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Hypoglycemic and Anti-hyperlipidaemia Effects of Methanolic Extract of *Zingiber officinale* and its Role in Ameliorating Oxidative Stress in Alloxan-induced Diabetic Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Author AOO designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author AA managed the analyses of the study and the literature searches. Author OSO wrote the final draft. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

This study was designed to examine the hypoglycemic and antioxidant defense by methanolic extract of *Zingiber officinale* on alloxan induced diabetes in Wistar rats.The preliminary study showed that *Z. officinale* extract was able to scavenge the 2,2-Diphenyl-1-picrylhydrazyl (DPPH) and 2,2,-azinobis (3-ethylbenzoline-6-sulphonic acid (ABTS) radicals and these radicals scavenging abilities were found to be dose-dependent. Alloxan induced diabetes mellitus in rats was accompanied by increases in serum glucose and activities of alkaline phosphatase (ALP), alanine transferase (ALT) and aspartate transferase (AST). Diabetic rats also exhibited lower activities of superoxide dismutase (SOD), catalase (CAT), and reduced glutathione (GSH) content and higher level of malondialdehyde (MDA) in hepatic and renal tissues as compared with normal rats. However, supplementation of diabetic rats with *Z. officinale* extract at the doses of 50 mg/kg and 100 mg/kg body weight for 7 days caused reversal of all these effects significantly, it also

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resulted in dose-dependent hypoglycaemic status and improved the lipid profile of the treated groups indicating that the high levels of triglyceride and total cholesterol associated with diabetes can also be significantly managed with the extract. These findings suggest that *Z. officinale* treatment exerts a therapeutic protective effect in diabetes by decreasing oxidative stress, and hepatic and renal damage. *Z. officinale* was found to contain high level of total phenolic content (52.42 mg/g in GAE/g dried weight) which maybe speculated to account for the observed pharmacological effects of the plant.

Keywords: Alloxan; diabetes; Zingiber officinale; lipid profile; hypoglycaemic; oxidative stress.

1. INTRODUCTION

Diabetes mellitus (DM) is a common metabolic disorder affecting people in both developed and developing countries. Diabetics can be divided into two main groups based on their requirements for insulin: insulin dependent diabetes mellitus (Type 1) and non – insulin dependent diabetes mellitus (Type 2) [1]. Type 1 commonly seen in juveniles is characterized by failure to produce insulin due to autoimmune destruction of beta-cells of the pancreas while type 2 is usually adult-onset and is associated with insufficient production of insulin and loss of responsiveness by cells to insulin [2]. Diabetes is characterized by symptoms such as weakness, polyuria, excessive thirst as well as ketonemia, ketouria and ketosis due to altered metabolism of lipids and proteins. It is associated with abnormalities such as kidney failure, nervous defect, impotence, blindness, stroke and heart diseases [3]. Abnormalities in lipid metabolism may contribute to excessive hepatic glucose through gluconeogenesis as well as abnormal drive from the autonomic nervous system [3]. Studies have confirmed that for the type 2 diabetes, effective control of blood glucose substantially decrease the risk of developing complications of diabetes [3,4].

Oxidative stress is known to play a pivotal role in development of diabetes [5]. An imbalance of oxidant/antioxidant in favour of oxidants contributes to the pathogenesis of diabetes. Hyperglycemia induced auto-oxidation of lipids and glycation of protein/glucose, result in formation of free radicals of oxygen (ROS) and nitrogen (RNS). Anti-oxidants provide protection to living organism from damage caused by uncontrolled production of ROS concomitant lipid peroxidation, protein damage and DNA strand breaking. Currently available synthetic antidiabetic agents produce serious side effects, such as hypoglycaemic coma and hepatorenal disturbances [6]. Moreover, they are not safe for use during pregnancy [7]. Use of these therapies is restricted by their pharmacokinetic properties, secondary failure rates and accompanying side effects [8]. Hence, the search for safer and more effective hypoglycaemic agents has continued. Recent awareness of therapeutic potential of several traditionally used plants has opened a new dimension for the study and research of medicinal plants.

Ethnomedical literature contains a large number of plants including *Z. officinale* that can be used against diseases, like diabetes, in which reactive oxygen species and free radicals play a major role. Many minor components of foods, such as secondary plant metabolites, have been shown to alter biological processes, which may reduce the risk of chronic diseases in diabetic humans. *Z. officinale*, commonly known as ginger, is cultivated in the tropics for its edible rhizome with the root stocks serving a variety of purposes, including culinary and medicinal [9,10]. Various reports have demonstrated that *Z. Officinale* has several medicinal properties [11,12]. This medicinal herb is considered to be an excellent candidate for oral therapy as it is effective, non-toxic and without serious side effects.

The aim of the present study was to investigate the efficacy of *Z. officinale* extract in ameliorating blood sugar and lipids levels as well conferring antioxidant effects in the hepatic and renal tissues of alloxan-induced diabetic rats.

