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Annals of Biological Research, 2014, 5 (6):26-31
(http://scholarsresearchlibrary.com/archive.html)

Annals of Biological Research
Scholars Research Library
ISSN 0976-1233
CODEN (USA): ABRNBW

Hypoglycemic Potentials of Ethanol Leaves Extract of Black Pepper (*Piper Nigrum*) on Alloxan-Induced Diabetic Rats

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ABSTRACT

The effect of ethanol leaves extract of Piper nigrum on some biochemical parameters in alloxan induced diabetic rats, was carried out. The graded doses of ethanol leaves extract of this plant was fed to alloxan-induced diabetic rats for a period of 21days and its effect on some biochemical parameters on the blood serum of the rats were assayed. Thirty (30) male albino rats were divided into six groups: normal rats (group I), diabetic untreated rats (group II), diabetic rats treated with glibenclamide (group III), diabetic rats treated with 100 mg/kg body weight of ethanol leaves extract (group IV), diabetic rats treated with 200 mg/kg body weight of ethanol leaves extract (group V) and diabetic rats treated with 300 mg/kg body weight of ethanol leaves extract (group VI). The result reviewed significant ($p<0.05$) decreases in fasting blood sugar (FBS) levels at day 7, 14 and 21 of the treated groups when compared to group 2 (diabetic untreated). There was a dose dependent non significant ($p>0.05$) decrease in sorbitol concentration in group IV when compared to group II while the glycosylated hemoglobin also had a non significant ($p>0.05$) decrease in all the treated groups when compared to group II. The result of this study indicates that ethanol leaves extract of Piper nigrum has hypoglycemic tendencies in diabetic conditions.

Keywords: *Piper nigrum*, Hypoglycemia, Alloxan, Glycosylation, Blood sugar.

INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by high levels of glucose in the blood due to impaired secretion of insulin or insulin insensitivity [1]. Diabetes mellitus affects approximately 4% of the population worldwide and is expected to increase by 5.4% in 2025 [2]

Glycosylated haeomglobin (HbA1c) concentrations have been shown to be related to physicians' ratings of diabetic control [3], fasting blood glucose concentrations, post prandial blood glucose concentrations, mean blood glucose concentrations during monitoring in hospital, peak blood glucose concentrations during monitoring in hospital, and home monitoring of blood glucose concentrations [4]. It thus reflects integrated glucose values over the preceding 6-8weeks in those patients. HbA1c is an index used in the management of patients with diabetes. In those patients with uncontrolled diabetes, the ratio of glycosylated haemoglobin to non-glycosylated haemoglobin is higher than in non-diabetics and diabetics in good control, or it may aid in the initial diagnosis of the disease. HbA1c measurements rely on a predictable effect of glucose concentration on haemoglobin (Hb) over a normal red blood cell (RBC) lifespan; however, any condition that alters RBC survival may invalidate HbA1c as an accurate measure of glycaemic control. Risk of misdiagnosis in those with iron deficiency anaemia is increased. Iron deficiency anaemia

is associated with higher concentrations of HbA1c among paediatric patients with type 1 diabetes despite similar levels of glycaemia [5].

According to India Herbal Medicine (Ayurveda), *Piper nigrum* (black pepper) possesses anti-tumorigenic, immuno-stimulatory, stomachic, carminative, anticholesterolaemic properties and again known for its strong phytochemcial activities [6]. 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 [7]. In respect to its numerous usages, the present study is aimed at evaluating the hypoglycemic effects of *Piper nigrum* ethanol leaves extract on alloxan induced 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.

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^{o}C.

Experimental induction of diabetes

The baseline blood glucose levels were determined before the induction of diabetes. The rats were fasted overnight prior to injection of alloxan dissolved in iced cold normal saline at a dose of 150 mg/kg body weight and the route of administration was intraperitoneal. Blood samples were taken from the tail vein 72 h after the alloxan injection to measure the blood glucose levels by ACCU-Check glucose meter. Animals with blood glucose levels (after fasting for 12 h) over 200 mg/dl were considered diabetic and used for the further study [8]. The treatment lasted for twenty one (21) days in which blood glucose levels of the animals were determined at the beginning and at the end of the study.

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 procedures 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 baseline blood glucose levels were determined before the induction of diabetes. The rats were fasted overnight prior to injection of alloxan dissolved in iced cold normal saline at a dose of 150mg/kg body weight intraperitoneally. After 3 days, rats with blood glucose levels greater than 200mg/dl were considered diabetic and used for the investigation [8]. The treatment lasted for twenty one (21) days in which blood glucose levels and body weight of the rats were taken on day 0,7,14 and 21. 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 (-20^0C) until estimation of some biochemical parameters.

