Effect of Hibiscus sabdariffa L., extract spiced with Piper nigrum L. and Zingiber officinale on lipid profile, liver function and oxidative stress indicators of alloxan-induced diabetic rats

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ABSTRACT

***Background:** Extensive studies have previously been carried out on the anti-diabetic activities of Hibiscus Sabdariffa extract (Zobo), However, little is known about its effect on diabetes when spiced with Zingiber officinale (ginger) and Piper nigrum (black pepper), as is the practice in traditional remedy.*

***Objective**: This study evaluated the effect of a combined aqueous extract of Zobo with ginger and black pepper on liver function indicators and lipid profile of alloxan-induced diabetic rats.*

***Methods:** Thirty (30) male Wistar rats (130 - 138 g) comprised of 24 diabetic and 6 non-diabetic, were divided into five groups of six each and treated thus: Groups 1 and 2 – normal and diabetic controls received distilled water, Groups 3 received 500 mg/kg of metformin and groups 4 and 5 received 250 mg and 500 mg/kg each of Zobo drink combined with ginger and black pepper. The treatment lasted for 21 days during which blood glucose and body weight changes were measured. At the end of treatment whole blood was collected, serum prepared and used for assay of lipid profile, liver enzymes and liver oxidative stress indicators.*

***Results:** The results obtained showed that the combined extracts lowered blood sugar levels progressively, over the treatment period and also maintained body weights of the rats. Serum cholesterol and triglyceride levels were found to decrease with a corresponding increase in High Density Lipoprotein (HDL) levels ($p < 0.05$). The liver enzyme (the aminotransferases) activities were both within the normal range of 40 U/L. Moreover, measured indicators of oxidative stress, malondialdehyde (MDA) and activities of superoxide dismutase (SOD) and catalase (CAT) were positively modulated.*

***Conclusions**: Taken together, data obtained from the study suggest that Zobo drink, commonly consumed in Nigeria, when spiced with ginger and black pepper, can attenuate the vascular system positively in a diabetic condition, including protecting the liver from injury, hence a promising functional food for diabetics.*

Keywords: Diabetes mellitus, *Hibiscus sabdariffa*, oxidative stress.

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INTRODUCTION

Diabetes, a group of metabolic disorders characterized by hyperglycemia, is a major global public health problem with an escalating incidence and prevalence, particularly in developing and newly industrialized countries (1). Diabetes results from defects in insulin secretion

or action, or both, and can cause serious health complications such as retinopathy, neuropathy, nephropathy, foot ulceration and heart disease amongst others (2)

Type I diabetes, is characterized by inability of the pancreas to produce insulin. Approximately 10% of all cases of diabetes that are diagnosed are Type I (3).On the other hand, Type II diabetes occurs when the body does not produce enough insulin or there is impaired insulin utilization in the body. Approximately 90% of all cases of diabetes worldwide are Type II. (3). The management of diabetes currently involves a combined use of medications, dietary control and exercise for an overall healthier lifestyle, as most of the oral anti-diabetic drugs (e.g. metformin) have severe side effects such as potential liver toxicity and skin rash. (4, 5). Therefore, in recent years, there has been a keen interest in searching for agents that can be used to manage diabetes especially traditional medicinal plants, because they strengthen the immune system, stabilize metabolism and contain many phytochemicals that act on different targets by various mechanisms (6). Spices especially, are becoming more popular because of their little to no side-effects and synergistic actions (4).

Zingiber officinale roscoe, commonly called ginger is a spice that has been used for a very long time for the treatment of respiratory diseases, rheumatism, heart diseases, and metabolic diseases such as diabetes, given its very low glycemic index. Ginger also has hypoglycemic potential and can reduce diabetic complications and pharmacological effects on the gastrointestinal tract, cardiovascular system, and blood pressure, blood clotting and antimicrobial effects (7).

Piper nigrum L. commonly called black pepper is a spice shown to contain an alkaloid called piperine that increases insulin sensitivity, hence a potential anti-diabetic agent. (8). *Hibiscus sabdariffa L.* locally known as zobo leaf is a health drink reported to contain phenolic compounds especially anthocyanins, complex compounds that have a high antioxidant function and protect the liver from damage (9). In addition, anthocyanins can lower blood sugar level of Alloxan-induced diabetic rats, thus increasing the activity of glutathione and the enzyme Roselle catalase. (10).

Globally, the rate of mortality and morbidity associated with diabetes is at an all-time high in developed countries. However, the developing world countries of Asia and Africa have the highest prevalence of diabetes and are predicted to remain so until 2030. (12, 2).

Dyslipidemia is common in people with diabetes, and is characterized by high total cholesterol, high triglycerides, low High Density Lipoprotein (HDL) and increased levels of Low Density Lipoprotein (LDL) (13). Diabetes has been linked with dyslipidemia and increase in some liver enzymes, the most common of which is Alanine Transaminases (14). Diabetes is an independent risk factor for the development of Coronary Heart Disease, atherosclerosis and myocardial infarction (15). The prevalence of Non-Alcohol Fatty Liver Disease (NAFLD) among people living with type II diabetes in Nigeria ranges from 21%-78%. In Type II diabetes, NAFLD follows a more aggressive clinical route with necro-inflammation and fibrosis, which could later progress to end-stage chronic liver disease (16). The management of liver disease linked with diabetes is a global problem and successful diagnosis and treatment is not yet widely available (17). Some of the drugs such as metformin, used in the management of diabetes, have adverse effects which include liver toxicity and skin rash (4), hence the need for alternative herbal treatments.

