

Ethanol Extract of *Piper nigrum* Leaves Ameliorates Serum Lipid Peroxidation and Antioxidant Enzymes Activities in Diabetic Rats

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Abstract: Diabetes is known to involve oxidative stress and changes in antioxidant enzymes. This study evaluated the effects of extract from *Piper nigrum* leaves on lipid peroxidation and antioxidant enzymes in rat model. The results confirmed that the untreated diabetic rats (group II) were subjected to oxidative stress as indicated by significant ($p < 0.05$) increase in MDA, significant ($p < 0.05$) decrease in protein levels and abnormal activities of their scavenging enzymes (low superoxide dismutase, catalase and glutathione activities) when compared with apparently healthy rats (group I). The ethanol extract of *Piper nigrum* leaves possessed antioxidant activity as shown by increased activities of superoxide dismutase and catalase and glutathione levels of the diabetic rats after 21 days treatment. The study indicates that *Piper nigrum* contains antioxidant potentials capable of restoring depleted antioxidant levels in oxidative stressed rats.

Key words: Oxidative Stress • *Piper nigrum* • Malondialdehyde • Antioxidant Enzymes

INTRODUCTION

Oxidative stress is increased in diabetes mellitus owing to an increase in the production of oxygen free radicals and insufficiency in antioxidant defense mechanisms [1, 2]. Alloxan is a toxic glucose analogue, which selectively destroys insulin-producing cells in the pancreas (that is beta cells) when administered to rodents and many other animal species [3]. Alloxan is selectively toxic to insulin-producing pancreatic beta cells because it preferentially accumulates in beta cells through uptake via the GLUT2 (Glucose transporter 2) [3]. Alloxan, in the presence of intracellular thiols, generates reactive oxygen species (ROS) in a cyclic reaction with its reduction product, dialuric acid [4]. The beta cell toxic action of alloxan is initiated by free radicals formed in this redox reaction [3].

Free radicals are capable of attacking the healthy cells of the body, causing them to lose their structure and function [4]. Fortunately, free radical formation is controlled naturally by various beneficial compounds known as antioxidants. It is when the availability of antioxidants is limited that this damage can become cumulative and debilitating [5, 6]. Antioxidants are our

first line of defense against oxidative damage and are critical for maintaining optimum health and well-being and are capable of stabilizing, or deactivating, free radicals before they attack cells. Antioxidants are absolutely, critical for maintaining optimal cellular and systemic health and well-being [7]. To protect the cells, organ and systems of the body against reactive oxygen species, humans have evolved a highly sophisticated and complex antioxidant protection system [8].

Piper nigrum (black pepper) possesses anti-tumorigenic, immuno-stimulatory, stomachic, carminative, anticholesterolaemic properties and again known for its strong phytochemical activities [9]. Piperine, a substance present in black pepper has been found to increase the absorption of selenium, B-complex vitamins, beta-carotene, curcumin as well as other nutrients from food. Piperine also inhibits pro-inflammatory cytokines that are produced by tumour cells. During that process, it interferes with the signaling mechanisms between cancer cells, thereby reducing tumor progression [10]. In respect to its numerous usages, the present study is aimed at evaluating the antioxidant effects of *Piper nigrum* ethanol leaves extract on alloxan induced diabetic rats.

MATERIAL AND METHODS

Plant Material: The leaves of *Piper nigrum* were used for this study. The leaves were purchased from Ogige market in Nsukka and were identified by Mr. Alfred Ozioko of the Bioresources Development Centre and Conservation Programme (BDCP) Research Centre, Nsukka, Enugu State.

Extraction of Plant Materials: The leaves of *Piper nigrum* were air-dried at room temperature for four weeks after which it was grounded into fine powder. The powdered leaves (500g) were macerated in 1.5 L of absolute ethanol for 48 h. The solution was filtered with Whatmann No.4 filter paper and the filtrate concentrated to a semi-solid residue in an oven at 60°C.

Experimental Design: All the animals used were obtained from the Animal House of the Faculty of Biological Sciences, University of Nigeria Nsukka. The rats were fed with standard growers mash rat pellets (Grand Cereals Ltd, Enugu) and water. The animals were acclimatized for 7 days under standard environmental conditions, with a 12 hour light/dark cycle maintained on a regular feed (Top feed; grower mash) and water. The ethical committee of the Department of Biochemistry for the care and use of laboratory animals approved the research.