Estimation of the chosen biochemical parameters
All the chosen biochemical parameters were estimated using biodiagnostic kits and the procedures were strictly followed as outlined in the manual guide.

Sorbitol determination
This was determined according to the method of [9]. A volume, 2ml of the buffer solution was pipetted into a test tube. One millilitre (1ml) of sample was added and then 0.1ml of Nicotinamide Adenine dinucleotide solution added. The tube was mixed and the extinction E1, at 340nm was measured.

A volume, 0.05m of sorbitol dehydrogenase was added and the tube mixed again and after, allowed to stand for 60mins after which the extinction E2 at 365nm was read again.

Glycosylated Haemoglobin
The method of [10] was used in the determination of the glycosylated haemoglobin levels. A volume, (0.5ml) of lysing reagent was added to tubes labeled as test and standard and 0.1ml of whole blood sample was also added. The tube was mixed and allowed to stand for 5mins to lyse. A volume, (3ml) of the ion –exchange resin was added to another tubes labeled as test and standard and 0.1ml of the lysate was added to the tubes. A resin separator was inserted into each tube, the tubes were mixed continuously or a rocker for 5mins and then allowed to settle. The supernatants were then poured out directly into a cuvette and the absorbances taken at 415nm against distilled water. Also, 5ml of distilled water was put into another tubes labeled as test and standard and 0.2ml of the lysate from the first step was added and mixed. The absorbance was taken to determine the total haemoglobin.

Calculation:

$$\text{Ratio of Test} = \frac{Abs\ of\ HbA1c}{Abs\ of\ THb}$$

HbA1c in % = Ratio of test x 10

Statistical Analysis
Data were reported as means ± SEM, where appropriate. Both one- and two- way analysis of variance (ANOVA) were used to analyze the experimental data and Duncan multiple test range was used to compare the group means obtained after each treatment with control measurements. Differences were considered significant when $p < 0.05$.

RESULTS

Results of the effect of the ethanol extract of *Piper nigrum* leaves on the fasting blood glucose levels of rats before and after induction of diabetes with treatments.
The figure below shows the mean fasting blood glucose levels of rats fed graded doses of ethanol extract of *Piper nigrum* leaves. The baseline glucose levels showed that the rats were non diabetic before the induction of diabetes. After the induction of diabetes, a significant decrease ($P<0.05$) was observed in the mean blood glucose levels of rats in group III,IV and V when compared to the group II (diabetic untreated) after day 7 and 14, while after day 21, the decrease was significant in all the test animals including group VI.

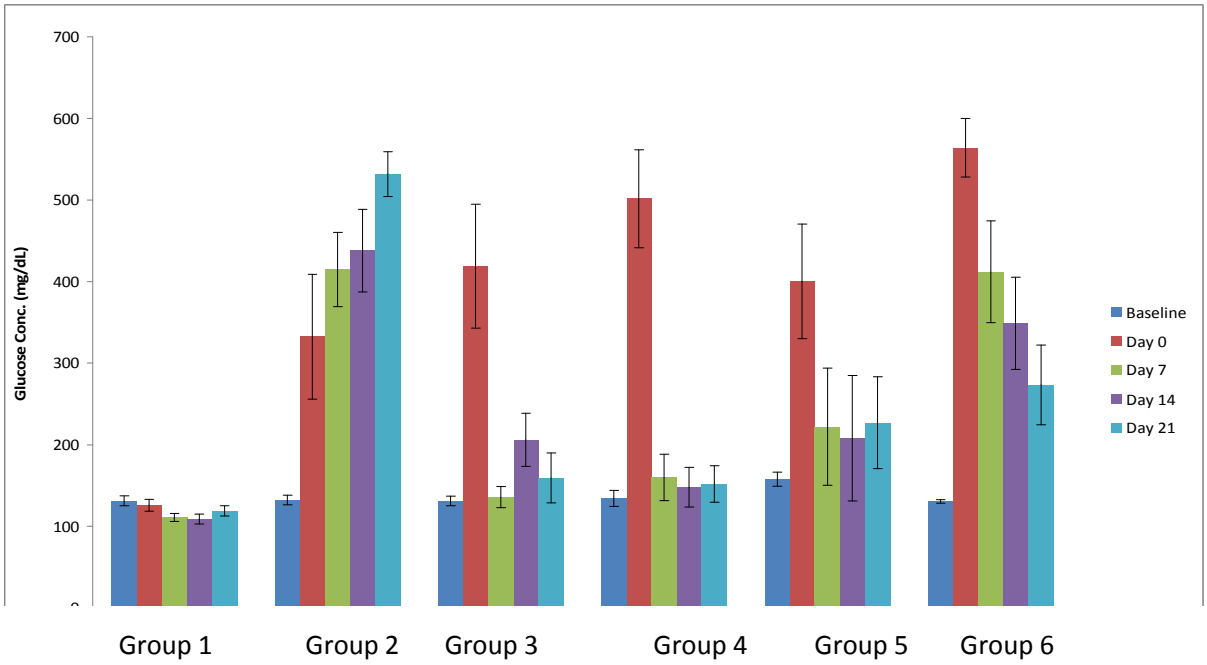

Fig.1: Bar chart showing the effect of the ethanol extract of *Piper nigrum* leaves on fasting blood glucose levels of rats before and after induction of diabetes.

