Evaluation of Anti-Diabetic Effect and Liver Enzymes Activity of Ethanol Extract of *Pterocarpus santalinoides* in Alloxan Induced Diabetic Albino Rats


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Abstract: Aim of the study is to evaluate anti-diabetic effect and liver enzymes activity of ethanol extract of *Pterocarpus santalinoides* in alloxan induced diabetic albino rats. Study design diabetes mellitus was induced in five groups of rats, one group was not treated while one group was treated orally with a standard drug (Metformin) and the other three groups were treated orally with *P. santalinoides* leaves extract at 100, 200 and 400 mg/kg body weight of rats twice for 1 week respectively. One group was not induced and received distilled water and feed only. The anti-diabetic and liver enzymes activities were determined from blood glucose and plasma transaminases activities of the rats. Place and duration of study department of Biochemistry Ebonyi State University, Abakaliki, Nigeria, October, 2014. Methodology eighteen (18) wistar albino rats weighing between 60-100g (4-6 weeks old) were used for this work. The animals were randomly assigned into six (6) different groups of three (3) rats each. Group A (Healthy control (HC)) was neither induced with diabetes nor given any treatment throughout the experiment and served as positive control, Group B (Diabetic control (DC)) was induced with diabetes but not given any form of treatment throughout the experiment and served as negative control, Group C (DM + Metformin) was induced with diabetes and treated with a standard drug (Metformin) at a dose of 100mg/kg, Group D was induced with diabetes and further subdivided into D1, D2 and D3 corresponding to 100, 200 and 400 mg/kg body weight of the *P. santalinoides* leaves extract. The treatment was done twice daily for seven days. The glucose and liver enzymes levels were determined using glucometeric and spectrophotometric methods respectively. Results the results revealed that there was significant reductions (P<0.05) in glucose level in rats treated with ethanol extract of *P. santalinoides* leaves compared to the diabetic control rats. There was no significant (P>0.05) reduction in Alkaline phosphatase and Aspartate aminotransaminase level between the controls and the treated groups. There was significant (P<0.05) increase in Alanine aminotransaminase level in rats treated with *P. santalinoides* leaves and standard drug. Conclusion the result showed that ethanol leaf extract of *P. santalinoides* has anti-diabetic effect and is relatively safe because it has the same effect with Metformin on liver enzymes. Hence, *P. Santalinoides* leaf consumption as vegetable should be encouraged for management of diabetes mellitus.

Key words: Anti-Diabetic · *Pterocarpus santalinoides* · Liver Enzymes · Metformin · Alloxan

INTRODUCTION

Diabetes is a chronic disease that occurs when pancreas does not produce enough insulin or alternatively, when the body cannot effectively use the insulin it produces [1]. Defective insulin secretion is the major cause for chronic hyperglycaemia resulting in impaired function or serious damage to many of the body’s systems, like eyes, kidneys, nerves, heart and blood vessels [2]. Permanent neonatal diabetes is caused by glucokinase deficiency and is an inborn error of the glucose-insulin signalling pathway [3].

Diabetes mellitus is a major worldwide health problem involving endocrine pancreas [4,5]. It is implicated in oxidative stress which induces insulin resistance in the peripheral tissue and impairs insulin resistance in the secretion from pancreatic β-cells [4,5]. It is a major cause of adult blindness, kidney failure, neuropathy, heart attack and strokes. It is also characterized by excessive disturbance of carbohydrates, proteins and lipid
metabolism, thickening of capillary basement throughout the body leading to micro-angiopathy, macro-angiopathy and long term complications which affect eyes, kidneys, nervous system and circulatory system [5].

One of the most potent methods to induce experimental diabetes mellitus is chemical induction by Alloxan [8]. It is a well-known diabetogenic agent that is used to induce Type I diabetes in experimental animals [9]. Alloxan is an urea derivative which causes selective necrosis of the β-cells of pancreatic islets. In addition, it has been widely used to induce experimental diabetes in animals such as rabbits, rats, mice and dogs with different grades of disease severity by varying the dose of alloxan used [8]. As it has been widely accepted that alloxan selectively destroys the insulin-producing β-cells found in the pancreas, hence it is used to induce diabetes in laboratory animals [8].

