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Effect of *Piper nigrum* Leaves Extract on Lipid Profile Status on Alloxan Induced Diabetic Rats

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Abstract: This study evaluated the effects of extract from *Piper nigrum* leaves on lipid profile in diabetic rats. The results confirmed that the untreated diabetic rats (group II) were subjected to oxidative stress as indicated by significant (p<0.05) increase in total cholesterol, low density lipoprotein (LDL), triglycerides and significant (p <0.05) decrease in serum high density lipoprotein (HDL) levels when compared with apparently healthy rats (group I). After 21 days treatment, the ethanol extract of *Piper nigrum* slight decreased the total cholesterol, LDL and triglycerides while the HDL concentration was not significantly (p >0.05) increased. These effects were dose dependent. The study indicates that *Piper nigrum* to some extend normalized the lipid profile status in alloxan induced diabetic rats.

Key words: Oxidative Stress · *Piper nigrum* · Total Cholesterol · HDL · LDL

INTRODUCTION

Free radicals, also known simply as radicals, are organic molecules responsible for ageing, tissue damage and possibly some diseases. These molecules are very unstable, therefore they look to bond with other molecules, destroying their health and further continuing the damaging process [1]. Free radicals are "free" because they float around until they stabilize and "radical" in the sense that there are a wide variety of molecules from which they can take an electron. The damage doesn't stop there, however, as the new molecule e.g. a piece of a cells wall, is now also missing an election and has become another free radical. This snowball effect can wreak havoc on healthy tissues [1]. Oxidative stress is increased in diabetes mellitus owing to an increase in the production of oxygen free radicals and insufficiency in antioxidant defense mechanisms [2].

Lipid peroxidation is the process in which free radicals "steal" electrons from the lipids in cell membranes, resulting in cell damage. This process proceeds by a free radical chain reaction mechanism [3, 4]. Lipid peroxidation is an autocatalytic free radical mediated process whereby poly-unsaturated fatty acids in cell membranes undergo degradation to form lipid hydro-peroxides [5]. Lipid peroxidation triggers the loss of membrane integrity causing increased cell permeability, enzyme inactivation and structural damage to DNA and cell death [6].

Piper nigrum (Black pepper) possesses anti-tumorigenic, immuno-stimulatory, stomachic, carminative, anticholesterolaemic properties and again known for its strong phytochemcial activities [7]. Piperine, a substance present in black pepper has been found to increase the absorption of selenium, B-complex vitamins, beta-carotene, circumin 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 [8]. In respect to its numerous usages, the present study is aimed at evaluating the effects of *Piper nigrum* ethanol leaves extract on lipid profile status in diabetic rats.

MATERIALS 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.

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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-sold 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.

Total Cholesterol Determination: In clinical chemistry, lipids have been associated with lipoprotein metabolism and atherosclerosis. The method of Allain *et al.* [1] was used. This involves hydrolysis of the cholesterol by hydrolysis of the cholesterol esters.

Principle: Cholesterol is determined after enzymatic hydrolysis and oxidation. The indicator quinonemine is formed from hydrogen peroxidase and 4 - aminoantipyrine in the presence of phenol and peroxidase.

Procedure of the Test: Three (3) test tubes were set up in a test tube rack and labeled blank, standard and sample respectively. To the blank was added 10ml of distilled water, 10ml standard to the standard tube and 10ml sample to the sample tube. To each of these tubes were added 1000ml of the cholesterol reagent. The tubes were thoroughly mixed and incubated for 10minutes at room temperature or 5minutes at 37°C in a water bath. The absorbances were taken at 540nm against the blank. Cholesterol mmol/L = $\frac{Abs\ of\ test}{Abs\ of\ std}$ x2 200 x 0.0259.

Low Density Lipoprotein (LDL)
Principle: LDL can be determined as the difference between total cholesterol and the cholesterol content of the supernatant after precipitation of the LDL fraction by polyvinyl sulphate (PVS) in presence of polyethyleneglycol monomethyl ether.

Procedure: Three (3) drops (i.e. 0.1ml) of the precipitant solution was added to a test tube and 0.2ml of the sample was added. The test tube was allowed to stand for 15minutes at room temperature after which it was centrifuged at 2000 xg for 15mins. The cholesterol concentration of the supernatant was then determined as was done for the total cholesterol.

LDL (mmo/L) = Total cholesterol (mmol/L) - 1.5 x supernatant cholesterol (mmol/L).

High Density Lipoprotein (HDL)
Principle: LDL and VLDL (Very Low Density Lipoprotein) are precipitated from serum by the action of a polysaccharide in the presence of divalent cations. Then, high density lipoprotein cholesterol (HDL) present in the supernatant is determined.