Results of the effects of ethanol extract of *Piper nigrum* leaves on sorbitol levels of rats

Fig.2 shows that there were no significant decrease (P>0.05) in the sorbitol levels of the animals in all the test groups with exception of group IV which showed a significant decrease (P<0.05) in sorbitol level when compared to groups I and II.

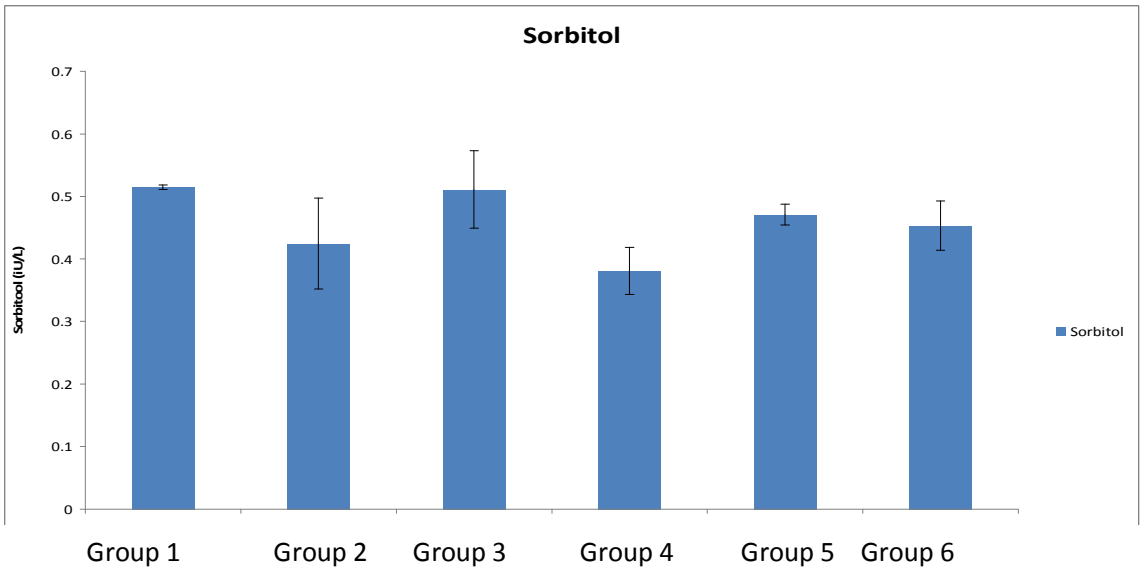

Fig. 2: Bar charts showing the effects of the ethanol extract of *Piper nigrum* leaves on sorbitol levels of rats.

Results of the effects of ethanol extract of *Piper nigrum* leaves on glycosylated haemoglobin levels of rats.

Figure 3 shows the bar chart of mean glycosylated haemoglobin levels of the rats after induction of diabetes. Those in group II showed a significant increase (P<0.05) in glycosylated haemoglobin levels when compared to group I.

There was a decrease in groups III, IV, V and VI when compared to that of group II but the decrease was not statistically significant.