Thirty (30) adult male Wistar albino rats weighing 125-220g were used for the study. They were acclimatized for fourteen (14) days with free access to feed and water. After acclimatization, they were evenly distributed into six (6) groups of five rats each. The treatment lasted for twenty one (21) days. The route of administration was via oral route with the aid of an oral intubation tube. The groups and doses administered are summarized below:

Group I: Control (Normal non-diabetic rats)

Group II: Positive control (Diabetic untreated rats)

Groups III: Diabetic rats treated with 2.5mg/kg body weight of glibenclamide.

Group IV: Diabetic rats treated with 100mg/kg body weight of the ethanol extract

Group V: Diabetic rats treated with 200mg/kg body weight of the ethanol extract

Group VI: Diabetic rats treated with 300mg/kg body weight of the ethanol extract.

At the end of the experimental period the rats were starved for 12 h and then sacrificed under ether anaesthetized. At the end of the experimental period the rats were starved for 12 h and then sacrificed under ether anaesthetized. Blood samples were received into clean dry centrifuge tube and left to clot at room temperature, then centrifuged for 10 minutes at 3000 r.p.m to separate serum. Serum was carefully separated into dry clean Wassermann tubes, using a Pasteur pipette and kept frozen at (-20°C) until estimation of some biochemical parameters.

Determination of Lipid Peroxidation (Malondialdehyde) Levels: Lipid peroxidation was determined spectrophotometrically by measuring the level of the lipid peroxidation product, malondialdehyde (MDA) as described by Niki *et al.* [11].

Procedure: A volume, (0.1ml) of serum was mixed with 0.9ml of H₂O in a beaker. To the beaker was added 0.5ml of 25% TCA (Trichloroacetic acid) in 0.3% NaOH. The mixture was boiled for 40 minutes in a water-bath and then cooled in cold water. Then 0.1ml of 20% sodiumdodecyl sulphate (SDS) was added to the cooled solution and then mixed properly. The absorbance was taken at wavelengths of 532nm and 600nm and the result calculated thus:

$$\% \text{ TBARS} = \frac{A_{532} - A_{600} \times 100}{0.5271 \times 0.1} (\text{mg} / \text{dl})$$

Total Serum Protein: The principle lies on the fact that at alkaline pH value of 7.0, proteins form a stable complex with Cu²⁺, which is photometrically measured. The total protein test was carried out as follows: three test tubes were labeled as blank, standard and sample respectively. To the sample tube was added 0.02ml of serum, 0.02ml of protein standard to the standard tube and 0.02ml of water to the blank. One millilitre (1ml) of the protein reagent was added to the three tubes. This was mixed and allowed to stand for 10minutes at room temperature (20-25°C). The absorbance was then taken at 540nm and the results calculated thus:

$$\text{Total serum proteins (g/dl)} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times 5$$

Antioxidant Enzymes Assay

Catalase Activity: This was done according to the method [12].

Procedure: A volume, (2ml) of hydrogen peroxide and 2.5ml of phosphate buffer were added to a beaker. Adequately, 0.5ml of the sample was also added and mixed. One millilitre (1ml) portion of the reaction mixture was added to 2ml of dichromate acetic acid reagent. The absorbance was read at 570nm at 1minute interval into four (4) places. Catalase activity was calculated using the following equation.

$$\text{Catalytic concentration (Unit/L)} = 0.23 \times \frac{\log \text{Abs1}}{\text{Abs2}} \div 0.00693$$

Superoxide Dismutase Assay: This was determined using the method of Soliman [13].

Procedure: To test tube, 0.9ml of distilled water was pipetted and 0.1ml of sample added. 0.1ml of this was mixed with 0.9ml of carbonate buffer and 75iL of xanthine oxidase added. The absorbance was determined at 500nm for 3mins at 20 seconds interval. The changing rate of absorbance was used to determine superoxide dismutase activity.

Glutathione: This was determined according to the method of Stanley and Venugopal [14].

A volume, (0.1ml) of the sample was mixed with 0.9ml of distilled water in a beaker. A volume, (0.02ml) of 2% sodium sulphite was added to the sample, shaken and allowed to stand for 2mins at room temperature. A known

volume, (0.02ml) of 20% Lithium sulphate, 0.2ml of phosphor-18- tungstic acid was also added to the beaker. It was shaken and allowed to stand for 4mins while observing it for maximum colour development. A volume, (2.5ml) of 2% sodium sulphite was added and the absorbance was taken at 680nm within 10mins against a blank of 0.1ml of water. Glutathione concentration was calculated from a standard cysteine curve.