2. MATERIALS AND METHODS

2.1 Reagents

6-Hydroxy- 2,5,7,8 tetramethylchroman-2-carboxylic acid (Trolox), 2,2-Diphenyl-1-picrylhydrazyl (DPPH), 2,2-azino-bis(3-ethylbenzthiazoline-6-sufonic acid) ABTS, Gallic acid, thiobarbituric acid (TBA), nicotinamide adenine dinucleotide reduced (NADH) were obtained from Sigma–Aldrich Chemical Co. Ltd. (England)Nitrobluetetrazolium (NBT), 5,5_-dithiobisnitro benzoic acid (DTNB) was obtained from Fluka (Buchs, Switzerland). All other chemicals used were analytical grade.

2.2 Plant Material (*Zingiber officinale*)

The dried rhizome of *Zingiber officinale* were bought from Sabo market, in Ogbomoso and authenticated at Department of Pure and Applied Biology of Ladoke Akintola University of Technology, Ogbomoso, by Prof. A.J. Ogunkunle and a specimen was deposited in the herbarium with voucher number UIH 735. The rhizomes were dried at room temperature and blended to a coarse powder.

2.3 Preparation of *Zingiber officinale* Extract

The coarse powder of *Zingiber officinale* (200 g) were soaked in 600 mL of methanol for 72 hours. The extract was filtered and the solvent was removed from the extract with a vacuum rotary evaporator at 45℃. The concentrated dried methanolic extract was then stored at -20℃ before use.

2.4 Determination of Total Phenolic Compounds in *Zingiber officinale*

The content of total phenolic compounds in *Zingiber officinale* was determined by Folin–Ciocalteu method as described by Miliauskas et al., [13]. Briefly, 1 ml aliquots of 0.024, 0.075, 0.0105 and 0.3 mg/ml ethanolic gallic acid solutions were mixed with 5 ml Folin-ciocalteu reagent (diluted ten-fold) and 4 ml (75 g/L) sodium carbonate. The absorption was read after 30 min at 20℃ at 765 nm and the calibration curve was drawn. One ml of *Z. officinale* (1 mg/ml) were mixed with the same reagents as described above, and after l h the absorption was measured for the determination of plant phenolics. All determinations were performed in triplicate. Total content of phenolic compounds in plant methanol extracts in gallic acid equivalents (GAE) was calculated by the following formula:

$$C = c \bullet V/m'$$

Where: C-total content of phenolic compounds, mg/g plant extract, in GAE; c-the concentration of gallic acid established from the calibration curve, mg/ml; V- the volume of extract, ml; m´- the weight of pure plant methanolic extract, g.

2.5 Trolox Equivalent Antioxidant Capacity (TEAC) Assay

The assay was performed essentially as described by Re et al., [14]. ABTS radical cation was produced by reacting 7 mM ABTS stock solution with 2.45 mM potassium persulphate and allowing the mixture to stand in the dark at room temperature for 12–24 h before use. The $ABTS^{\bullet+}$ solution was diluted with water and adjusted to an absorbance of 0.700 ± 0.020 at 734 nm. For the photometric assay, 1 ml of the $ABTS^{\bullet+}$ solution and various concentrations of the extracts were mixed for 45 seconds and measured immediately after 1 minute at 734 nm. The antioxidant activity of the extracts was calculated by determining the decrease in absorbance at different concentrations by using the following equation.

$$\% \text{ antioxidant activity} = ((A_{(ABTS^{\bullet+})} - A_{(Extracts)}) / (A_{(ABTS^{\bullet+})}) \times 100.$$

2.6 DPPH (2, 2-Diphenyl-1-picrylhydrazyl) Radical Scavenging Activity

The assay was performed as previously described by Schelesier et al., [15]. The radical solution is prepared by dissolving 2.4 mg $DPPH^{\bullet}$ in 100 ml methanol. For the photometric assay 1.95 ml $DPPH^{\bullet}$ solution and 50 µl antioxidant solution were mixed. At first, the absorbance of the disposable cuvette with 1.95 ml $DPPH^{\bullet}$ was measured as blank, then the antioxidant solution was added and mixed. The reaction was measured at 5 min interval at 515 nm until $\Delta A=0.003\ min^{-1}$. The anti-oxidative activity was calculated by determining the decrease in absorbance at different concentrations by using the following equation:

$$\%\text{Inhibition activity} = ((A_{(DPPH^{\bullet})} - A_{(Extracts)}) / (A_{(DPPH^{\bullet})}) \times 100$$

2.7 Animals and Induction of Diabetes

Twenty four Wistar albino rats (180-220 g) were obtained from the animal house at LAUTECH Agricultural Department, Ogbomoso, Oyo state and they were maintained under standard environmental conditions and had free access to feed and water. Animal studies were approved by the Committee for Ethical Animal Care and Alternatives to Animal Use in Research, Testing, and Education of 1986. The rats were acclimatized in the animal house of the department for two weeks prior to the commencement of the experiment. For the induction of diabetes, rats were fasted overnight and diabetes was induced by a single intraperitoneal injection of a freshly prepared solution of alloxan tetrahydrate (150 mg/kg body

weight). Blood glucose concentration was checked by Glucometer after 4 days of alloxan injection. The rats with glucose concentration exceeding 230 mg/dl were considered diabetic.

