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EFFECT OF GINGER RHIZOME (*ZINGIBER OFFICINALE*) METHANOLIC EXTRACT ON SOME OXIDATIVE STRESS MARKERS IN ALLOXAN-INDUCED DIABETIC RATS

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ABSTRACT

The prevalence of diabetes mellitus is rising all over the world. Much evidence suggests major role of reactive oxygen species/oxidative stress in development and progression of diabetic complications. In the current study, the antioxidant potentials of methanolic extract of ginger rhizome (*Zingeber officinale*) in alloxan-induced diabetic rats were evaluated. Thirty (36) healthy albino rats were randomly divided into six groups of six rats each: positive control group (PC), normal control group (NC), metformin control group (MC) and experimental groups i.e Groups 1, 2, and 3 which were orally administered with methanolic extract of ginger rhizome on daily doses of 1500mg/Kg, 1000mg/Kg and 500mg/kg body weight respectively for six weeks. Results of the study showed significant ($p<0.05$) increase in serum levels of reduced glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT) activities of GR1 (9.0±1.3 µmol SOD/min/mg of protein), (28.3±2.2 mg/dl) and (31.4±3.1 IU/l) respectively, GR2 (8.6±2.4 µmolSOD/min/mg of protein), (25.1±3.2 mg/dl), and (31.1±3.9 IU/l) respectively, and GR3 (8.2±2.3 µmolSOD/min/mg of protein), (24.7±4.0 mg/dl) and (28.1±2.2 IU/l) respectively when compared with that of positive control group (4.2±0.5 µmolSOD/min/mg of protein), (19.4±2.3 mg/dl), and (22.9±2.1 IU/l) respectively. A significant ($p<0.05$) decrease was found in the level of malondealdehyde (MDA) in GR1, GR2, and GR3 (54.8±5.2 nmol/L, 65.3±5.5 nmol/L, and 72.9±4.3 nmol/L respectively) when compared with that of positive control group (101.8±8.7 nmol/l). Thus, ginger has the potentials to lower the effect of oxidative stress in alloxan-induced diabetic rats.

Keywords: Diabetes Mellitus, Ginger Rhizome, Oxidative Stress

INTRODUCTION

The prevalence of diabetes mellitus is rising all over the world and has been associated with an increased risk of mortality and prevalence of cardiovascular disease. Atherosclerotic cardiovascular disease is the main source of morbidity and mortality in patients with diabetes (Bray, 2000). In addition, oxidative stress may occur as a consequence of abnormalities in glucose and lipid metabolism, which favour hyperglycaemia and dyslipidaemia (Chertow and Edwards, 2004). It causes several adverse effects on cellular physiology.

Management of diabetes mellitus by insulin therapy has several draw backs such as insulin resistance. Anorexia nervosa, brain atrophy, and fatty liver are encountered in

chronic treatment with insulin. For oral hypoglycemic drugs, sulphonylureas and biguanide are being used effectively in controlling hyperglycaemia, however, their use could exert some side effects such as hepatotoxicity, abdominal pain, flatulence, diarrhoea and hypoglycaemia. Drug resistance to these medicines could also occur after prolonged period of treatment.

Diabetes remedy that is gaining popularity today is herbal treatment, with a variety of plant- derived preparations being promoted as capable of controlling blood sugar levels. Several herbs can help to reduce blood sugar, high blood cholesterol concentrations, provide some protection against cancer and stimulate the immune system. Therefore, this study was aimed at evaluating the effect of methanol extract of ginger rhizome (Zingiber officinale) on some oxidative stress markers in alloxan induced diabetic rats.

MATERIALS AND METHODS

Collection and Identification of Plant Material

Fresh ginger rhizome was obtained from Malumfashi market at Malumfashi local government, Katsina State, Nigeria. The sample was botanically identified and authenticated by the taxonomist in the Department of Plants Science, Faculty of Biological Sciences, Bayero University, Kano. A voucher specimen number BUKHAN 296 was assigned to the sample.