Across the world, there has been a dramatic increase in the use of traditional and alternative medicines in treating various diseases because of their *naturality*, effectiveness, availability, and affordability (18). Although the anti-diabetic properties of many herbs and spices have been investigated, little work has been known about combined effect of *Zobo* leaf drink, with local spices including ginger and black pepper as the practice is in Nigerian. Hence the current study, investigated the effect of zobo spiced with Ginger and black pepper spiced in diabetic rats.

2 MATERIALS AND METHODS

2.1 Preparation of extracts: *Hibiscus sabdariffa L. leaves, Piper nigrum L. fruits,* and *Zingiber officinale rhizomes* were purchased from Ilishan

market in Ogun state, Nigeria. Extracts of these plants were prepared according to standard guidelines and protocols. The plant samples were washed, first with running tap water and then with distilled water (at three rounds) after which they were dried under low heat (40 degree centigrade). 1000g freshly dried leaves were then ground to powder and dissolved in 1000mL of 100% methanol (CH_3OH) overnight. The extract was filtered and the filtrate was transferred to clean vessels and evaporated to dryness, the extracts of 32mg and 80mg were dissolved in 147ml of distilled water.

2.2 Chemical and reagents: The methanol used in this study was a product of Merck, Germany. Randox Liquizyme assay kits (AST, ALT, ALP,) were used to determine the biochemical parameters. Other chemicals and reagents used were of analytical grade and were also obtained from Sigma-Adrich Co., St Louis, USA.

2.3 Experimental animals: The experiment was carried out in the Babcock University Experimental Animal Facility (BUEAF), Ilishan-Remo, Ogun State, Nigeria. The experimental subjects were thirty sub-adult (8-10 weeks old) male Wistar rats of average body weight (130g to 138g). Ethical clearance for the study was obtained from the Babcock University Research, Health, and Ethical Committee (BURHEC), and the study was conducted in line with recommended national and institutional guidelines for the care and use of animals in scientific studies (19).

2.4 Experimental design: Diabetes was induced in the animals using Alloxan and metformin as the standard anti-diabetic drug. At outset, the rats were fasted for 12 hours thereafter 65mg/kg body weight of alloxan in 0.1M cold citrate buffer, PH 4.5 was injected intra-peritoneal. Blood was collected from the tail vein after 72 hours and glucose levels were determined using glucometer test kit (ACCU-CHECK, BG-CHECK). Animals with blood glucose ≥ 200 mg/dl were considered diabetic (20). The rats were treated with the extract by oral gastric intubation once every 24 hours as follows. Doses of 250 mg/kg and 500mg/kg were selected for treatment. The experimental rats were divided into five groups of 6 animals each and assigned different treatments that lasted for 21 days. Group 1 animals (Normal control) were orally administered with normal saline; Group II animals (negative control) were induced with alloxan and received water only. In Group III (CT 250) animals were induced with diabetes and treated with *Zobo* and spice (ginger and black pepper) mixture extract at 250mg/kg body weight/day. Group IV animals (CT 500) were also induced and received the combined plant extracts of zobo and spice (ginger and black pepper) at 500 mg/kg body weight/day. Lastly group V (Standard control) received the standard treatment drug (metformin) 500 mg/kg after being induced with alloxan. Body weights and blood glucose were measured intermittently during and at the end of study.

2.5 Blood collection: On the twenty first day, animals were sacrificed and blood samples collected via ocular puncture, into heparinized bottles for biochemical analysis.

2.6 Analysis of plasma lipid profile: Plasma lipid profile including total cholesterol, triglycerides and high-density lipoprotein (HDL)-cholesterol were assayed by enzymatic colorimetric methods using commercially available kits (Randox assay kit) following the manufacturer's directions. VLDL-cholesterol was estimated as TG/5, and LDL-cholesterol as LDL (mg/dl) = TC − (HDL+VLDL) LDL (mg/dl) = TC − (HDL+VLDL). (21).

2.7 Biochemical Parameters: Estimation of Alanine Aminotransferase (ALT), Aspartate aminotransferase (AST) was carried out using standard UV methods (21). Standard methods were used in the determination of Catalase (CAT), and Superoxide dismutase (SOD) activities (22) while Malondialdehyde was determined using the thiobarbituric acid reactive substances (TBARS) procedure (23).

2.8 Statistical Analysis: Data was analysed using GraphPad Prism version 8.00 (GraphPad

Software, San Diego, California). Results are expressed as mean ± SEM. The difference between the means was analyzed statistically using one-way analysis of variance (ANOVA; 95% confidence interval).

3. RESULTS

3.1 Effect of combined extracts on blood glucose levels: The treatment of diabetic rats with combined leaf extract of *H. Sabdariffa, P. Nigrum L.* and *Z. officinale* as well as standard drug caused a significant p-value ($p < 0.05$) decrease in the elevated fasting blood glucose concentration in comparison with diabetic untreated rats which showed a progressive increase in fasting blood sugar (Figure 1).