2.8 Grouping of Animals

The rats were divided into four groups, six rats in each group and treated as follows:

Group 1: Normal Control: This group of rats were not injected with alloxan and served as non-diabetic control animals.

Group 2: Diabetic control (Alloxan 150 mg/kg body weight): Alloxan was given intraperitonially for the induction of diabetes to this group.

Group 3: Diabetic plus *Z. officinale* treatment: diabetic rats treated with methanolic extract of *Z. officinale* (50mg/kg body weight) for a period of 7 days.

Group 4: Diabetic plus *Z. officinale* treatment: diabetic rats treated with methanolic extract of *Z. officinale* (100mg/kg body weight) for a period of 7 days.

After completion of 7 days of treatment, the animals were sacrificed by diethylether anaesthesia. Blood samples of each rats were collected by heart puncture and were allowed to clot for 45 min at room temperature. Serum was separated by centrifugation at 4000 × g for 5 min and stored at -20℃ for biochemical analysis. Liver and kidney tissues were excised at -4℃. The tissues were washed with ice-cold saline and immediately stored at -20℃ for further biochemical analysis.

2.9 Preparation of Liver and Kidney Homogenates

Prior to biochemical analyses, the liver and kidney samples were cut into small pieces and homogenized in Phosphate buffer saline (PBS) with a homogenizer to give a 10% (w/v) liver and kidney homogenate. The homogenates were then centrifuged at 12,000 rpm for 15 min. The supernatant obtained was used for assay of superoxide dismutase, catalase, reduced glutathione, thiobarbituric acid reactive substances (TBARS) content, and protein estimation.

2.10 Biochemical Analysis

Alkaline phosphatase (ALP), aspartate aminotransefrase (AST), alanine aminotransferase (ALT), cholesterol (CHO), triglyceride (TAG) and high density lipoprotein-cholesterol (HDL-C) in serum were determined using enzymatic kits (Labkit, Spain) according to the manufacturer's instructions.

2.11 Superoxide Dismutase Activity Assay

SOD activity was measured according to method of Kakkar et al. [16]. Assay mixture contained 0.1 mL of supernatant, 1.2 mL of sodium pyrophosphate buffer (pH8.3; 0.052 M), 0.1 mL of phenazine methosulphate (186 µM), 0.3 mL of nitro blue tetrazolium (300 µM) and 0.2 mL of NADH (750 µM). Reaction was started by addition of NADH. After incubation at 30℃ for 90 s, the reaction was stopped by addition of 0.1 mLof glacial acetic acid. Reaction mixture was stirred vigorously with 4.0 mL of n-butanol. Colour intensity of the chromogen in the butanol was measured spectrophotometrically at 560 nm. One unit of enzyme activity was defined as that amount of enzyme which caused 50% inhibition of NBT reduction/mg protein.

2.12 Catalase Activity Assay

Catalase activity was measured by the method of Aebi [17]. An aliquot (10 µl) of each tissue supernatant was added to cuvette containing 1.99 µl of 50 mM phosphate buffer (pH 7.0). Reaction was started by addition of 1000 µl of freshly prepared 30 mM H_2O_2. The rate of decomposition of H_2O_2 was measured spectrophotometric ally at 240 nm. Activity of catalase was expressed as U/mg of protein.

2.13 Determination of Reduced Glutathione

GSH was assayed by the method of Jollow et al. [18], with slight modification. An aliquot of 0.5 mL of each tissue homogenate was precipitated with 0.5 mL of trichloroacetic acid (10% w/v). The precipitate was removed by centrifugation. 0.8 mL of the filtered sample was mixed with 0.3 DTNB (4 mg/mL) and 0.9 mL phosphate buffer (0.1 M, pH 7.4). The yellow colour developed was read at 412 nm. Reduced glutathione was expressed as µg/mg of protein.

2.14 Measurement of Hepatic and Renal Lipid Peroxidation

MDA levels were measured by the double heating method as reported by Draper and Hadley [19]. The method is based on spectrophotometric measurement of the purple

colour generated by the reaction of thiobarbituric acid (TBA) with MDA. Briefly, 0.5 mL of liver and kidney homogenates were mixed with 2.5 mL of tricholoroacetic acid (TCA, 10%, w/v) solution followed by boiling in a water bath for 15 min. After cooling to room temperature, the samples were centrifuged at 3000 rpm for 10 min and 2 mL of each sample supernatant was transferred to a test tube containing 1mL of TBA solution (0.67%, w/v). Each tube was then placed in a boiling water bath for 15 min. After cooling to room temperature, the absorbance was measured at 532 nm with respect to the blank solution. The concentration of MDA was calculated based on the absorbance coefficient of the TBA–MDA complex ($\varepsilon = 1.56\times10^5$ $cm^{-1}M^{-1}$) and it was expressed as µmol/mg protein.