Statistical Analysis: Data were presented as mean of three replicates \pm SD. Statistical analysis was carried out using Statistical Package for Social Sciences (SPSS) version 19. One way analysis of variance was adopted for comparison and the results were subject to post hoc test using least square deviation (LSD). The data were expressed as mean \pm standard deviation. $P < 0.05$ was considered significant.

RESULTS

Effect of the Ethanol Extract of *Piper Nigrum* Leaves on Lipid Peroxidation (Malondialdehyde) Leaves of Rats:

Fig.1 shows the effect of the ethanol extract of *Piper nigrum* leaves on MDA levels of the rats after induction and treatment of diabetes. The was significant ($P < 0.05$) increase in level of MDA in group II (Diabetic untreated) when compared to group I (Normal control) indicating that the animals were oxidatively stressed but the administration of graded doses of *Piper nigrum* significantly ($P < 0.05$) decreased the MDA levels in groups IV, V and VI in a dose dependent manner when compared to group II.

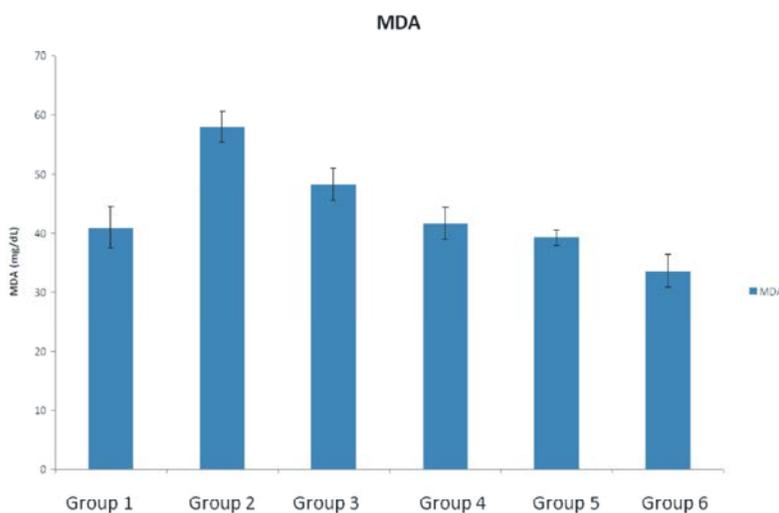


Fig 1. The effect of the ethanol extract of *Piper nigrum* leaves on MDA levels of rats.

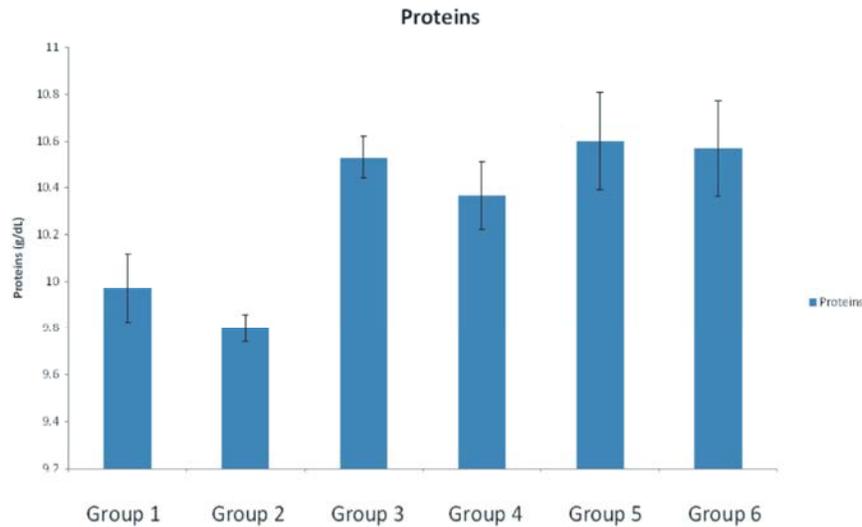


Fig. 2: The effect of ethanol extract of *Piper nigrum* leaves on total serum proteins of rats.