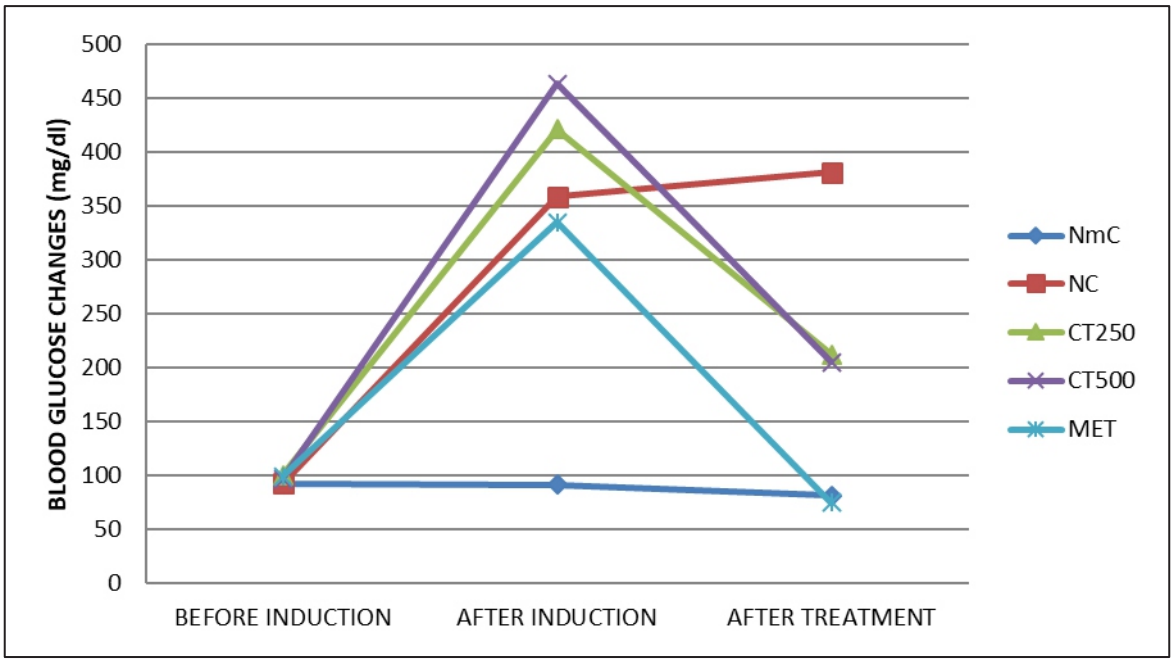

Figure 1: Blood glucose level of the normal control group, negative control group and chemo therapeutic groups during the oral glucose tolerance test. Results are presented as Mean ± SEM.

NmC: Normal (normal glycemic) control group
NC: negative control group
CT200: chemotherapeutic diabetic group treated with 200mg/kg body weight of extract
CT500: chemotherapeutic diabetic group treated with 500mg/kg body weight of extract
SC: standard control (metformin) group

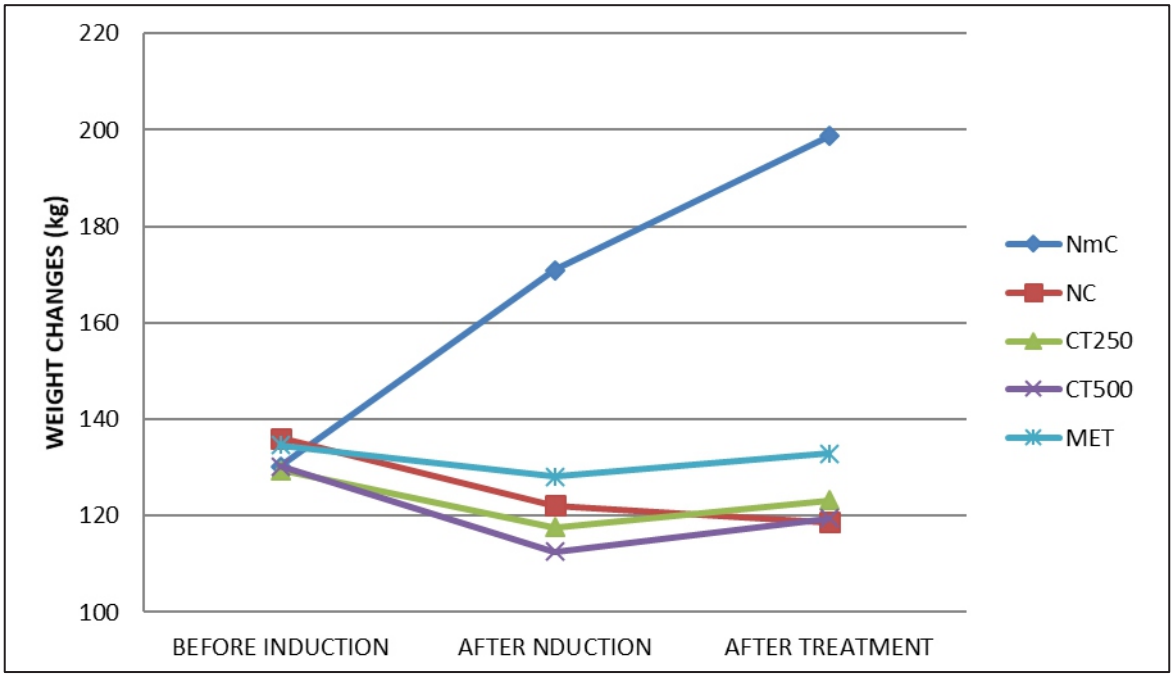

Figure 2: Weight gain levels of the normal control group, negative control group and chemo therapeutic groups. Results are presented as Mean ± SEM.
NmC: Normal (normal glycemic) control group
NC: Negative (diabetic) control group
CT250: chemotherapeutic diabetic group treated with 250mg/kg body weight of extract
CT500: chemotherapeutic diabetic group treated with 500mg/kg body weight of extract
SC: Standard control group treated with metformin