The Liver is the largest internal organ which has more functions than any other organ and sustains life even when only 10-20% of liver tissue is functioning. Its roles include: purification, synthesis, storage and transformation. It is therefore very obvious that any disease condition or adverse physiological conditions, which affect the hepatocytes, will cause concerted and tremendous metabolic derangement [10]. When tissue damage occurs, cellular enzymes may be released into the serum and the elevation of certain enzymes is often associated with damage to specific tissue or organs. Although the liver enzymes are present in tissues throughout the body, their elevation (Particularly in combination) is most often associated with liver injury or disease [11]. Almost any medication, including herbal preparation and illicit drugs, may cause a transient elevation of the aminotransferases. It is therefore very pertinent to ascertain the effect of any ingestible food or drug on the serum activities of the liver enzymes so as to ensure the hepatoprotectiveness of such food or drug. This can be achieved through liver function tests, which include estimation of plasma protein, aspartate aminotransferase (AST), alkaline phosphatase (ALP), alanine aminotransferase (ALT) and bilirubin [12].

The management of diabetes mellitus is considered a global problem and a successful treatment is yet to be discovered [13]. Metformin is considered a cornerstone in the treatment of diabetes and is the most frequently prescribed first line therapy for individuals with Type 2 diabetes [14]. Metformin major effect is to decrease hepatic glucose output [15]. In addition, metformin decreases glucose absorption in the small intestine, increases insulin-mediated glucose utilization in peripheral tissues (Such as muscle and liver), and has an anti-lipolytic effect that lowers serum free fatty acid concentrations, thereby reducing substrate availability for gluconeogenesis [16].

Plants have been the major source of drugs for the treatment of diabetes mellitus in some countries of the world like India and China [5]. The importance of anti-diabetics plants in the development of economic and effective treatment for diabetes currently estimated to affect over 30 million people worldwide has been recognized by the World Health Organization [17]. Most of the anti-diabetic plants have been found to contain substance like glycosides, alkaloids, terpenoids, flavonoids and so on Loew and Kaszkin [18]. One of such plant species used for the management of diabetes is Pterocarpus santalinoides [19, 20].

Pterocarpus santalinoides L’Herit ex DC (Family: Fabaceae-papilionoidae) is a shade-tolerant tree 9-12m tall, with low straggling branches, commonly found along riverine forests in Africa and tropical South America. It is native to Brazil, Cameroon, Ghana, Nigeria and Senegal [21]. The plant is commonly referred to as Red Sandal wood in English, “Gundurugyadar Kurmi” in Hausa, “Uturukpa” in Igbo and “Gbenghe” in Yoruba [22]. Various morphological parts of P. santalinoides are used in traditional medicine, in many African countries, to treat an array of human ailments. The fresh leaves of P. santalinoides are consumed locally, in soups, by the Igbos of South East Nigeria and are reputed to be useful in the treatment of diarrhoea and other gastrointestinal disorders [22].

MATERIALS AND METHODS

Materials
Equipment and Instrument: The equipment and instruments are of analytical standards.

Chemicals and Reagents: The chemicals and reagents are of analytical grades.

Collection of Plant Materials: Fresh leaves of fully grown Pterocarpus santalinoides were collected from Mgbalukwu in Onicha local government area of Ebonyi State between the month of August and September, 2014. The plant sample was identified by a taxonomist, Prof. S. S. Onyekwelu of the department of Applied Biology, Ebonyi State University, Abakaliki, Nigeria. A voucher specimen was deposited at the herbarium in the Department of Applied Biology, Ebonyi State University, Abakaliki, Nigeria, for reference purposes.
Experimental Animals: Eighteen wistar albino rats weighing between 60-100g (4-6-weeks old) were obtained from the animal house of the faculty of Veterinary Medicine University of Nigeria, Nsukka, Nigeria. They were acclimatized for seven days in stainless steel cages under good laboratory conditions. They were fed with commercial poultry growers mash feed (Vital feed®, Jos, Nigeria). Clean water was provided daily and access was free. The animals were weighed using triple beam weighing balance. Handling, management and use of animals for the experiment were as such that allowed minimal stress. Ebonyi State University Animal Ethical Committee approved the animal studies.

