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In Vivo Anti-Diabetes Potential of Huntera Umbellata Seed

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ABSTRACT

The possible use of ethanol extract of H*unteria umbellata* seed as a curative agent in alloxan-induced diabetic rats was investigated. A total of forty-eight adult albino Wistar rats (male: weighed 150 g – 250 g; aged 16 – 19 weeks) were used for this study. Completely randomized design was used for this experiment. Standard methods were used to ascertain the blood glucose levels, kidney function test, serum lipid profile, and liver enzymes. Also, the radical scavenging activity was carried out using the DPPH (2,2-diphenyl-1-picyhydrazyl) method. The results showed that the administration of alloxan has led to significantly increased blood glucose levels in rats. The diabetic control group had constant high blood glucose level that was >250 mg/dl, whereas a significant decrease in blood glucose level was witnessed when the rats were pre-treated with the seed extract. Again, the extract was found to reduce certain biochemical parameters such as overall cholesterol, triglyceride, low density lipoprotein, alanine aminotransferase, alkaline phosphatase, urea and creatinine. while high density lipoprotein was increased. Also, the radical scavenging activity (RSA) study revealed that the seed has inhibitory concentration (IC_{50}) of 227.34 µg/ml as compared to the IC_{50} of superoxide dismutase (389.73 µg/ml).The observed effects of the extract could be possibly due to its ability to reduce oxidative damage to insulin-producing β-cell membranes, thus, resulting in increased insulin concentration and sensitivity in the cells. Therefore, in this study it was suggested that ethanol extracts of osu seeds could be a potential candidate for the management of diabetes due to its hypoglycaemic effects.

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Introduction

Diabetes mellitus is a group of metabolic disorders characterized by hyperglycemia and an annual increase in recorded cases across the globe [1]. In addition to the long term complications affecting the retina, the kidney, and the nervous system, diabetes mellitus is associated with disturbances of glucose, protein, and fat metabolism, together with long term complications affecting the retina, the kidney, and the nervous system [2]. Center for Disease Control in their National Diabetes Statistics Report, posited that 133 million Americans have diabetes or prediabetes – almost half of the United States population, and 84 % of people with prediabetes do not know they have it. More so, IDF reported that in Nigeria, over 3 million people are living with diabetes, and the number was estimated to increase to 8 million by the year 2045 [3-4].

Impaired glucose metabolism is a chronic disease of diabetes mellitus. If the pancreas does not make enough insulin to cover all of the blood glucose absorbed by cells or if there is insufficient use in the body of manufactured insulin, this may result in reduced glucose metabolism [5]. This results in a condition known as hyperglyceamia (high blood glucose level) which can trigger oxidative damage of cell membranes through the production of oxidative radicals such as superoxide anions, thus generating hydroxyl radicals via Haber-Weiss reaction, including protein glycations and membrane lipid peroxidation [1] .These radicals can result in necrosis of the cells and can further cause derangement of proteins, carbohydrates, lipids and DNA [5]. Consequently, herbal preparation with anti-oxidative properties will be useful in the management of diabetes. Mostly, this is because of the many phytochemicals, minerals and vitamins they contain, coupled to their safety, affordability and effectiveness [6-7].

Hunteria umbellata (K. Schum.), commonly known as "Osu", "Npokiri" and "Abeere" in Edo, Igbo, and Yoruba parts of Nigeria respectively is of the Apocynaceae plant family and consists of 4555 species of trees, shrubs, woody vines, and herbs. It is common in West and Central African countries of Cameroon, Senegal, Ghana, Gabon, Congo, Liberia, Guinea Bissau, Sierra Leone, Ivory Coast, and Nigeria [8]. Numerous local and folkloric medicinal uses have been documented for the plant. The seeds and barks are prepared as infusions and decoction that have been shown to be effective in fighting fever, leprosy, menstrual

disturbances, infertility, yaws, intestinal worms, abdominal colic, indigestion or stomach ache [9].

Method

Plant Sample Collection and Preparation

H. umbellate seed was collected from a farm in Onipako village, Ododanre Local Government Area of Ondo State, Southwest Nigeria. The fresh seed was cleaned, air dried under a shed, and pulverized using pestle and mortal.