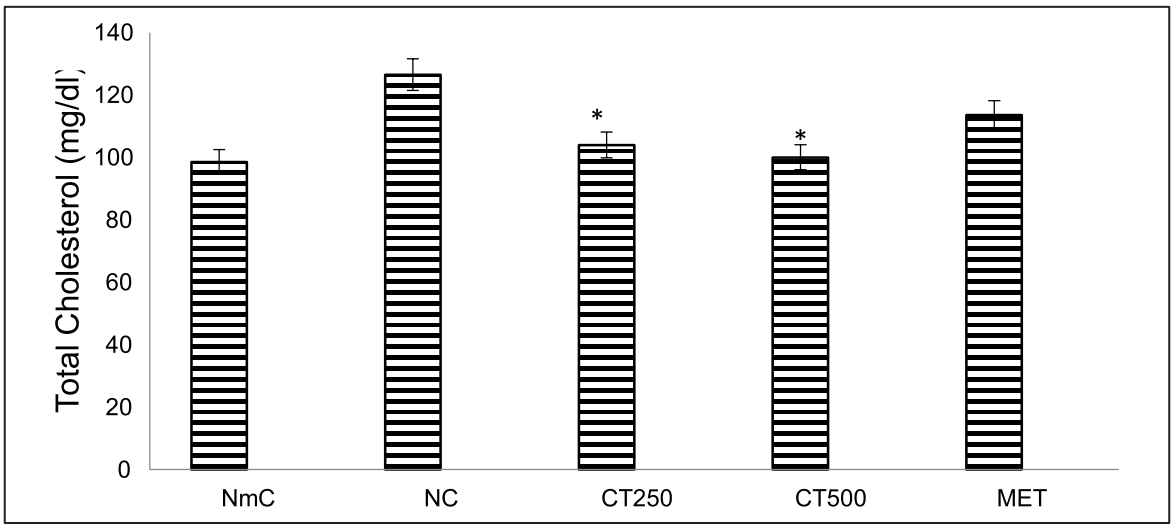

FIGURE 3: plasma total cholesterol levels of the normal control group, negative control group and chemo therapeutic groups. Results are presented as Mean ± SEM.
NmC: Normal (normal glycemic) control group
NC: Negative (diabetic) control group
CT250: chemotherapeutic diabetic group treated with 250mg/kg body weight of extract
CT500: chemotherapeutic diabetic group treated with 500mg/kg body weight of extract
SC: Standard control group treated with metformin

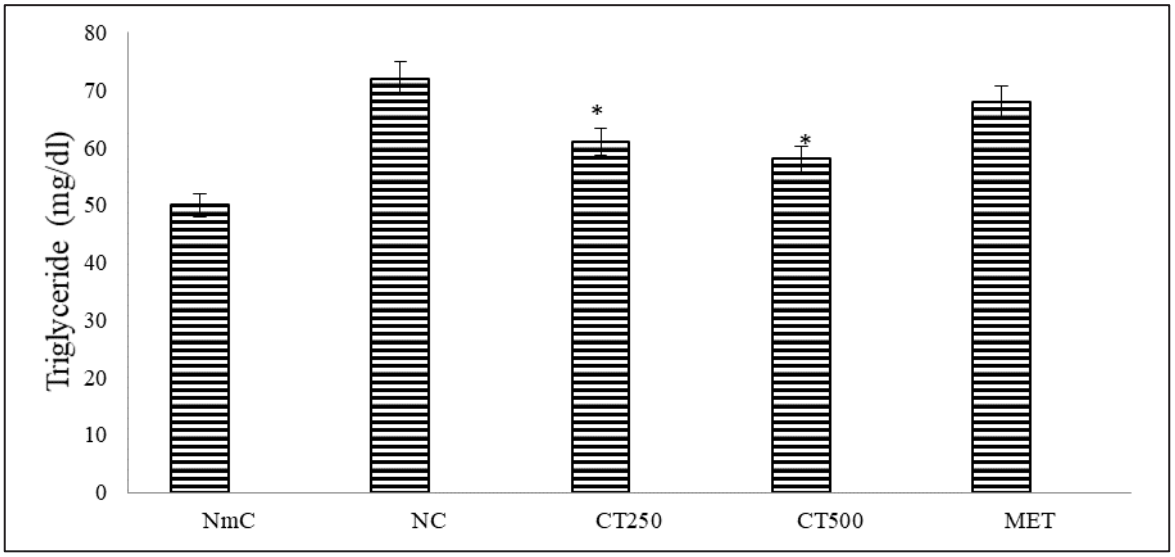

FIGURE 4: Plasma triglycerides levels of the normal control group, negative control group and chemo therapeutic groups. Results are presented as Mean ± SEM.
NmC: Normal (normal glycemic) control group
NC: Negative (diabetic) control group
CT250: chemotherapeutic diabetic group treated with 250mg/kg body weight of extract
CT500: chemotherapeutic diabetic group treated with 500mg/kg body weight of extract
SC: Standard control group treated with metformin