Experimental Design: At the end of the seven days acclimatization period, the animals were randomly assigned into six different groups of three rats each. Each group was kept on different cages. The grouping was done as follows: Group A, healthy control (HC) were neither induced with diabetes nor given any treatment throughout the experiment rather they received water and feed only and served as positive control. Group B, diabetic control (DC) were induced with diabetes but not given any form of treatment throughout the experiment, they served as negative control. Group C were induced with diabetes and treated with a standard drug (Metformin) at a dose of 100mg/kg. Group D were further subdivided into D1, D2 and D3 corresponding to 100, 200 and 400 mg/kg body weight of the extract. Group D1 (DM + P. santalinoides 100mg/kg) were induced with diabetes and treated with P. santalinoides ethanol leaf extract at dose of 100mg/kg. Group D2 (DM + P. santalinoides 200mg/kg) were induced with diabetes and treated with P. santalinoides ethanol leaf extract at 200 mg/kg body weight. Group D3 (DM + P. santalinoides 400mg/kg) were induced with diabetes and treated with P. santalinoides ethanol leaf extract at 400mg/kg body weight. The treatment was done twice daily for seven days.

Methods
Preparation of Plant Extract: The leaves were rinsed in clean water and air dried under room temperature. The dried leaves were pulverized to fine granules using electric blender. Powdered plant leaves (93g) were macerated in 400ml of 95% ethanol at room temperature for 24 hours. After 24 hours the extract obtained was then filtered using sieve cloth. The filtrates were pooled and evaporated at 40°C-50°C and the residue weighed. The yield of the ethanol extract of P. santalinoides (EEPS) was 17.2%. The ethanol extract (3g) was subsequently dissolved in distilled water (30ml) to get stock solution of 0.1g/ml. The stock solution was stored in the refrigerator.

Preparation of Standard Drug (Metformin): Metformin (Glucophage) tablets were obtained from Clonal Pharmacy Outlet at Abakpa Market, Abakaliki, Ebonyi State, Nigeria. The tablets (5g) were then ground into fine powder. The powder was then dissolved in 26.0ml of distilled water to get stock solution of 0.192g/ml which was stored in the refrigerator.

Induction of Diabetes Mellitus: 3g of alloxan were dissolved in 30ml of distilled water. The animals in groups B, C and D were weighed and 100 mg/kg body weight of the alloxan was injected via the intra-peritoneal cavity with the insulin syringes. Diabetes Mellitus was confirmed after 72hrs by the symptoms of polydipsia, polyuria, glycosuria and by testing the fasting blood sugar concentration in the blood obtained from the tail vein of the animals using glucometer [11]. The animals with sugar level more than 180mg/dl were considered as experimental diabetic [10].

Administration of Drugs: Both the metformin and P. Santalinoides were administered via the oral route with the aid of oropharyngeal cannular. The rats were handled appropriately to restrict movement and prevent trauma during drug administration. The drugs were administered twice daily for the period of seven days.

Determination of Blood Glucose Level: All blood samples for monitoring of blood glucose level in situ were taken from the tail vein of the rats using 24 gauge needles at intervals of 0, 3 and 7 days. Blood glucose level was determined by the glucose oxidase method using reactive strips and a single touch glucometer [10].

Blood Collection and Preparation: After overnight fast, the animals were sacrificed on the 8th day under mild chloroform anaesthesia and blood was obtained via femoral vein. Blood samples were transferred into plain centrifuge tubes and allowed to clot at room temperature. They were then centrifuged within 1 hour of collection at 4000x g for 10min on a Centrifuge to separate the sera from the clot. The resultant sera samples were stored frozen at-20°C. Prior to liver enzyme assay, frozen sera were completely thawed and well mixed and all reagents were allowed to attain room temperature.
Determination of Liver Enzymes: Aspartate aminotransferase (AST), Alanine aminotransferase (ALT) and Alkaline phosphatase (ALP) were determined by method described by Carl and Edward [22] and Reitman and Frankel [23].