Procedure: To one drop of the precipitant solution was added 0.3ml of the serum sample. The tube was mixed thoroughly and allowed to stand for 15mins at room1

temperature. It was then centrifuged at 2000 x g for 15mins after which the cholesterol concentration of the supernatant cholesterol was determined as for the total cholesterol.

Triglycerol Determination

Clinical Significance: Triglycerol measurements are used in the diagnosis and treatment of diseases involving lipid metabolism and various endocrine disorders e.g. diabetes mellitus, nephrosis and liver obstruction.

Principle: The triglycerols are determined after emzymatic hydrolysis with lipases. The indicator is a quinoneimine formed from hydrogen peroxide, 4 - aminophenazone and 4 - chlorophenol under the catalytic influence of peroxidase.

$$\text{Triglycerol} + H_2O \xrightarrow{\text{lipases}} \text{Glycerol} + \text{fatty acids}$$

$$\text{Glycerol} + ATP \xrightarrow{GK} \text{Glycerol - 3 - phosphate} + ADP$$

$$\text{Glycerol - 3 - phosphate} + O_2 \xrightarrow{GPO} \text{Dihydroxyacetone phosphate} + H_2O_2$$

$$2H_2O_2 + \text{4 - aminophenazone} + \text{4 - chlorophenol} \xrightarrow{POD} \text{Quinoneimine} + Hcl + 4H_2O.$$

Method: A known volume, (1.0ml) of the triglycerol reagent was put into 3 tubes labeled as blank, standard and sample. Ten microlitres (10µL) of water was added to blank, 10µL of standard solution to the standard tube and 10µL of sample to the sample test tube. The tubes were allowed to stand for 10mins at room temperature for 5mins at 37°C in a water bath. The absorbances were read at 540nm against the blank and the triglycerol concentration calculated thus:

$$\text{TAG concentration (mmol/L)} = \frac{\text{Abs of sample}}{\text{Abs of standard}} \text{ x concentration of standard (mmol/L).}$$

Statistical Analysis: Data were presented as mean of three replicates ± 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 ± standard deviation. $P< 0.05$ was considered significant.

RESULTS

Effect of Ethanol Extract of *Piper Nigrum* Leaves on Serum Total Cholesterol of Rats: Fig. 1 shows the effect of the ethanol extract of *Piper nigrum* leaves on total cholesterol concentrations of the rats after treatment of diabetes. There was a non significant ($P<0.05$) increase in level of total cholesterol concentrations 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 total cholesterol concentration in group IV when compared to group II. Groups III, V and VI were also non significantly ($P >0.05$) decreased when compared to group II.

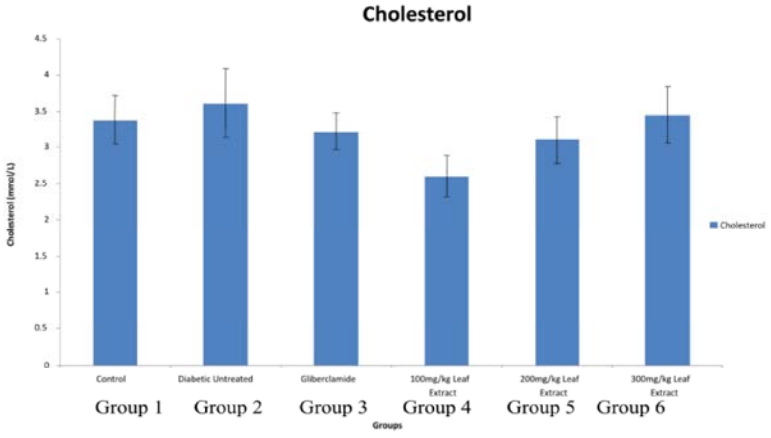

Fig. 1:The effects of ethanol extract of Piper nigrum leaves on serum total cholesterol of rats.