The sample was extracted in ethanol using the Soxhlet apparatus, and about 0.5 kg of extract was obtained[10]. Fifty grams (50 g) of the powdered sample was measured using electronic weighing balance, wrapped using whatmann filter paper and inserted in the tube of the soxhlet apparatus. A 250 ml of 70 % ethanol was poured inside the round bottom conical flask of the apparatus and was heated at 70 oC. The extraction was carried out for 3 h. The remaining ethanol in the extracts was evaporated to get crude extract. This was done in vacuum using a rotary evaporator.

Experimental Animals

Forty-eight (48) adult albino Wistar rats (male; weighed 150 g – 250 g; aged 16 – 19 weeks) were used for this experiment. The rats were bought from the Department of Biochemistry, Faculty of Sciences, University of Nigeria Nsukka, Nigeria. The animals, upon arrival, were left to acclimatize for 7 days in an animal house at the Faculty of Science, Ebonyi State University, Abakaliki, Nigeria. The rats were randomly assigned to 6 groups of 8 rats. The rats were fed standard pellet diet and water *ad libitum* and left under good laboratory conditions.

Toxicity Test

The up and down method of acute toxicity study was used for this study [11]. Six (6) rats were randomly selected, weighed and divided into 2 groups (A and B) of 3 rats each. The first group was used as the control group and received only vehicle, whereas, the second group received fixed dose of 2,000 mg/kg of the extract. Oral treatment of the rats with pawpaw seed through intraperitoneal injection was done after the rats have been fasted overnight. The rats were observed over a 48-hour period for signs of toxicity and mortality.

Experimental Design

A complete randomized design was used in the used. Six treatment groups of 8 rats each were created (Table 1) and each group allowed access to feed and water. A single intraperitoneal injection of freshly prepared alloxan monohydrate (150mg/kg bw and at a concentration of 20mg/ml) was used to induced diabetes in overnight fasted rats [12]. After 3 days of administering the alloxan, the plasma glucose level of each rat was determined to confirm that diabetes has been successfully induced. The rats whose plasma glucose levels greater than 250 mg/dl were considered to be diabetic and used in the experiment. Treatment of the rats with the extract commenced 3 days after alloxan injection, and this was recorded as the Day 1 of treatment. Treatment continued for the next 28 days.

Blood Collection

At the 29th day of the experiment, the rats were fasted overnight (16 h), and sacrificed by cervical decapitation as contained in the ethical norms of Ebonyi State University, Abakaliki. The blood was collected from the trunk in separation tubes containing clot activator and centrifuged at 2,000 x g for 10 min in a refrigerated centrifuge at 4 0C to remove the blood clot. A polypropylene tube was used to store the resulting supernatant (serum) at 4 0C prior to analysis [1].

Biochemical Analysis

The serum level of triglyceride (TG) was determined using method by Bucolo and David (1973). The methods according to Allain et al. (1974), Lund-Katz et al. (2003), and Nauck et al. (2002) were used for total cholesterol (TC), high density lipoprotein (HDL), and low-density lipoprotein (LDL) respectively. The Biuret method with kits as described by Dawnay et al. (1991) was used in the determination of serum Total protein (TP), whereas, standard diagnostic kits described by Shah et al. were used to determine serum glutamic oxaloacetic transaminase (SGOT/AST), serum glutamic pyruvic transaminase (SGPT/ALT), alkaline phosphatase (ALP), urea and creatinine[13].

Determination Of 2,2-Diphenyl-1-Picrylhydrazyl (Dpph)

A 2 g of the sample was dissolved in 10ml of distilled water. 0.2 ml of the solution was collected and mixed with 1.8 ml of 0.1 Mm DPPH solution in methanol and left in the dark at room temperature for 60 mins. The mixture was measured at a wavelength of 517 nm with UV-spectrophotometer using methanol as a blank.

$$\text{Dpph} = \frac{\text{Absorbance (control)} - \text{Absorbance (sample)}}{\text{Absorbance (control)}} \times 100$$

Statistical Analysis

Analysis of variance (ANOVA) for Completely Randomized Design (CRD) was carried out using Statistical Package for Social Sciences (SPSS). Values were presented as mean ± standard deviation (mean ± standard error of mean). Differences between means were separated using the Least Significant Difference (LSD), with a p-value of less than 0.05 ($p < 0.05$) as the level of significance.

Results

Toxicity Test

Oral administration of 2,000 mg/kg extract and equal volume of distilled water produced no death or any sign of toxicity after 48 h.