2.15 Statistical Analysis

Results are expressed as means ± SEM. Statistical analyses were performed using oneway analysis of variance followed by Tukey's test. All analyses were done using Graph Pad Prism Software Version 5.00 and $p < 0.05$ was considered statistically significant.

3. RESULTS

3.1 Trolox Equivalent Antioxidant Capacity (TEAC) Assays [mmo]l^{-1} of Three Antioxidants; Trolox, Gallic Acid and *Zingiber officinale*

In TEAC assay, the TEAC value of trolox is 1.00. Gallic acid responded as the strongest antioxidant in the assay while *Z. officinale* responded lowest (Table 1 and Fig. 1).

3.2 Diphenyl-1-picrylhydrazyl (DPPH) Scavenging Activity of *Zingiber officinale*

The *Z. officinale* demonstrated a concentration and time dependent scavenging activity by quenching DPPH radicals (Fig. 2) and was compared with gallic acid, as a positive control. The IC50 values (defined as the concentration of test compound required to produce 50% inhibition) for DPPH scavenging by *Z. officinale* and gallic acid were 121.66 ± 5.32 µg/dL and 16.33 ± 1.50 µg/dL respectively (Table 1).

Table 1. Total phenolic content, DPPH radical scavenging value and trolox equivalent antioxidant capacity (TEAC) of *Zingiber officinale*

Sample	Total phenol[a]	DPPH scavenging activity (IC 50)[b]	Troloxequivalent antioxidant capacities (TEAC)[c]
Trolox	ND	ND	1.00
Gallic	ND	16.32 ± 1.50	4.25 ± 0.12
Zingiber officinale	51.42 ± 1.62	121.66 ± 5.32	0.15 ± 0.03

Each value represents the mean±SEM (n=3)
a Total phenolic content was expressed as mg gallic acid equivalents/g dried extract.
b Expressed as µg/mL
c Expressed as mmol/L

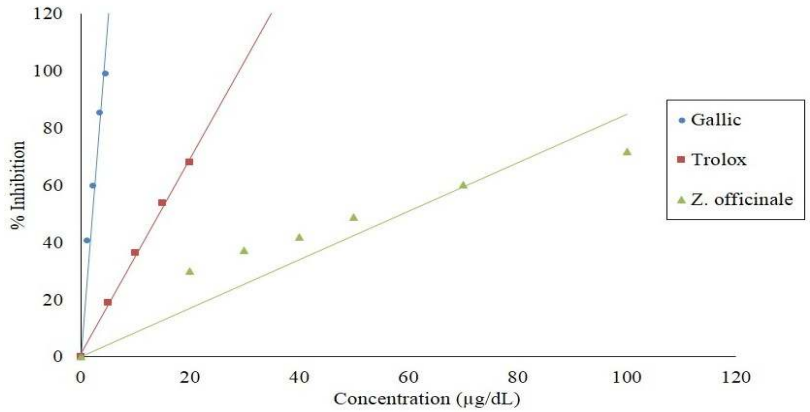

Fig. 1. The effects of different concentrations of Gallic, Trolox and *Z. officinale* on the inhibition of the ABTS radical
Values are the means of three experiments ± SEM

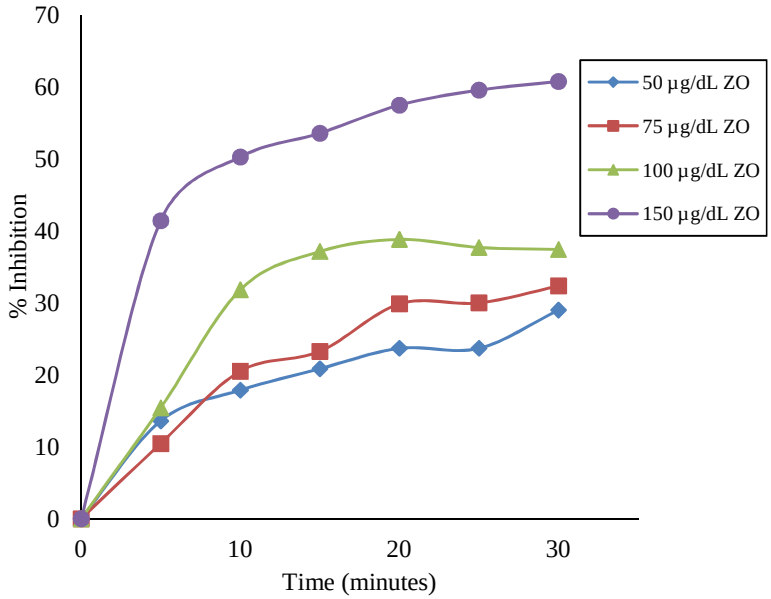

Fig. 2. The effects of time on different concentration of methanolic extract of *Z. officinale* on inhibition of DPPH radical

3.3 The Total Phenolic Content of *Zingiber officinale*

The phenolic content of *Z. officinale* was determined using Folin-Ciocalteu assay and by constructing a standard curve using gallic acid. The total amount of phenolic compounds present in *Z. officinale* was found to be 51.42 ± 1.62 mg/g in Gallic acid equivalent (Table 1).