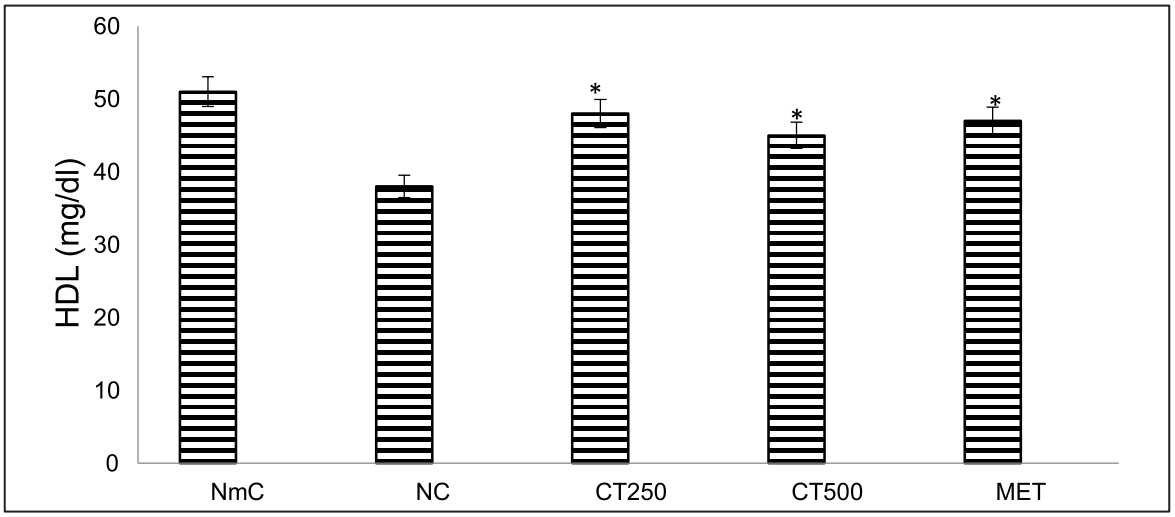

FIGURE 5: plasma HDL levels of the normal control group, negative control group and chemo therapeutic groups. Results are presented as Mean ± SEM.
NmC: Normal (normal glycemic) control group
NC: Negative (diabetic) control group
CT250: chemotherapeutic diabetic group treated with 250mg/kg body weight of extract
CT500: chemotherapeutic diabetic group treated with 500mg/kg body weight of extract
SC: Standard control group treated with metformin

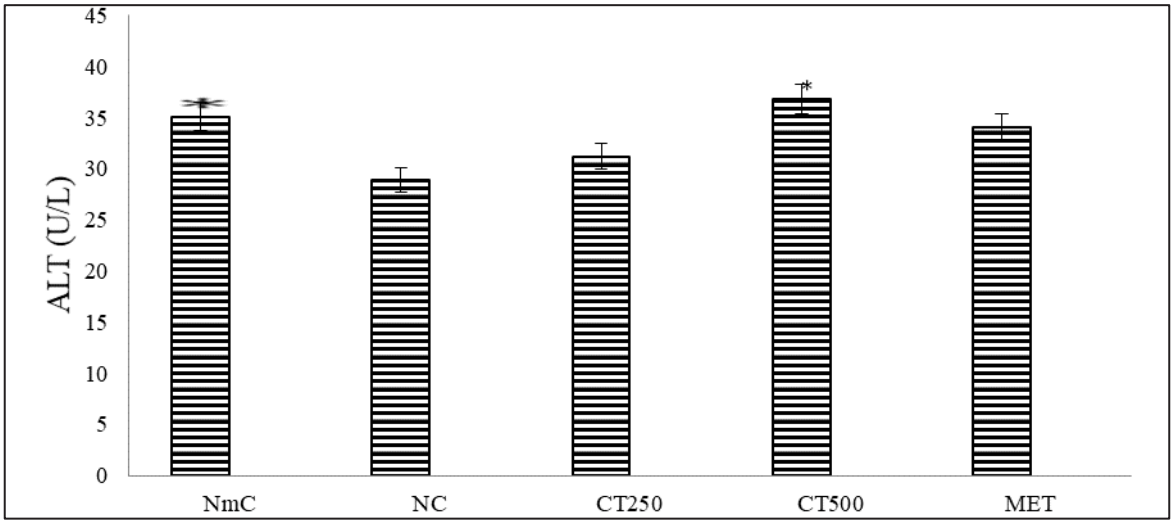

FIGURE 6: plasma ALT levels of the normal control group, negative control group and chemo therapeutic groups. Results are presented as Mean ± SEM.