Effect of The Extract on Plasma Glucose Level

Figure 1: Shows the results of the plasma glucose level of the rats on different time intervals of the experiment. It was observed that the glucose levels significant increased ($p<0.05$) in all groups after inducing the rats with alloxan (Day 1) compared to the normal control (group 1) that was not induced with alloxan. Again, the blood glucose level of the diabetic control group (group 2) was significantly ($p<0.05$) higher than the blood glucose level of the normal control (group 1) throughout the experimental period. Moreover, it was observed that the the different concentrations of the extract significantly decreased ($p<0.05$) the plasma glucose levels of the diabetic rats. Only Group 6 recorded a blood glucose level that was significantly lower ($p<0.05$) on the Day 7, when compared to the diabetic control (Group 2).

Effect of The Extract on The Lipid Profile

The result of the serum total cholesterol, triglyceride, low density lipoprotein and high density lipoprotein are presented in Figure 2. The result shows that the diabetic control group (group 2) had the highest total cholesterol (6.45±0.495 mmol/L), which was significantly ($p<0.05$) higher than the total cholesterol level of Group 6 (3.9±0.283 mmol/L). the total cholesterol values of the groups treated with the extracts were not significantly different ($p\geq0.05$) from the total cholesterol value of the positive control group (group 3). Also, it was observed from this study that the diabetic control group (Group 2) had the highest serum triglyceride level (2.5±0.424 mmol/L), but this value was not significantly

different ($p \geq 0.05$) from the serum triglyceride levels of the other groups.

The high-density lipoprotein increased with increasing concentration of the extract. The result shows that the group treated with 200 mg/kg of the extract (group 6) had the highest high density lipoprotein level, with a value of 1.15±0.071 mmol/L. The high-density lipoprotein value of group 6 was significantly different ($p<0.05$) from the high density lipoprotein value of the diabetic control group (group 2), but was not significantly different ($p \geq 0.05$) from the high density lipoprotein value of the other groups. The diabetic control group (group 2) was observed to have the highest low density lipoprotein, with a value of 7.4±0.424 mmol/L which was significantly ($p<0.05$) higher than the low density lipoprotein values recorded for other groups. The groups treated with the extract had low density lipoprotein values that were not significantly different ($p \geq 0.05$) from the low density lipoprotein value of the positive control group (group 3).

Effect of The Extract on Liver Enzymes

In this study, the diabetic control group (group 2) was observed to have the highest values of Alkaline Phosphatase (ALP), Alanine Transaminase (ALT), Aspartate Aminotransferase (AST) as shown in Table 2 below. The alkaline phosphatase value of the diabetic control group (group 2) (53.5±0.707 U/L) was significantly ($p<0.05$) higher than the alkaline phosphatase values of the other groups. The alkaline phosphatase values of the groups pre-treated with the extracts were not significantly different ($p \geq 0.05$) from the alkaline phosphatase value of the positive control group (group 3). Similarly, alanine transaminase and aspartate aminotransferase values of the groups pre-treated with the extracts, were not significantly different ($p \geq 0.05$) from the ALT and AST values of the positive control group (group 2).

Effect of The Extract on Kidney Profile

The results show that serum urea and serum creatinine values of the rats increase with the increasing concentration of the extracts as shown in Table 3 below. The group pre-treated with 200 mg/kg of the extract recorded the highest urea and creatinine values of 6.15±0.354 mg/dl and 90.0±1.414 mg/dl respectively. The urea values of the groups pre-treated with the extracts were not significantly different ($p \geq 0.05$) from the urea value of the positive control group (group 3). Nevertheless, the creatinine value of the positive control group (group 3) was significantly ($p<0.05$) lower than the creatinine values of the groups pre-treated with 100 mg/kg and 200 mg/kg of the extract, but was significantly ($p<0.05$) higher than the creatinine value of the group pre-treated with 50 mg/kg of the extract.

Anti-Oxidants Content of the Extract

The antioxidant potentials of the seed of *H. umbellata* are presented in Figure 3. The fruit and seed of the plant were observed to possess radical scavenging effect with IC_{50} of 227.34 µg/ml as compared to the reference, superoxide dismutase with IC_{50} of 389.73 µg/ml. The radical scavenging activities of the plant seed increased with increasing concentration of the extract.

Discussion

Diabetes mellitus, a globally health problem, increases progressively with about 6.4 % prevalence rate among adults opined that, between 2010 and 2030, there would be a possible upshot of the prevalence rate of diabetes among adults to 69 % in developing countries, and 20 % in the developed countries [12, 14, 15]. Also, WHO (2016) projected that the disease would be the 7th leading cause of death in 2030. Many orthodox antiglycaemic agents have been tried, yet failed to provide the desired prolonged antidiabetic effects. In this light, medicinal herbs and their extracts have been widely recommended as antidiabetics because of their many pharmacological properties, safety, and affordability. The present study illustrated the anti-hyperglycaemic potentials of ethanol extracts of osu seed in alloxan-induced diabetic rats.