3.4 Effect of *Zingiber officinale* Extract on the Blood Glucose Levels

The results obtained in this study showed a significant (P< 0.05) increase in blood glucose levels in diabetic rats. However, treatment of diabetic rats with *Z. Officinale* extract significantly decreased the blood glucose levels when compared to the diabetic control rats (Table 2).

3.5 Effect of *Zingiber officinale* Extract on the Levels of AST, ALT and ALP Activities

Induction of diabetes with alloxan resulted in significant (p < 0.05) rise in the levels of AST, ALT and ALP when compared to the normal rats. Oral administrations of *Z. Officinale* extract at two different doses (50mg/kg and 100mg/kg) to diabetic rats for 7 days lower the levels of these marker enzymes, namely, AST, ALT and ALP significantly (p < 0.05) (Fig. 3).

Table 2. Effects of *Zingiber officinale* treatments on blood glucose level in alloxan-induced diabetic rats

Parameter	Normal rats	Diabetic control	Diabetic + 50 mg/kg *Z. officinale*	Diabetic + 100 mg/kg *Z. officinale*
DAY 1	75.44± 1.68	239.25 ± 6.83*	238.72 ± 5.52	243.6 ± 7.33
DAY 4	76.62 ±1.73	240.42 ±7.83*	209.28 ±4.85	173.19 ± 4.72**
DAY 7	75.48 ±1.20	238.37 ±6.52*	163.33 ±4.64**	130.33 ± 3.17**

Values are expressed as mean ± SEM of six rats.
**Significant at P<0.05when diabetic control was compare with normal rats.*
***Significant at P<0.05when Z. officinale treated rats was compare with diabetic control rats*

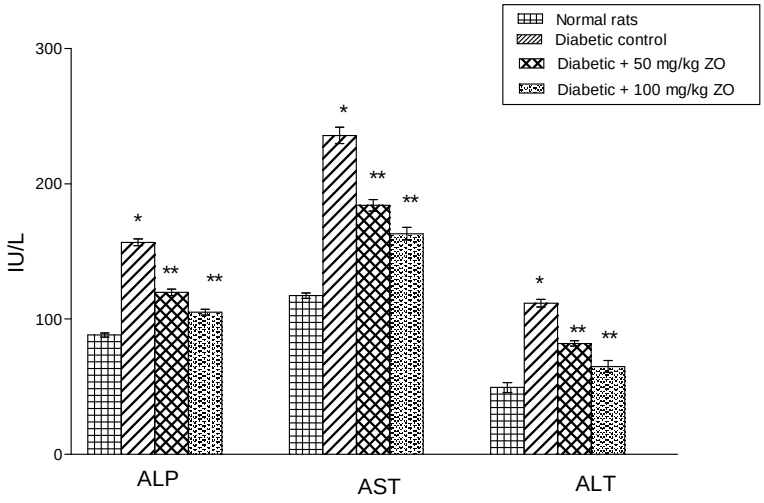

Fig. 3. Effect of *Z. Officinale* extract on serum levels of AST (IU/L), ALT (IU/L) and ALP (IU/L) in alloxan induced diabetic rats

*Values are mean ± SEM. * Group 2 (diabetic control rats) compared with Group 1 (normal rats). ** Groups 3 and 4 (Z. officinale treated rats) compared with Group 2 (diabetic control rats)*

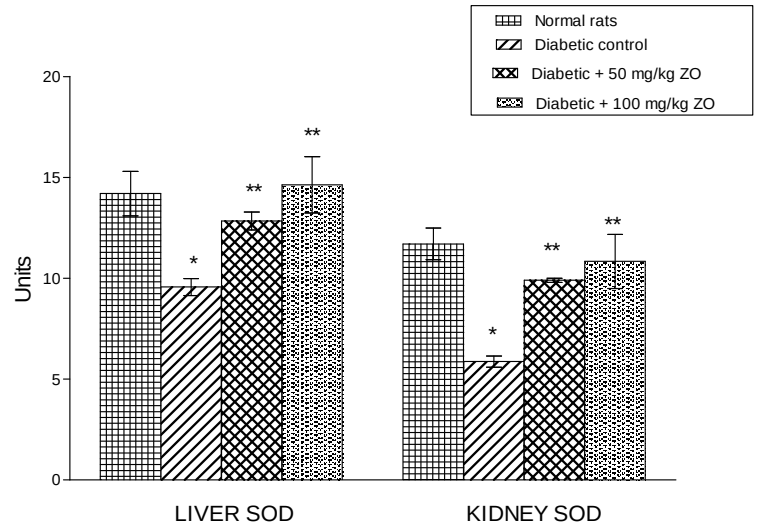

Fig. 4. Effect of *Z. Officinale* extract on hepatic and renal levels of SOD (U/mg protein) in alloxan induced diabetic rats