Sample Preparation

Fresh ginger rhizome was thoroughly washed with distilled water several times to remove dust, sand and stones. It was then cut into smaller pieces with table knife and air-dried under shed for a period of one week. The dried ginger was then grinding into powder using a domestic grinder (mortar and pestle). Powder of ginger (40 g) was soaked in 400ml of methanol and stored in a dark place at room temperature for 48 hours, after which the extract was filtered using a whatchman filter paper. The filterate was then concentrated under a reduced pressure using Rotove-flash evaporator at a temperature of 45^{oC} and a pressure of 700mmHg.

Experimental Design

Thirty (36) apparently healthy young Wister albino rats of both sexes weighing between 100 – 200 g were used in this study. They were kept at animals' house in Department of Biological Sciences, Bayero University, Kano under normal environmental conditions and maintained with free access to pelletized growers feed, and access to water *ad libitum.* They were allowed to acclimatize for two weeks (14 days).

The rats were randomly selected and divided into six (6) equal groups (i.e each group contained 6 rats): Diabetic control group (PC), Normal control group (NC), Group one (GR1), Group two (GR2), Group three (GR3), and metformin control group (MC). Methanolic extract of ginger rhizome was administered orally every morning by intubation using intravenous cannula tube at different doses (GR1 = 1500mg/Kg, GR2 = 1000mg/Kg and GR3 = 500 mg/kg) by single forced oral feeding once per day for a period of 42 days. While rats in the MC group were administerd with 500 mg/kg body weight Metformin using the same procedure. 24 hours after the last treatment, the animals were subjected to 12

hours fasting after which they were anaesthetised. They were then removed from the jar and blood samples collected from them through decapitation. Serum reduced glutathione (GSH) level (Beutler *et al*. 1963), lipid peroxidation (LPO) (Varshney and Kale, 1990), Catalase activity (Claiborne, 1985) and Superoxide Dismutase (SOD) activity (Misra and Fridovich, 1972) were determined.

Statistical Analysis

Results were expressed as mean ± standard deviation and analyzed using one-way analysis of variance (ANOVA) using the Statistical Package for Social Sciences (SPSS) version 20. P value of 0.05 or less was considered to be significant.

RESULTS

Result of this study shows that all fractions of ginger rhizome extract have an antioxidant capacity compared with ascorbic acid standard. The methanolic fraction was found to have highest activity of 53.89% at a concentration of 1.00 mg/ml (figure 1) followed by ethylacetate fraction with a highest activity of 33.86% at a concentration of 1.00 mg/ml, then the chloroform fraction with a highest activity of 20.00% at a concentration of 1.00 mg/ml and lastly the hexane fraction with highest activity of 15.47% at same concentration with the others. It was observed that the methanolic fraction has the highest percentage inhibition on DPPH reagent when compared with the ascorbic acid standard.