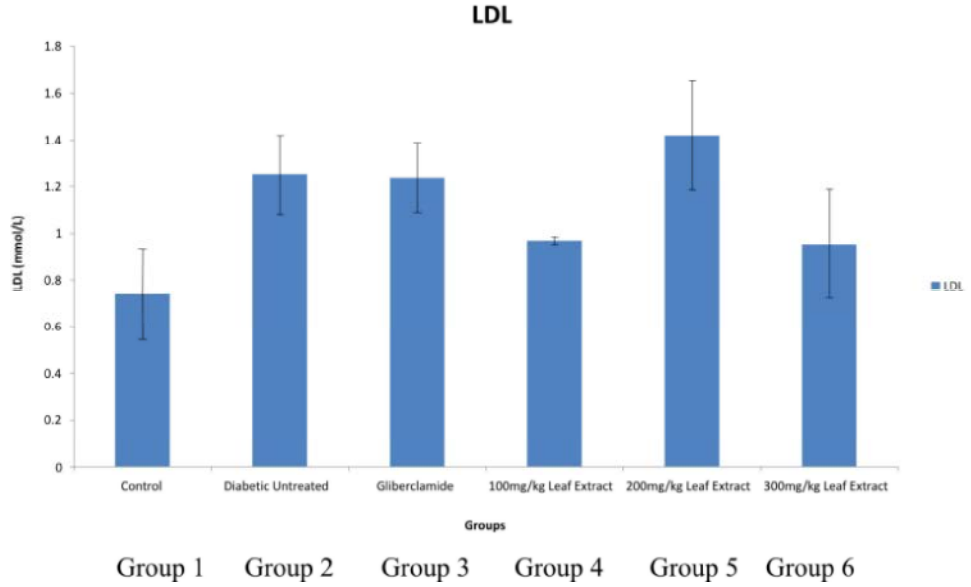

Fig. 2: The effects of the ethanol extract of Piper nigrum leaves on serum low Density Lipoprotein (LDL) of rats.

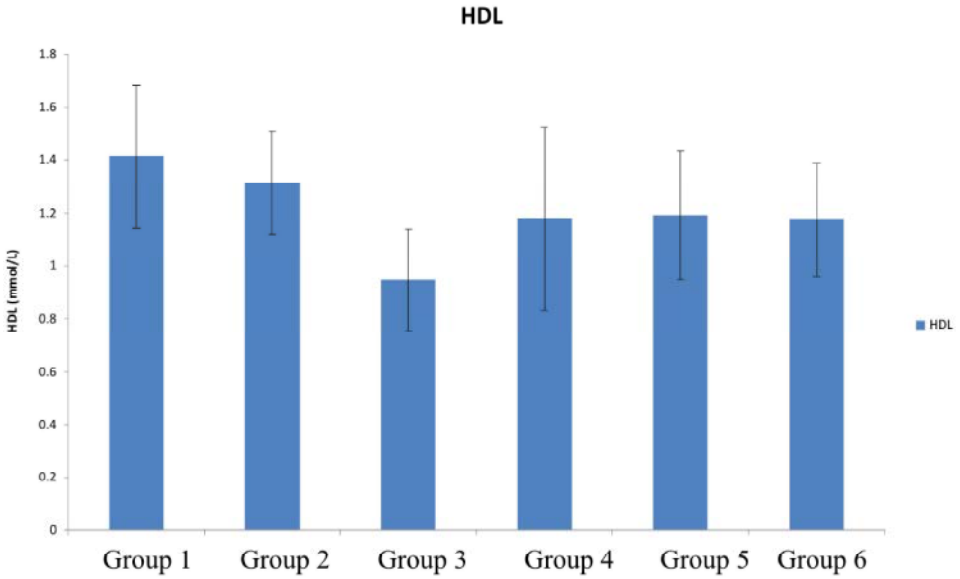

Fig. 3: The effects of ethanol extract of piper nigrum leaves on High Density Lipoprotein (HDL) of rats.

Effects of the Ethanol Extract of *Piper Nigrum* Leaves on Serum Low Density Lipoproteins (Ldl) of Rats: Fig. 2 shows the effect of the ethanol extract of *Piper nigrum* leaves on LDL concentrations of the rats after treatment of diabetes. There was a significant (P <0.05) increase in level of LDL concentrations 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* non significantly (P >0.05) decreased the LDL concentration in groups IV and VI when compared to group II. Group V showed significantly (P <0.05) increase when compared to groups I and II.