NmC: Normal (normal glycemic) control group
NC: Negative (diabetic) control group
CT250: chemotherapeutic diabetic group treated with 250mg/kg body weight of extract
CT500: chemotherapeutic diabetic group treated with 500mg/kg body weight of extract
SC: Standard control group treated with metformin

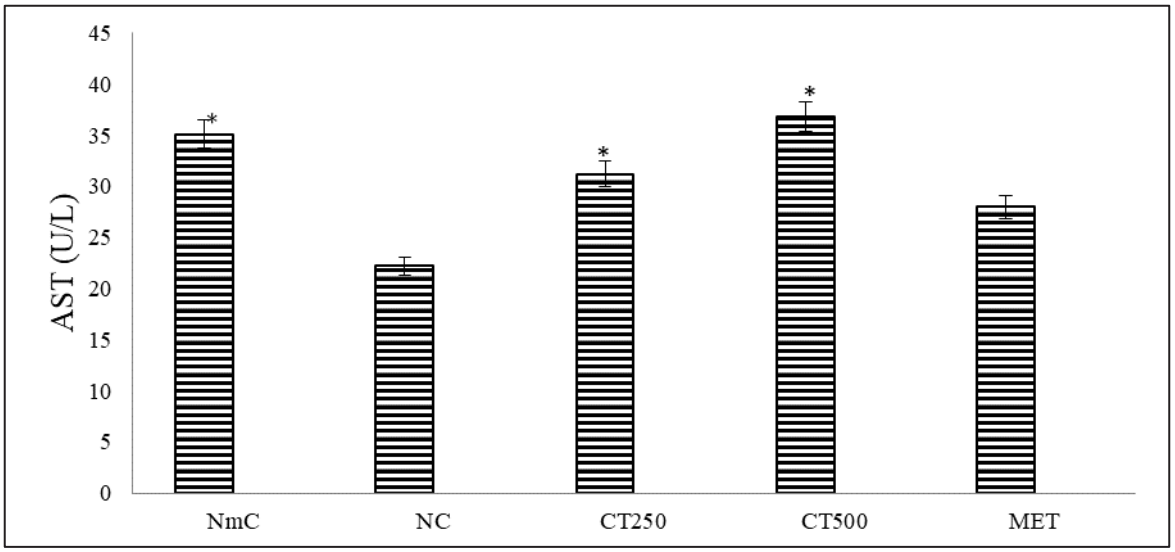

FIGURE 7: plasma AST levels of the normal control group, negative control group and chemo therapeutic groups. Results are presented as Mean ± SEM.

NmC: Normal (normal glycemic) control group
NC: Negative (diabetic) control group
CT250: chemotherapeutic diabetic group treated with 250mg/kg body weight of extract
CT500: chemotherapeutic diabetic group treated with 500mg/kg body weight of extract
SC: Standard control group treated with metformin.

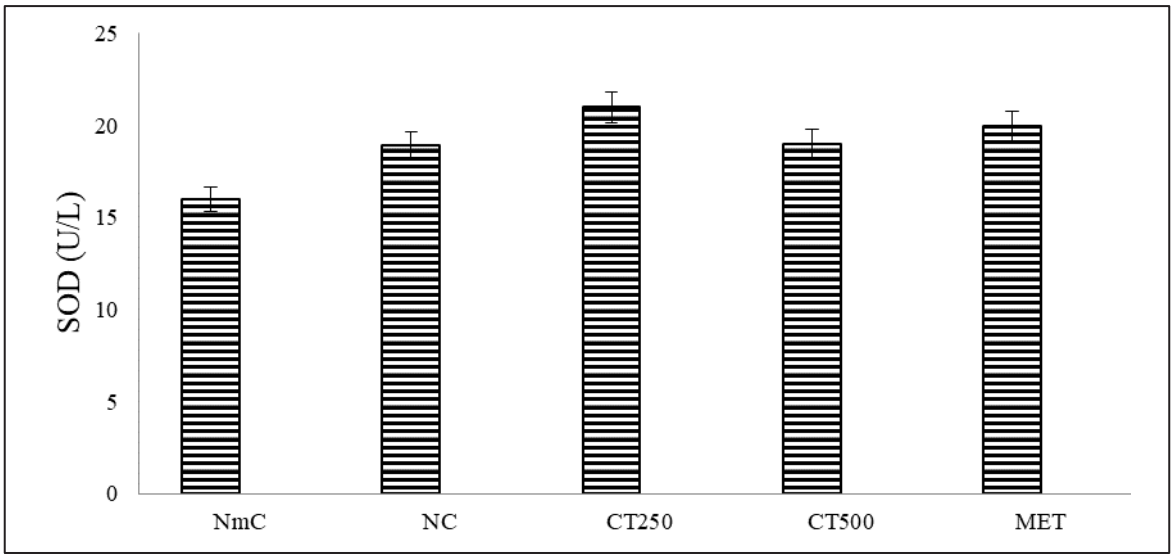

FIGURE 8: SOD levels of the normal control group, negative control group and chemo therapeutic groups. Results are presented as Mean ± SEM.

NmC: Normal (normal glycemic) control group
NC: Negative (diabetic) control group
CT250: Test diabetic group treated with 250mg/kg body weight of extract
CT500: Test diabetic group treated with 500mg/kg body weight of extract
SC: Standard control group treated with metformin.