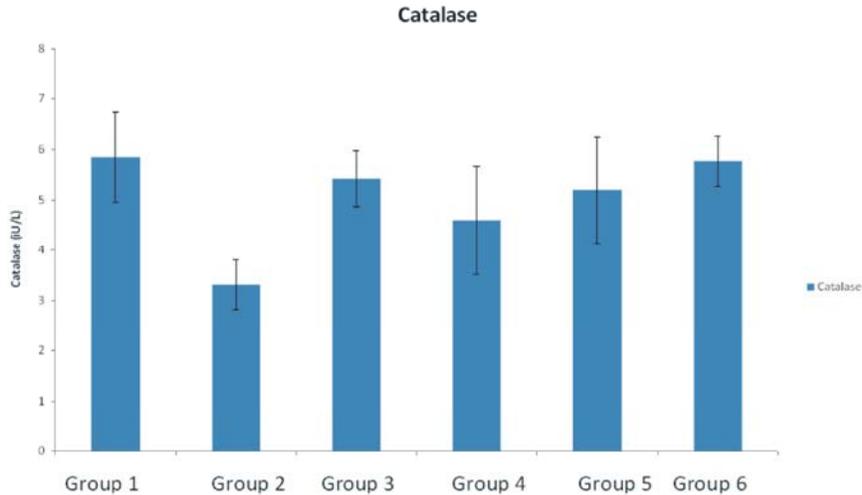


Fig. 3: The effect of ethanol extract of *Piper nigrum* leaves on serum catalase activity in rats.

Effect of the Ethanol Extract of *Piper Nigrum* Leaves on Total Serum Protein of Rats: Figure 2 shows the mean total serum protein of the rats after induction and treatment of diabetes. There was a significant ($p > 0.05$) decrease in the total serum protein of group II (Diabetic untreated) when compared to group one (normal control) which indicates peroxidation of protein moieties but the administration of *Piper nigrum* extract and the standard drug (Glibenclamide) significantly ($P < 0.05$) increased and restored to normalcy the total serum protein of the rats in groups III, IV, V and VI when compared to that of group II.

Effect of the Ethanol Extract of *Piper Nigrum* on the Catalase Activity in Rats: Fig. 3: shows that there was a significant ($p > 0.05$) decrease in the serum catalase activity

of group II (Diabetic untreated) when compared to group one (Normal control) which indicates depletion of antioxidant enzyme catalase but the administration of *Piper nigrum* extract and the standard drug (Glibenclamide) significantly ($P < 0.05$) increased in a dose dependent manner and restored to normalcy the serum catalase activity of the rats in groups III, IV, V and VI when compared to that of group II.

Effect of Ethanol Extract of *Piper Nigrum* Leaves on Serum Superoxide Dismutase (SOD) Activity in Rats: Fig. 4 shows that there was a significant ($p > 0.05$) decrease in the serum catalase activity of group II (Diabetic untreated) when compared to group one (Normal control) which indicates depletion of antioxidant enzyme SOD but the administration of *Piper nigrum* extract and the

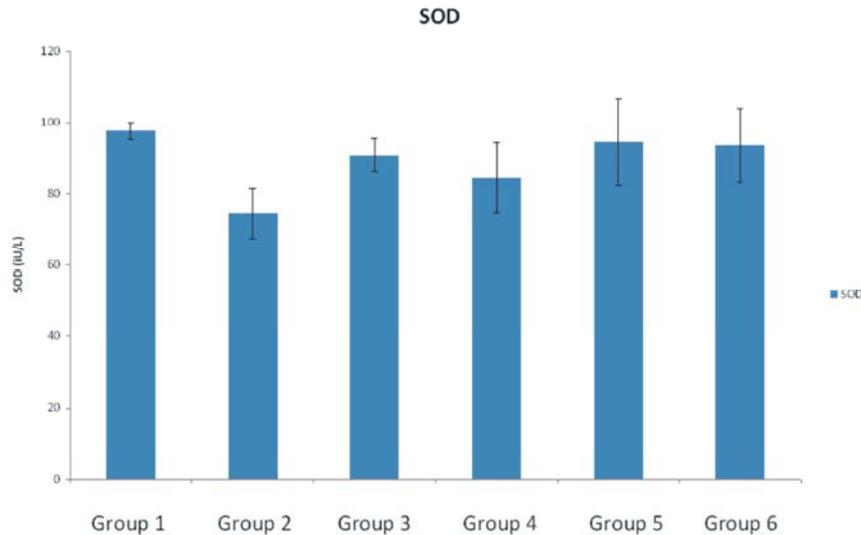


Fig. 4: Effect of the ethanol extract of *Piper nigrum* leaves on serum Superoxide Dismutase (SOD) activity in rats.

