemic agent as well as its ability to induce weight reduction in obese Wistar rats and they concluded that the report suggested that these effects were as a result of phytochemical, rich in the plant. These reports are in agreement with the result obtained in this current study, in which unlike the previous studies considered the effect of a combined meal of the different plants on the studied parameters, in streptozotocin (STZ) induced diabetic Wistar rats.

5.0 Conclusion

Vernonia calvoana and *Solanum gilo* have many bioactive constituents which are believed to have contributed to the positive effects on the liver enzymes, and the histology of the liver affected by streptozotocin-induced Wistar rats complication. The of *venonia calvoana and solannum gilo* at combined and separate forms were capable of improving liver structure and function, as well as anti-oxidant effect on diabetic Wistar rats and could be used as a remedy for the management of diabetes.

Conflict of interest: There is no conflict of interest.

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Authors' Declaration: The authors' hereby declare that the work presented in this article is 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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