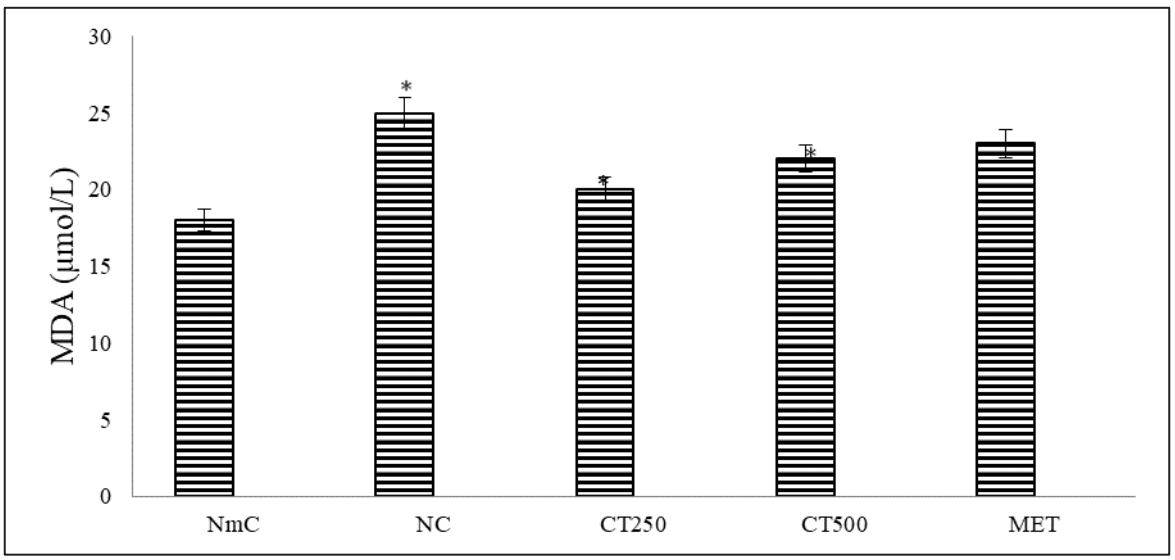

FIGURE 9: MDA levels of the normal control group, negative control group and chemo therapeutic groups. Results are presented as Mean ± SEM.

NmC: Normal (normal glycemic) control group
NC: Negative (diabetic) control group
CT250: chemotherapeutic diabetic group treated with 250mg/kg body weight of extract
CT500: chemotherapeutic diabetic group treated with 500mg/kg body weight of extract
SC: Standard control group treated with metformin.

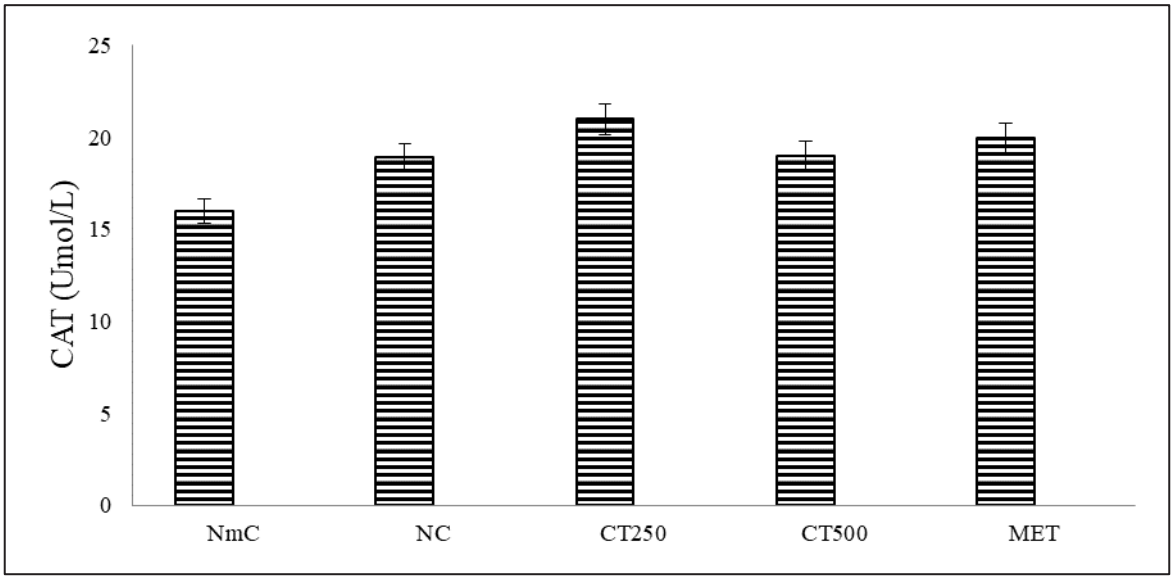

FIGURE 10: CAT levels of the normal control group, negative control group and chemo therapeutic groups. Results are presented as Mean ± SEM.