*Values are mean ± SEM. * Group 2 (diabetic control rats) compared with Group 1 (normal rats). ** Groups 3 and 4 (Z. officinale treated rats) compared with Group 2 (diabetic control rats)*

3.6 Effect of *Zingiber officinale* Extracton Hepatic and Renal SOD Levels

SOD levels was significant ($p < 0.05$) decreased in the liver and kidney of diabetic control rats when compared with normal rats. Administration of 50 mg/kg and 100 mg/kg body weight of *Z. Officinale* extract for 7 days significantly increased liver SOD level by 34.11% and 52.92% respectively and increased kidney SOD by 68.59% and 84.40% respectively when compared with diabetic control rats (Fig. 5).

3.7 Effect of *Zingiber officinale* Extracton Hepatic and Renal CAT Levels

As presented in Fig. 6. injection of alloxan significant (p <0.05) decreased catalase levels in

the liver and kidney of diabetic control rats when compared with normal animal. Administration of 50 mg/kg and 100 mg/kg body weight of *Z. officinale* extract for 7 days significantly increased liver catalase level by 74.23% and 95.45% respectively and also increased kidney catalase by 50.78% and 100.00% respectively when compared with diabetic control rats.

3.8 Effect of *Zingiber officinale* Extract on Hepatic and Renal GSH Levels

GSH levels was significant ($p < 0.05$) decreased in the liver and kidney of diabetic control rats when compared with normal rats. Administration of 50 mg/kg and 100 mg/kg of *Z. officinale* extract for 7 days significantly ($p < 0.05$) increased liver GSH level by 36.47% and 57.28% respectively and increased kidney SOD level by 82.59% and 84.78% respectively when compared with diabetic control rats (Fig. 7).

3.9 Effect of *Zingiber officinale* Extract on the Levels of Serum TAG, CHO and HDL-C

As presented in Fig. 4. injection of alloxan increased serum CHO and TAG levels of control diabetic rats significantly ($p < 0.05$) above the normal levels while it reduced HDL-C below the normal level. Treatment of diabetic rats with 50 mg/kg and 100 mg/kg *Z. officinale* extract significantly decreased serum triglyceride by 24.54% and 39.50% respectively, decreased serum cholesterol by 27.76% and 42.16% respectively and increased serum HDL-C by 38.99% and 70.54% respectively when compared with diabetic control rats.

3.10 Effect of *Zingiber officinale* Extracton Hepatic and Renal TBARS Levels

Injection of alloxan increased Lipid peroxidation level (LPO) levels in the liver and kidney of diabetic control rats when compared with normal rats. Oral administration of 50 mg/kg and 100 mg/kg of *Z. Officinale* extract for 7 days significantly ($p < 0.05$) decreased liver LPO level by 30.57% and 40.49% respectively and decreased kidney LPO level by 25.75% and 42.22% respectively when compared with diabetic control rats (Fig. 8).

4. DISCUSSION

Diabetes mellitus, a product of defects in insulin secretion or action or both, is characterized by hyperglycaemia, glycosuria, and polyuria [20]. Persistent hyperglycaemia during diabetes resulted in increased production of free radicals especially reactive oxygen species (ROS), for all tissues from glucose auto-oxidation and protein glycosylation [21]. Although many traditional plant remedies are available for the treatment of diabetes, only a few among them have been scientifically evaluated [4]. Therefore, this study investigated the effect of *Z. officinale* on biomarkers of oxidative stress, and lipid peroxidation (LPO) in liver and kidney of alloxan-induced diabetic rats.

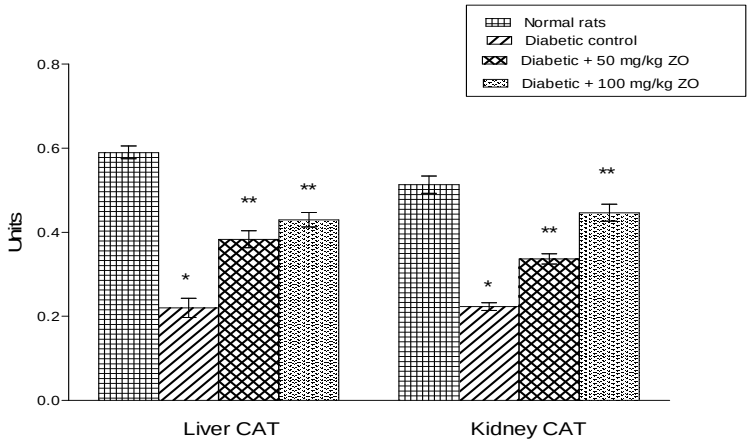

Fig. 5. Effect of *Z. Officinale* extract on hepatic and renal levels of catalase (U/mg protein) in alloxan induced diabetic rats