In this study, visible signs of diabetes such as frequent urination, excessive drinking of water, and increased blood glucose levels were observed in the alloxan-induced diabetic rats. Furthermore, the diabetic control group (group 2) was observed to eat more than the other groups, and had a blood glucose level that was greater than 250 mg/dl throughout the experimental period. This finding is in line with the reports of Oko et al. (2022) that alloxan induces diabetes in rats. A blood glucose of >250 mg/dl and <80 mg/dl suggests hyperglycaemic and hypoglycaemic conditions respectively [1]. So, there was a progressive decrease in their blood glucose levels of the diabetic rats when they were pre-treated with the extract. The results show that 100 mg/kg and 200 mg/kg extract were able to restore the blood glucose level of the rats to values less than 250 mg/dl and greater than 80 mg/dl from the Day 14 of the experiment till the last day. These effects were comparable to that of glibenclamide, a commercial antidiabetic agent. These findings suggest that osu seed extract can enhance the peripheral utilization of insulin in diabetic rats.

Again, the serum lipid profile, which often increases in diabetes mellitus, was observed to increase in the diabetic control group (group 2), and as such, suggests a possible increase in lipids prompts coronary illnesses [16]. The hypercholesterolemia, hypertriglyceridemia, high LDL and low HDL observed in the diabetic control group (group 2) were markedly diminished by the pre-treatment with osu seed extracts. Also, this is evident that the extract can aid in ameliorating the problems of lipid profile.

Similarly, diabetes may induce hepatic malfunctioning through increasing serum AST, ALT and ALP profiles, which are essential hepatic enzymes [16]. In line with our findings in the diabetic control group (group 2), increased ALT, AST and ALP has been reported for diabetic conditions [1,7, 17]. Which might suggest spillage of these enzymes from the liver cytosol into the blood stream [18], which is an implication of the hepatotoxic effect of alloxan [16]. Nevertheless, pre-treatment with osu seed extract resulted in notable decrease in the liver enzymes, which is in accordance with the findings of Ohaeri et al., Karuna et al., and Taher et al. for treatment of diabetes in rats with medicinal herbs [1, 17, 19].

Again, the principal markers of renal dysfunction (elevated levels of serum urea and creatinine) are usually observed in alloxan-induced diabetes rats [20]. The findings of this study are consistent with this assertion since there was a significant increase in serum urea and creatinine levels in the diabetic control group (group 2), proving that diabetes may expedite renal dysfunction. Nevertheless, pre-treatment of the rats with the extract resulted in a markedly decrease in serum urea and creatinine which was comparable to the urea and creatinine levels of the group pre-treated with glibenclamide. Similarly, the observed radical scavenging activity of the extract, with a lower IC50 value, further confirms the usefulness of osu seed as anti-hyperglycaemic agent.

Conclusion

Ethanol extract of osu seed exerts antidiabetic activities. This was suggested by the observed lowering effect of the extract on serum cholesterol, triglyceride, low density lipoprotein, liver

enzymes, improved renal function and subsequent increase in high density lipoprotein. These antidiabetic activities could be attributed to a possible increase in the insulin concentration and insulin sensitivity in the cells, as a result of the anti-oxidative property of the extract. Therefore, this study demonstrates that ethanol extract of osu seed could serve as a potential natural agent for the management of diabetes. Nevertheless, this study has only elucidated the potential of the extract in the management of diabetes, but has failed to detail the exact mechanism and point of action of the extract due to financial constraints. It is therefore recommended that more scientific research is carried out to ascertain the exact mechanism and point of action of the extract before trial on human subjects [21-27].

Ethics and Animal Welfare

Animal care and procedures were performed by following the guidelines of good experimental practices according to the Code of Practice for Housing and Care of Animals Used in Scientific Procedures by Faculty of Science, Ebonyi State University, Abakaliki Ebonyi State, Nigeria. This includes a detailed description of the efforts made to provide environmental enrichment and to avoid the animals undergoing any unnecessary suffering, including humane endpoints and the guidelines for euthanasia.

Note: this manuscript does not contain clinical studies or patient data.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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