Data Analysis: Results were expressed as mean standard deviation. The one-way analysis of variance (ANOVA) was used to analyze the data followed by post-hoc tests. The results are considered significant at P<0.05.

RESULTS

Percentage Yield of Extract of Pterocarpus santalinoides Leaves: The percentage yield of ethanol extract of Pterocarpus santalinoides Leaves gave 17.2% as shown in the table 1. This means that ethanol is a good solvent for some of the active ingredients.

Result of the Body Weight of Albino Rats during Seven (7) Days of Treatment with Ethanol Extract of Pterocarpus santalinoides Leaves: The body weight of animals is an excellent physical interpretation of effect of drugs or foods on the biochemical profile of the animal. Thus, the body weight of albino rats during seven (7) days of treatment with ethanol extract of Pterocarpus santalinoides leaves and standard drug (Metformin) were recorded. The result showed a significant (P<0.05) increase in the body weight of the treated group as illustrated in the Fig. 1.

Result of the effect of Ethanol Extract of Pterocarpus santalinoides Leaves on Glucose Level on Alloxan-Induced Diabetic Albino Rats: The result of the effect of ethanol extract of Pterocarpus santalinoides leaves on glucose level on alloxan-induced diabetic albino rats showed a significant (P<0.05) decrease in the glucose level of rats in the treated groups as shown in the fig. 2.

Result of the effect of Ethanol Extract of Pterocarpus santalinoides Leaves on Liver Enzymes in Alloxan-Induced Diabetic Albino Rats: The result of effect of ethanol extract of Pterocarpus santalinoides leaves on liver enzymes in alloxan-induced diabetic albino rats showed no significant (P>0.05) increase in levels of AST and ALP in the rats of the treated groups when compared to the control. There was significant (P<0.05) increase in the ALT levels in rats in treated groups compared to the healthy and diabetic controls as illustrated in the Fig. 3.

Table 1: Percentage yield of ethanol extract of Pterocarpus santalinoides Leaves

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Used</th>
<th>Plant part</th>
<th>Mass of pulverized dried leaves before extraction (g)</th>
<th>Mass of pulverized dried leaves after extraction (g)</th>
<th>Volume of the solvent (ml)</th>
<th>Mass of the extract (g)</th>
<th>Percentage yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanol</td>
<td>Pterocarpus santalinoides Leaves</td>
<td>93</td>
<td>77</td>
<td>400</td>
<td>16</td>
<td>17.2</td>
<td></td>
</tr>
</tbody>
</table>
Fig. 3: Liver enzymes level in alloxan-induced diabetic albino rats treated with ethanol extract of *Pterocarpus santalinoides* leaves

**DISCUSSION**

The effect of ethanol extract of *Pterocarpus santalinoides* leaves on body weight was measured from 1<sup>st</sup> to 7<sup>th</sup> day of post induction. The result showed initial reduction in mean body weight of all the groups after induction except in the healthy control. After treatment the mean body weight of the treated groups were restored to near that of the healthy control group while that of the diabetic control group were drastically reduced (Figure 1). This showed that as the glucose level decreases, the body weight improves also. Aja et al. [10], reported a significant (P<0.05) reduction in the mean body weight of rats in diabetic control compared to positive group while rats in treated groups showed significant (P<0.05) increase in their mean body weight compared to diabetic control group in work done earlier on anti-diabetic effect of aqueous extract of *Moringa oleifera* and *Bridelia ferruginea* leaves in alloxan-induced diabetic albino rats. Grover et al. [24], had also reported that aqueous extract of *Aeglemarmelose* leaves was equally effective in comparison to insulin in restoring blood glucose and body weight to normal levels.