NmC: Normal (normal glycemic) control group
NC: Negative (diabetic) control group
CT250: Test diabetic group treated with 250mg/kg body weight of extract
CT500: Test diabetic group treated with 500mg/kg body weight of extract
SC: Standard control group treated with metformin

DISCUSSION

This study was aimed to evaluate the effects of Hibiscus esculentus extract on glucose and lipid profile of diabetic
rats. By using alloxan monohydrate with a dose of 120 mg/kg in rats, we were able to make diabetes in rats. Alloxan causes the increase of plasma glucose with the destruction of the beta cells of the Langerhans islets. This finding is attuned with the findings of previous published ones (11,12) This study was aimed to evaluate the effects of Hibiscus esculentus extract on glucose and lipid profile of diabetic rats. By using alloxan monohydrate with a dose of 120 mg/kg in rats, we were able to make diabetes in rats. Alloxan causes the increase of plasma glucose with the destruction of the beta cells of the Langerhans islets. This finding is attuned with the findings of previous published ones (11,12) This study was aimed to evaluate the effects of Hibiscus esculentus extract on glucose and lipid profile of diabetic rats. By using alloxan monohydrate with a dose of 120 mg/kg in rats, we were able to make diabetes in rats. Alloxan causes the increase of plasma glucose with the destruction of the beta cells of the Langerhans islets. This finding is attuned with the findings of previous published ones (11,12)

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This study investigated the effect of combined extracts of *H. Sabdariffa, P. Nigrum L.* and *Z. officinale* on diabetic rats**. A**lloxan was used to induce diabetes - increased plasma glucose level as a result of the destructive action it exerts on the beta cells of islet of langerhans (24, 25). Asides beta cells destruction, alloxan also increases the free oxygen radical, thereby leading to oxidative stress (26). Therefore in this research, the plasma glucose was evaluated; the results showed significant increase of blood glucose of diabetic control rats, compared to normal control, along with other symptoms including weight loss. This is in agreement with (27). Interestingly, after treatment, it was observed that there was a decline in the plasma levels of glucose of animals that received the combined extract and metformin. The implication of this finding is that our combined extracts remedied the glucose level imbalance observed in diabetic rats possibly through the repair of the damage caused to the pancreatic beta cells (27). Following the induction of diabetes with alloxan, there was a significant decrease in the weight of the animals, even though they were still consuming the same relative amount of food and water. This showed that weight loss is an accurate symptom of diabetes and this finding corroborates with (28), whose research showed a decrease in body weight of alloxan induced diabetic rats. There was however, a noticeable increase in the weight of the rats upon commencement of the treatment compared to the negative non-diabetic group (NC). This shows that the extract of *H. Sabdariffa, P. Nigrum L.* and *Z. officinale* helps to control body weight.

In recent years, there has been a growing interest in the possible role of nutrition in the management of diseases, as natural ingredients like herbs and spices have been observed to exert little or no side effects on the human systems.. Type 2 diabetes (T2DM), which is characterized by the inefficiency or insufficiency of insulin produced, has been said to be associated with a clinical spectrum of liver abnormalities known as Non-Alcoholic Fatty Liver Disease (NAFLD). (16). In this study, Plasma triglyceride and total cholesterol levels were significantly reduced in the chemotherapeutic groups treated with 250mg/kg body weight and 500mg/kg body weight doses of extract respectively, compared to the diabetic untreated (NC) group. The result also showed that the standard treatment group also had lower values of plasma total cholesterol and triglycerides compared to the negative control group though the difference was not significant. It has been reported that increase in the level of blood glucose correlates with increase in lipid levels and plasma lipoprotein (27) therefore the high levels of plasma TG and Total cholesterol in diabetic untreated rats are expected occurrences and the decreased level of these lipids in animals that received the combined extract could be a pointer that the extracts have antihyperlipidemic potential. These findings agree with a report on the lipid profile levels associated with T2DM (28). High density lipoprotein (HDL) levels were significantly increased in the chemotherapeutic groups treated with 250mg/kg body weight, 500mg/kg body weight doses of extract and metformin treated diabetic group respectively, compared to the diabetic untreated (NC) group.

The liver enzymes Alanine aminotransferase and Aspartate aminotransferase were within the normal range for all rats (40U/L) hence there was no indication of the onset of liver disease, a finding similar to the report given by (29).There is a significant decrease in the liver enzymes levels of the positive diabetic untreated group when compared to the chemotherapeutic groups and the metformin group and this does not concur with the reports by (29), who said that in a diabetic environment, the activity of the liver enzymes are said to be abnormally elevated.

The difference in the plasma SOD and Catalase levels of the chemotherapeutic groups treated with 250mg/kg body weight, 500mg/kg body weight doses of extract as well as metformin treated diabetic group respectively were not significant when compared to the diabetic untreated (NC) group. This therefore suggests the presence of reactive oxygen species (ROS). MDA levels were significantly lower in the chemotherapeutic groups treated with 250mg/kg body weight and 500mg/kg body weight doses of

extract, compared to the diabetic untreated (NC) group, except for the metformin treated diabetic group where no significant difference was observed. This showed that the combined extract of *H. Sabdariffa, P. Nigrum L.* and *Z. officinale* helps to reduce levels of MDA, thereby reducing reactive oxygen species. This finding corroborates the report by (30), who suggested that increased levels of MDA causes increase in oxidative stress.

Conclusion: Put together, data from this study, showed that combined extracts of *H. Sabdariffa, P. Nigrum L.* and *Z. Officinale* exerts anti-oxidant, anti-hyperglycemic and anti-hyperlipidemic activity in diabetes, thus validating their use in traditional setting. Further studies are however recommended to elucidate the mechanisms through which these combined extracts act.

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