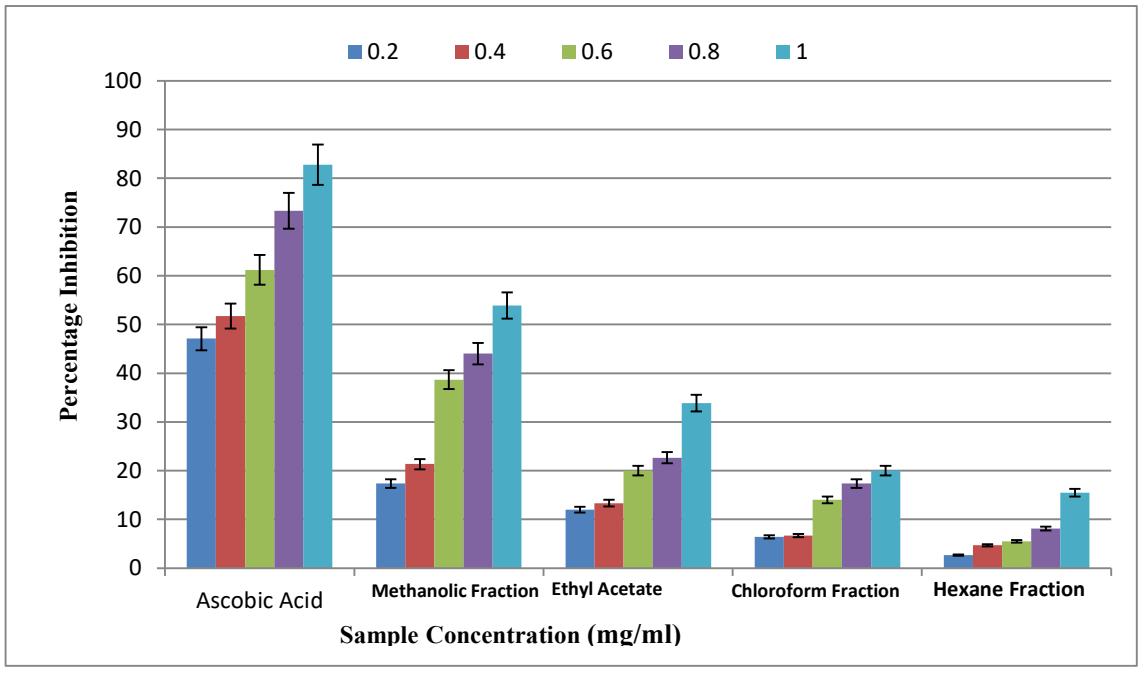

Figure 1: Antioxidant potential of Different Fractions of ginger rhizome Extract

Table 1, indicates significant decrease (p<0.05) in serum levels of reduced glutathione (GSH), superoxide dismutase (SOD) and Catalase activities in the positive control group (PC) when compared with the normal control group (NC). It was observed that there was significantly increase in serum malondialdehyde (MDA) level in the diabetic control group (PC) compared with that of the normal control group (NC). The effect of treatments with different concentrations (1500, 1000, and 500 mg/Kg) of the methanolic extract of ginger rhizome for six weeks resulted in significant (p<0.05) increase in serum levels of reduced glutathione (GSH), superoxide dismutase (SOD) and Catalase activities as well as significant (p<0.05) decrease in serum level of MDA when compared with the diabetic control group (PC). The serum superoxide dismutase (SOD), reduced glutathione (GSH) and Catalase activities of GR1 treated group was found to be (9.0±1.3 µmol SOD/min/mg of protein), (28.3±2.2 mg/dl) and (31.4±3.1 IU/l) respectively. The levels of these same parameters in GR2 were (8.6±2.4 µmolSOD/min/mg of protein), (25.1±3.2 mg/dl), and (31.1±3.9 IU/l) respectively, while that of GR3 were found to be (8.2±2.3 µmolSOD/min/mg of protein), (24.7±4.0 mg/dl) and (28.1±2.2 IU/l) respectively. All of which were significantly (p<0.05) higher than that of the diabetic control group (PC) where the serum superoxide dismutase (SOD), reduced glutathione (GSH) and Catalase activity were found to be (4.2±0.5 µmolSOD/min/mg of protein), (19.4±2.3 mg/dl), and (22.9±2.1 IU/l) respectively.

The serum MDA levels of the treated groups were found to be (54.8±5.2 nmol/L), (65.3±5.5 nmol/L), and (72.9±4.3 nmol/L) for GR1, GR2, and GR3 respectively, which are significantly (p<0.05) lower than that of the diabetic control group (PC) where the serum MDA level was found to be (101.8±8.7 nmol/l). The result also showed that, there was no significant difference in serum superoxide dismutase (SOD) catalase activity, reduced glutathione and MDA levels when the Normal and Metformin control group are compared.

It was also observed that the results of the serum levels of reduced glutathione in GR1 treated group was statistically the same (no significant (p>0.05) difference) as compared with the normal and Metformin control groups (NC & MC).

DISCUSSION

From the results of this study, it was observed that methanolic fraction of ginger has the highest percentage inhibition on DPPH reagent when compared with ascorbic acid standard (figure 1). This is in agreement with the findings of Stoilova *et al.* (2007), who studied the antioxidant activity of alcohol extract of ginger from Vietnamand and found that the DPPH radical inhibition reached up to 90.1%. Hinneburg *et al.* (2006) in Finland found high antioxidant action of the aqueous extracts of ginger, where the IC50 value for inhibition of DPPH radical was ~9 mg/ml. Oxidation of biological molecules induces a variety of pathological diseases including atherosclerosis and cancer. These damages are caused due to presence of free radicals. For that reason, the concept of pharmacological supplements to defend against free radicals with antioxidants has become an intense area of research (Gounder and Lingmallu, 2012). According

to Atashak (2014), [6]-gingerol and [6]-shogaol have displayed strong antioxidant activity in vitro. It is known that the antioxidant activity of plant extracts containing polyphenol components is due to their capacity to be donors of hydrogen atoms or electrons and to capture free radicals.