*Values are mean ± SEM. * Group 2 (diabetic control rats) compared with Group 1 (normal rats). ** Groups 3 and 4 (Z. officinale treated rats) compared with Group 2 (diabetic control rats).*

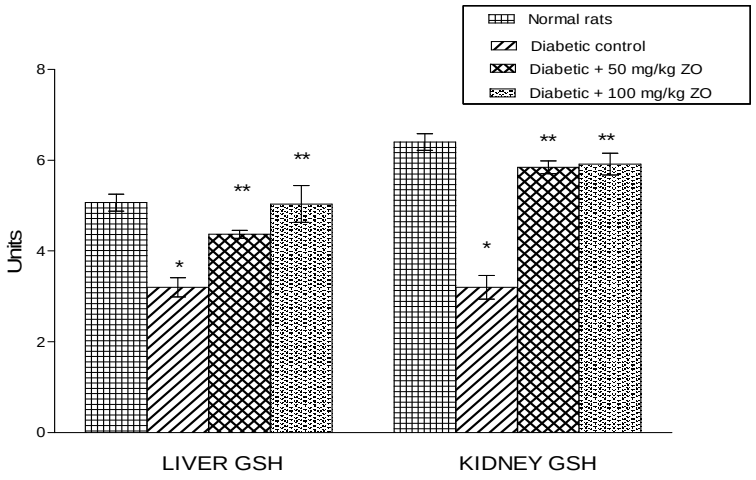

Fig. 6. Effect of *Z. Officinale* extract on hepatic and renal levels of GSH (µg/mg protein) in alloxan induced diabetic rats

*Values are mean ± SEM. * Group 2 (diabetic control rats) compared with Group 1 (normal rats). ** Groups 3 and 4 (Z. officinale treated rats) compared with Group 2 (diabetic control rats)*

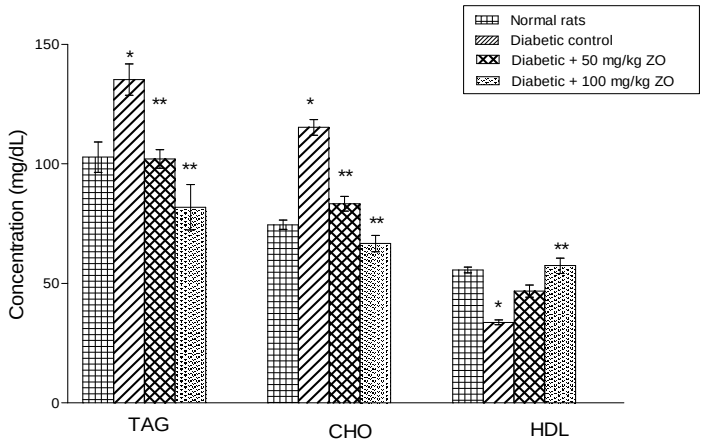

Fig. 7. Effect of *Z. Officinale* extract on serum levels of CHO (mg/dL), CAT (mg/dL) and HDL-c (mg/dL) in alloxan induced diabetic rats

*Values are mean ± SEM. * Group 2 (diabetic control rats) compared with Group 1 (normal rats). ** Groups 3 and 4 (Z. officinale treated rats) compared with Group 2 (diabetic control rats)*

Preliminary study evaluated the in-vitro antioxidant potential of *Z. officinale* extract against $ABTS^{\bullet+}$ and DPPH radicals. The extract showed significant $ABTS^{\bullet+}$ and DPPH radicals scavenging activity in a concentration dependent manner, which can be attributed to hydrogen donating ability of the extract. *In-vivo*, alloxan was used for the induction of diabetes mellitus. Alloxan is known for its selective pancreatic islet β – cell cytotoxicity and has been extensively used to induce diabetes mellitus in animals [3,22]. In the present study, after the induction of alloxan, it is evident that the diabetic rats had much higher blood glucose level when compared with the normal rats. Generalised increase in the level of blood glucose during diabetes have been consistently reported both in animal models [3,22] and humans especially those suffering from insulin dependent diabetes mellitus [23]. Methanolic extract of *Z. officinale* significantly reduced blood glucose levels in a dose dependent manner, probably by stimulating insulin production from the pancreatic islets or by increasing peripheral utilization and inhibition of

the proximal tubular reabsorption mechanism for glucose in the kidney which have a glucose lowering effect [12].