The effect of ethanol extract of *Pterocarpus santalinoides* leaves on glucose showed that normal rats feed has no effect on the blood glucose in healthy control and diabetic control group. The diabetic control animals (Group B) exhibited gradually increased glucose level. There was a significant (P<0.05) elevation in glucose level in the diabetic control (Group B) when the values for days 0, 3 and 7 were compared to the corresponding values in the normal control rats. The values were shown in Figure 2. The intra-peritoneal induction of alloxan in the rats showed significant (P<0.05) increase in glucose level as earlier asserted by Bamidele et al. [11]; they observed that after 72 hours of injecting 100mg/kg of alloxan dissolved in distilled water the glucose level of the albino rats were significantly (P<0.05) elevated. Aja et al. [10] had earlier reported a significant (P<0.05) decrease in glucose level in alloxan induced diabetic albino rats treated with aqueous extracts of *Moringa oleifera* and *Bridelia ferruginea* leaves.

Oral administration of metformin (100mg/kg/day) significantly (P<0.05) reduced the elevated levels of blood glucose of the albino rats in Group C on the 7<sup>th</sup> day of treatment. This is in line with the work of Okonkwo and Okoye [25] as they reported that the oral administration of metformin (250mg/kg/day) significantly (P<0.05) reduced the elevated levels of blood glucose on the 5th, 10th and 15th day of treatment compared to the corresponding values in the untreated diabetic control (P<0.05).

On the other hand, oral administration of the extract at the doses of (100 and 200mg/kg/day) showed a significant (P<0.05) decrease in blood glucose level on 7<sup>th</sup> day of treatment whereas the reduction observed at the dose of (400mg/kg/day) was not significant (P>0.05). The results of this experiment thus validate the anti-diabetic effects of *Pterocarpus santalinoides* and as such lend credence to its glucose lowering properties earlier reported by Okwuosa and Okoye [25]. The reduction in blood glucose level observed in this present work had been reported by Bamidele et al. [11] and Grover et al. [24] in other species of plant. Grover et al. [24], reported that the aqueous extract of *Aeglemarmelose* leaves (1 gm/kg
for 30 days) significantly controlled blood glucose of alloxanized (60 mg/kg IV) rats as compared to controls and this effect was similar to insulin treatment. On the other hand, Bamidele et al. [11], reported that at the dose of 200mg/kg the anti-diabetic effect of aqueous leaf extract of *Basella alba* was comparable to that of metformin-treated alloxan-induced diabetic albino rats. The reduction of blood glucose level by *P. Santalinoides* leaves extract may be due to its numerous bioactive compounds such as saponins, flavonoids, phenols, triterpenoids and tannins [26]. Some of these bioactive compounds may exert their hypoglycemic effects by, reducing insulin resistance, increasing release and decreasing glucagon's secretion, slowing the digestion and absorption of carbohydrates or by decreasing hepatic glucose production [27].

The effect of ethanol extract of *Pterocarpus santalinoides* leaves on level of liver enzymes has no significant (P>0.05) increase in the levels of ALP in diabetic rats compared to rats in normal control group (Figure 3) while rats in treated groups showed no significant (P>0.05) reductions compared to rats in diabetic groups. There was significant (P<0.05) increase in the ALT levels in rats in treated groups compared to the healthy and diabetic controls. There were no significant (P>0.05) increase in the levels of AST in diabetic rats compared to rats in normal control group while rats in treated groups showed no significant (P>0.05) reductions compared to rats in diabetic groups (Figure 3). This results correlates excellently well with results obtained from similar work done by Aja et al. [10] on the effects of aqueous extract of *Moringa oleifera* and *Bridelia ferruginea* leaves on the level of liver enzymes in alloxan-induced diabetic albino rats.

**CONCLUSION**

Based on the findings of this current work it suggest that *Pterocarpus santalinoides* leaves have anti-diabetic effect on alloxan-induced diabetic albino rats and it may be safe at the treated doses based on the effect on the liver enzymes. Thus, its leaf consumption as vegetable should be encouraged to manage diabetes mellitus.

**REFERENCES**