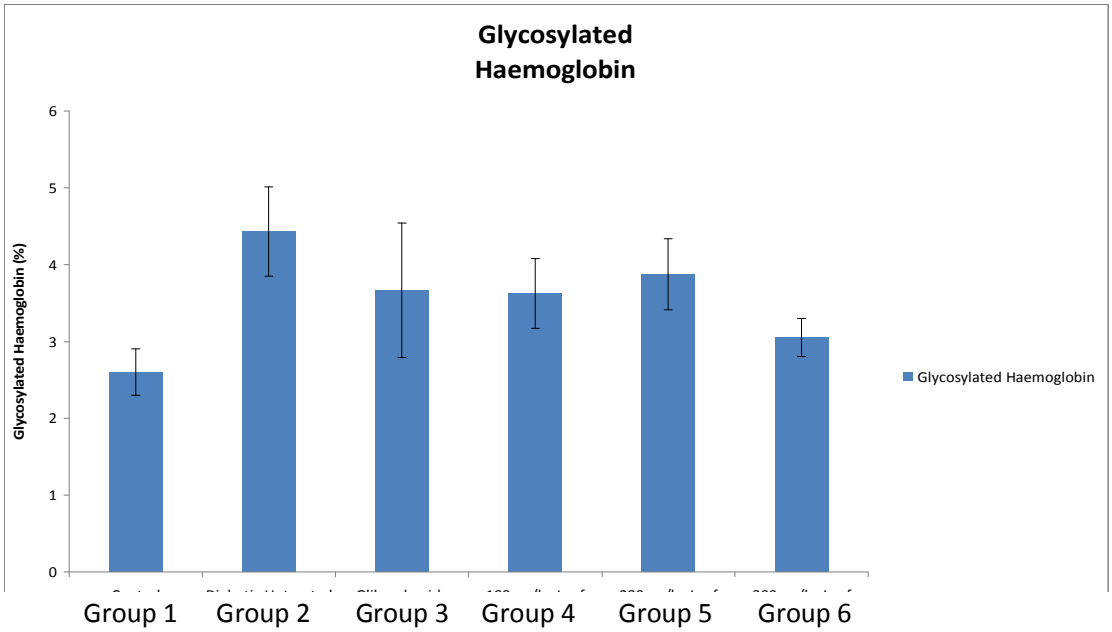

Fig.3: Bar charts showing the effects of ethanol extract of *Piper nigrum* leaves on glycosylated haemoglobin level s of rats.

DISCUSSION

Glucose level increases in diabetic condition have been previously reported by other researchers [11][12]. Also, increase in glucose level is known to be associated with oxidative stress [13]. Thus, the increased blood glucose level in group II (diabetic not treated) may be as a result of intrinsic oxidative stress in diabetic condition caused by alloxan injection as shown in Figure 1. The reduction in the blood glucose level in the test groups which was significant ($p<0.05$) suggesting that the extracts contain antioxidants which possibly countered oxidative stress in the experimental animals. Earlier report by Dekker *et al*. [14] showed that antioxidant vitamins (A, C, and E) act synergistically to reduce blood glucose level. This observation laid credence to the report of Chung *et al.* [15] that these nutrients played protective roles against oxidative stress in alloxan induced diabetic rats. Hyperglycemia is associated with the generation of ROS causing oxidative damage particularly to the heart, kidney, eyes, nerves, liver, small and large vessels and gastro intestinal system [16].

Twenty one (21) days treatment with 100, 200 and 300mg/kg body weight of the extract and a standard drug (glibenclamide) lowered elevated fasting blood sugar level, which was reported high in diabetic control animals. The significant reduction ($P<0.05$) in the blood glucose levels of the test animals suggested that the extract which has already been shown to contain some antioxidants was able to lower blood sugar possibly because of its antioxidant properties. This observation is in line with the report of Gulcin, [6] who stated that these nutrients played protective roles against oxidative stress in alloxan induced diabetic rats. Thus, the ethanol extract of *Piper nigrum* proved to have hypoglycaemic activity in diabetic rats, which was comparable to the standard drug (glibenclamide) used.

The possible mechanisms of hypoglycaemic action may be by increasing either the pancreatic secretion of insulin from β-cell of islet of Langerhans or its release from pro-insulin form [17]. Often hyperglycaemia is associated with the generation of ROS causing oxidative damage and also increased production of sorbitol which also causes damage to the kidney, eyes, nerves and cardiovascular system. [18]. The significant decrease ($P<0.05$) in sorbitol levels in group IV showed that *Piper nigrum* extract at the concentration of 100mg/kg body weight was able to reduce the production of sorbitol in diabetic conditions (Figure 2) which is advantageous in reducing diabetic complications [19]

Reactive oxygen species has also been implicated in the mechanism of red cells damage [20]. During diabetes the excess glucose present in blood reacts with haemoglobin. So the total hemoglobin level is decreased in alloxan diabetic rats [13]. The glycosylated haemoglobin level is usually increased and thus the glycosylated haemoglobin (HbAlc) is an index for monitoring the control of diabetes [21]. The non-significant decrease ($P>0.05$) in glycosylated haemoglobin in the tested animals when compared to that of the diabetic control showed that *Piper nigrum* extract was not able to sufficiently lower the HbAlc levels. This may be because of the time interval of the application of the extract since the lifespan of Rbcs is one hundred and twenty days, so the red cells that were already glycated had not died off before the analysis were carried out as indicated by Selvin *et al*. [22]. Glycosylated haemoglobin is used as a marker for estimating the degree of protein glycation in diabetes mellitus [4]. HbAlc was found to increase in patients with diabetes mellitus and the amount of increase is directly proportional to the fasting blood glucose level [23].

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. In this study, the leaves of the plant have been shown to possess anti-diabetic properties. This provides scientific evidence that the plant can safely be used in the treatment and management of diabetes.

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