It has been reported that diabetic complications exhibited in alloxan-induced animals are free radical mediated [24]. In this study, alloxan-induced animals developed a significant hepatic damage and oxidative stress, which was observed from a substantial increase in the activities of serum AST, ALT and ALP. Increase in these liver enzymes is an indication of liver damage [25] therefore the decrease in the serum levels of these enzymes following the administration of methanolic extract of *Z. officinale*o btained in this study is an indication of the stabilization of serum membrane as well as repair of liver damage caused by alloxan-induced oxidative stress. This observation is in agreement with the commonly accepted view that serum levels of transaminases return to normal with healing of hepatic parenchyma and the regeneration of hepatocytes [26].

Antioxidant enzyme activities were significantly decreased in diabetic rats in this study. Both enzymatic antioxidants (SOD, CAT) and non-enzymatic antioxidants (GSH) showed lower activities in liver and kidney during diabetes and the results agree well with the earlier published data [27,28]. The decreased activities of SOD and CAT as well as decreased GSH level in liver and kidney tissues of diabetic rats may be due to their increased utilisation against reactive oxygen species [29]. However, supplementation of diabetic rats with methanolic extract of *Z. officinale* reversed SOD, CAT and GSH to normal levels, this shows that *Z. officinale* has an antioxidant property.

Diabetes lead cardiovascular complications is associated with increased levels of triglycerides and cholesterol. In the present study, alloxan-induced diabetic mellitus caused a significant rise in serum lipids which mean increased risk of atherosclerosis and coronary artery diseases. Methanolic extract of *Z. officinale* used in this study significantly reduces triglyceride and total cholesterol levels with increase of HDL cholesterol in diabetic rats as compared to diabetic control rats. These changes are beneficial in preventing diabetic complications as well as in improving lipid metabolism in diabetics [30]. Oxidative stress is associated with diseases and occurs in alloxan-induced diabetic rats seen as an increase in malondialdehyde (MDA), an end product of lipid peroxidation [30]. There was far more increase in lipid peroxidation in the diabetic control rats than in the rats treated with methanolic extract of *Z. officinale*, suggesting that *Z. officinale*has antioxidant activity, an attribute required in the treatment of diseases.

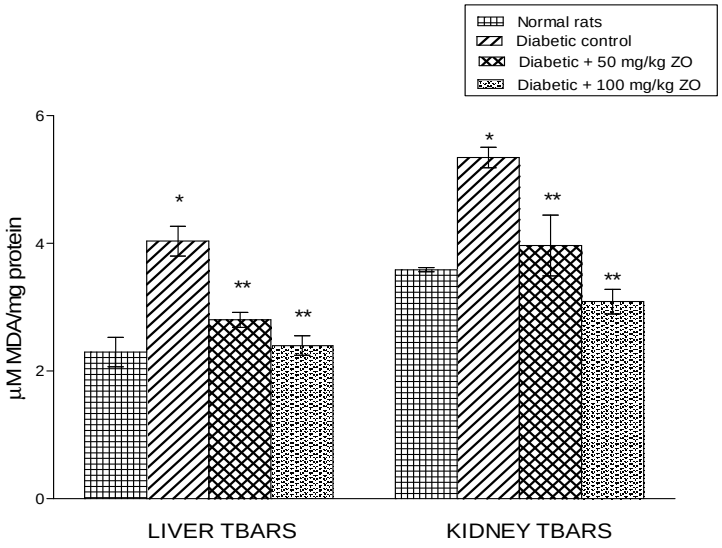

Fig. 8. Effect of *Z. Officinale* extract on hepatic and renal levels of thiobarbituric acid reactive substance (TBARS) (µM/mg protein) in alloxan induced diabetic rats

*Values are mean ± SEM. * Group 2 (diabetic control rats) compared with Group 1 (normal rats). ** Groups 3 and 4 (Z. officinale treated rats) compared with Group 2 (diabetic control rats)*

Our present study revealed that *Z. officinale* contain a considerable amount of phenolic content and exhibited strong free radical scavenging property. A number of researchers have reported that phenolic compounds in plants extract have antioxidant properties in various experimental models [11,31,32]. Consequently, the ability of the methanolic extract of *Z. officinale* to protect the rats against alloxan-induced liver and kidney damaged may be attributed to the high antioxidant potentials exhibited in this study. This finding justifies the use of *Z. officinale* in traditional medicine in Africa for the treatment of diabetes millitus and supports the use of *Z. officinale* in the prevention of free radical mediated diseases.

5. CONCLUSION

There was a profound reduction in glucose levels and other biochemical parameters assayed in the rats treated with methanolic extract of *Z. officinale* when compared to diabetic control rats. Although our study suggests that *Z. officinale* is rich in phenolic compounds which may account for this pharmacological property, however further study is needed to identify the active constituent (s) responsible for this beneficial effect.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No.85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the Faculty of Basic Medical Sciences, LAUTECH, Ogbomoso ethics committee.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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