Effects of the Ethanol Extract of *Piper Nigrum* Leaves on Serum High Density Lipoprotein (HDL) of Rats: Fig. 3 shows the effect of the ethanol extract of *Piper nigrum* leaves on HDL concentrations of the rats after treatment of diabetes. There was a non significant (P >0.05) decrease in level of HDL concentrations in group II

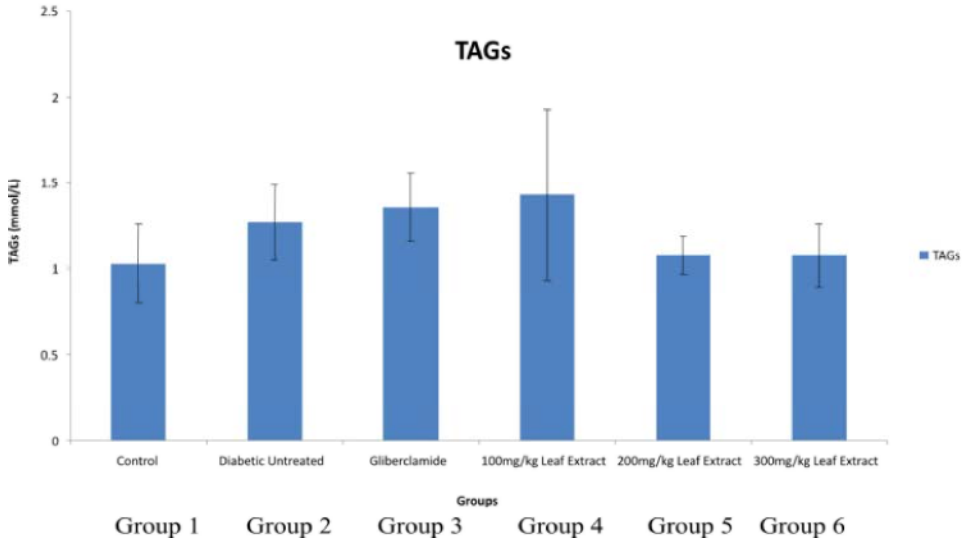

Fig. 4: The effects of ethanol extract of Piper nigrum leaves on serum triglyceride concentrations of rats.

(Diabetic untreated) when compared to group I (Normal control). The administration of graded doses of *Piper nigrum* did not significantly (P >0.05) increase the HDL concentration in the treated groups when compared to groups I and II.

Effects of Ethanol Extract of *Piper Nigrum* Leaves on Serum Triglycerides Concentrations of Rats: Fig. 4 shows the effect of the ethanol extract of *Piper nigrum* leaves on serum triglycerides concentrations of the rats after treatment of diabetes. There was a non significant (P >0.05) increase in level of serum triglycerides concentrations in group II (Diabetic untreated) when compared to group I (Normal control). The administration of graded doses of *Piper nigrum* did not significantly (P >0.05) decrease the serum triglycerides concentration in the treated groups (V and VI) when compared to group I.

DISCUSSION

The increased risk of coronary artery disease in subjects with diabetes mellitus can be partially explained by the lipoprotein pattern abnormalities associated with diabetes mellitus [9]. Hypertriglyceridaemia and low levels of high density lipoprotein (HDL) are the most common lipid abnormalilties [10]. The non-insulin dependent diabetic (NIDDM) patient with mild fasting hyperglycaemia commonly has mild hypertriglyceridaemia due to overproduction of triglyceride-rich lipoproteins in the liver, associated with decreased HDL cholesterol levels [11]. The most typical lipoprotein pattern in diabetes also known as diabetic dyslipidaemia or atherogenic dyslipidaemia, consists of moderate elevation in triglyceride (TAG) levels, low HDL cholesterol values and elevated low density lipoprotein (LDL) cholesterol [12].

A variety of alterations in metabolic and regulatory mechanism, due to insulin deficiency or due to insulin resistance are responsible for the observed accumulation of lipids [13]. Alloxan-induced diabetes also developed hyperlipidaemia which is in agreement with our previous observations [14]. In the present study, the extract did not significantly reduce (P >0.05) the total cholesterol (Fig. 1), LDL (Fig. 2) and TAG (Fig. 4) with no significant increase in HDL (Fig. 3) in treated rats compared to the untreated diabetic rats. This is in contrast with the report of Vijayakumar *et al.* [15] who fed rats with high fat diet. The increased level of serum lipid in diabetes represents a great risk for coronary heart disease [16]. In insulin deficient diabetes, the concentration of serum free fatty acids is elevated as a result of free fatty acids outflow from fat depots. Thus an excess fatty acid in the plasma produced by alloxan induced diabetes promotes the conversion of excess fatty acids into phospholipids and cholesterol in the liver, TAGs formed in the liver may be discharged in the liver in form of lipoproteins [17].

CONCLUSION

Diabetes mellitus has been a serious disease in Africa and the whole world. Many plants have been neglected and underutilized with no or little knowledge of their usefulness in the field of medicine and *Piper nigrum* is

one of these plants. This study indicates that *Piper nigrum* to some extend normalized the lipid profile status in alloxan induced diabetic rats and could be used as an adjunct in the management of hyperlipidemic conditions in diabetic cases.

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