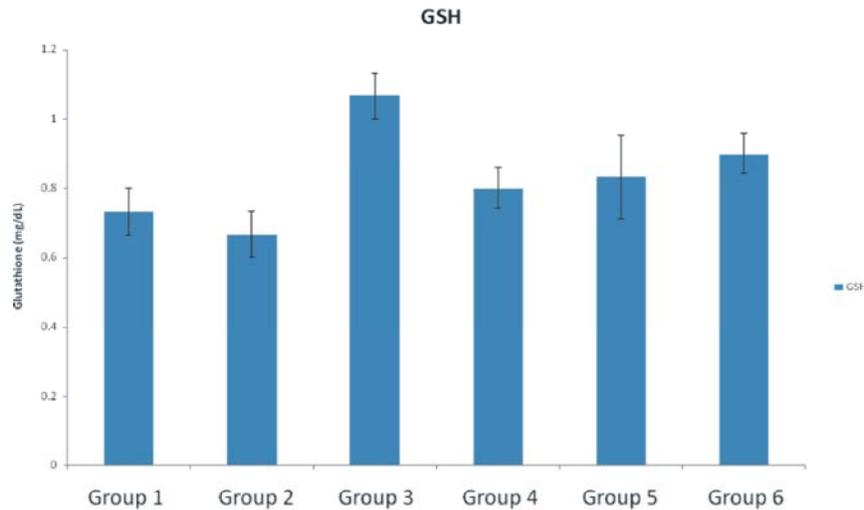


Fig. 5: The effect of ethanol extract of *Piper nigrum* leaves on serum glutathione (GSH) concentration of rats.

standard drug (Glibenclamide) non significantly ($P > 0.05$) increased in and restored to normalcy the serum catalase activity of the rats in groups III, IV, V and VI when compared to that of group I and II.

Effect of the Ethanol Extract of *Piper Nigrum* Leaves on Serum Glutathione (GSH) Concentration of Rats: Fig. 5 shows that there was a non significant ($p > 0.05$) decrease in the serum GSH level of group II (Diabetic untreated) when compared to group one (Normal control) which indicates depletion of GSH level but the administration of *Piper nigrum* extract non significantly ($p < 0.05$) increased in a dose dependent manner and restored to normalcy the serum GSH level of the rats in groups III, IV, V and VI when compared to that of group II except

group III (Glibenclaminde) which showed a significant increase ($p < 0.05$) when compared to Groups I and II.

DISCUSSION

Serum MDA has been used as a biomarker for lipid peroxidation and has served as an indicator of free radical damage. Additionally, MDA can interact with several functional groups on proteins and lipoproteins, altering their chemical behaviour and possibly contributing to carcinogenesis and mutagenesis [15]. Due to its highly reactive nature, MDA also functions as an electrophile that can cause toxic stress within the cell and is, therefore a potent maker for measuring the overall level of oxidative stress within an organism [16, 17].

The increased lipid peroxidation during diabetes as found in the present study (Figure 1) could be due to the inefficient antioxidant system seen in diabetes. The status of lipid peroxidation (Figures 1 and 2) as well as altered levels of certain endogenous radical scavengers is taken as direct evidence for oxidative stress [18]. Free radicals are scavenged by antioxidant enzymes like SOD and catalase and these protect the body from oxidative stress [19].

The decrease in the activity of the antioxidant enzymes in the present study could be attributed to the excessive utilization of these enzymes in attenuating the free radicals generated during the metabolism of alloxan. Similar reports have also shown an elevation in the status of lipid peroxidation in the liver after alloxan induction [20]. The increase in the concentration of the antioxidant enzymes as shown in figures 3 and 4 and non antioxidant enzyme (Figure 5) after 21 days of administration of the extract could be attributed to the ability of the enzymes to scavenge ROS, thus preventing further damage to membrane proteins. Therefore, the antioxidant properties of *Piper nigrum* extract might have resulted in the recoument of the activities of the enzymic antioxidants (SOD and catalase). Non enzyme antioxidants act synergistically to scavenge the free radicals formed in the biological system [21]. After 21 days of treatment, *Piper nigrum* extract effectively restored the depleted level of this non-enzyme antioxidant caused by alloxan induction. Increase in GSH level in turn contributes to the recycling of other antioxidants such as vitamin C and E [22].

CONCLUSION

The finding of this study shows that *Piper nigrum* extract ameliorates free radicals generated by alloxan induction and improves the antioxidants status of the stressed animals.

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