Table 1: Serum Levels of Some Oxidative Stress Markers in Rats Treated with Methanolic Extract of Ginger Rhizome for Six Weeks.

Group	MDA (nmol/L)	SOD (µmolSOD/min/mg of protein)	GSH reduced (mg/dl)	CAT (IU/L)
NC	39.8±4.4^{a}	15.4±4.8^{a}	31.7±3.1^{a}	38.6±2.4^{a}
PC	101.8±8.7^{b}	4.2±0.5^{c}	19.4±2.3^{b}	22.9±2.1^{d}
MC	39.2±7.3^{a}	13.8±3.6^{ab}	30.8±2.3^{a}	35.9±5.3^{a}
GR1	54.8±5.2^{c}	9.0±1.3^{b}	28.3±2.2^{ac}	31.4±3.1^{c}
GR2	65.3±5.5^{cd}	8.6±2.4^{b}	25.1±3.2^{cb}	31.1±3.9^{c}
GR3	72.9±4.3^{d}	8.2±2.3^{b}	27.7±4.0^{cb}	28.1±2.2^{cd}

Values are expressed as mean ± S.D., Mean values having different superscript letter in the same column are significantly different at (p<0.05).

Key: NC: Normal Control, PC: Positive Control, MC: Metformin Control (500 mg/kg) body weight of rat; GR1; treatment group with high dose(1500 mg/Kg), GR2; treatment group with medium dose (1000 mg/Kg) and GR3; treatment group with low dose (500 mg/Kg) body weight of rat.

In the current study, it was observed that there was in increase in the level of the oxidative stress markers in alloxan-induced diabetic control rats (table 1). The results indicated that the alloxan-induced diabetic control group (PC) has lower level of serum reduced glutathaione (GSH), superoxide dismutase (SOD) and Catalase activities, but has higher level of serum MDA (a marker of lipid peroxidation). The increase in lipid peroxidation as revealed by the high level of MDA formed in the alloxan-induced diabetic rats compared to that of normal control rats, suggests that the natural antioxidant defense mechanism to scavenge excessive free radical has been compromised in rats induced with diabetes (Pratibha *et al*., 2004). Decrease in antioxidant enzyme activity as well as increased MDA observed in diabetes mellitus might be due to an altered intracellular ratio between free radicals and antioxidant capacity, because, the reactive oxygen species (ROS), which are excessively produced in diabetes are able to overwhelm the endogenous defense systems leading to oxidative stress (Omolola, 2014). ROS are considered important independent risk factors developed in diabetes mellitus via what is known as “auto-oxidative glycosylation”, a process which is relevant at elevated blood glucose level. Hyperglycemia may also raise aldose reductase activity which depletes NADPH cell stores, thus perturbing the defense system. The elevated blood glucose level can also cause non-enzymatic glycation of plasma proteins

leading to the production of more powerful oxidizing species which can bind with most normal cellular components to "pair up" its unpaired electrons; thus, they react with the unsaturated bonds of membrane lipids, denature the proteins, and attack nucleic acids, resulting in cellular oxidative damage. This in turn may lead to the development of cardiovascular diseases (He *et al*., 2017). Results of the effect of treatments with different doses (1500, 1000, and 500 mg/Kg) of the methanolic extract of ginger rhizome after six weeks of treatment showed elevation in the serum levels of reduced GSH, superoxide dismutase and CAT activity in comparison with that of the diabetic control (PC) group.

CONCLUSION

Oxidative stress plays an important role in progression and development of diabetes and its complications. The result of the current study showed that methanolic extract of ginger rhizome has the highest antioxidant potential. However, all the graded doses of methanolic extract of ginger rhizome studied, have the potentials to manage the oxidative stress in alloxan-induced diabetic rats by increasing serum levels of reduced glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT) activities and also by decreasing serum level of malondealdehyde (MDA). Thus, it can be concluded that ginger rhizome could be a good therapy in the management of diabetes mellitus.

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