THE EFFECT OF ETHANOL LEAF EXTRACT OF *PTEROCARPUS SANTALINOIDES* (NTRUKPA) ON THE LIPID PROFILE OF ALLOXAN-INDUCED DIABETIC ALBINO RATS

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ABSTRACT

The antidiabetic and antihyperlipidemic effect of ethanol extract of *Pterocarpus santalinoids* on alloxan-induced diabetes in albino rats were studied. Phytochemical and proximate analyses were done using standard methods. Twenty eight rats were used for acute toxicity studies which were placed in seven groups of four rats each. For biochemical studies sixteen male albino wistar rats were divided into four groups of four rats each. Group 1 (control group rats) was not induced with diabetes while groups 2, 3 and 4 were induced with diabetes using alloxan. The results of the qualitative phytochemical analysis of *Pterocarpus santalinoids* showed that ethanol leaf extract contains alkaloids, glycosides, flavonoids and steroids. The results showed that the leaf extract of *Pterocarpus santalinoids* contains moisture, crude fiber, carbohydrate, fats, protein and ash contents. No death was recorded in the acute toxicity studies. The results showed that there was a significant decrease (p<0.05) in blood glucose level (mg/dl) following the administration of ethanol extract of *Pterocarpus santalinoids* in groups 3 and 4 (124.07 ± 6.24mg/dl and 113.44±5.20mg/dl) when compared to the diabetic control group 2 (428.59±2.36mg/dl). The results also revealed that administration of 200 and 400 mg/kg body weights of ethanol leaf extract of *Pterocarpus santalinoids* significantly decreased (p<0.05) total cholesterol, triglycerides and low density lipoprotein cholesterol (LDL-C) when compared with non diabetic control. But, there was a significant increase (p<0.05) of high density lipoprotein cholesterol on the administration of 200 and 400 mg/kg body weights of ethanol leaf extract of *Pterocarpus santalinoids* in groups 3 and 4 rats when compared to group 2 diabetic control rats. Our results suggest that the ethanol leaf extract of *Pterocarpus santalinoids* at 200 and 400 mg/kg body weights may possess anti-diabetic and antihyperlipidemic effects and can be useful in the treatment and management of diabetes mellitus.

Keywords: *Pterocarpus santalinoids*, diabetes, phytochemical and hyperlipidemia.
INTRODUCTION

Diabetes mellitus is a serious chronic condition which has several complications including diabetic nephropathy, diabetic neuropathy, coronary heart disease and hypertension. These complications may be delayed, lessened or prevented by maintaining blood glucose level close to normal [1]. Symptoms of diabetes mellitus include polyuria (production of large volume of urine), polydipsia (abnormally intense thirst) polyphagia (increased appetite) general fatigue and weight loss [2].

*Pterocarpus santalinoids* commonly known as Red Sandalwood belongs to the family *Fabaceae*. It is used in India and other parts of the world, with illegal harvest being a key threat. The plant is renowned for its characteristic timber of exquisite color and beauty [3]. The Red Sandalwood has natural dye (santalin) which is used as coloring agent in pharmaceutical preparations and food stuffs. In the traditional system of medicine, the decoction from the heartwood is attributed various medicinal properties. It is used in ulcers, eye diseases, inducing vomiting and mental aberrations [3, 4, 5, 6 and 7]. The heartwood is known to have antihyperglycaemic activity, antipyretic, anti-inflammatory, antihelmintic, tonic, hemorrhage, dysentery, aphrodisiac, diaphoretic activities and also used as a cooling agent [8]. It has been reported that wood in combination with other drugs is prescribed for snake bites and scorpion stings [9].

Phytochemical studies of this plant indicate that it contains substances such as alkaloids, phenols, saponins, glycosides, flavonoids, triterpenoids, sterols and tannins. In addition, heart wood contains isoflavone, glucosides and two antitumour lignans, viz., savinin and calocedrin. This review explores the phytochemical and pharmacological effects of the *Pterocarpus santalinoids* Linn and compiles vital information that may assist researchers on what is known about this plant for further investigation [10]. However, the species has remained unexplored for many pharmacological activities claimed. Hence, the present research was to determine the anti-diabetic and anti-lipidemic effects of ethanol leaf extract of *Pterocarpus santalinoids* on alloxan induced diabetes in rats.
AND OBJECTIVES

Aim: The aim of the research was to investigate the anti-diabetic and lipid profile of ethanol leaf extract of *Pterocarpus santalinoids* on alloxan induced diabetes in rats.

SPECIFIC OBJECTIVES

- To determine the chemical constituent of ethanol leaf extract of *Pterocarpus santalinoids*.
- To carry out acute toxicity test of ethanol leaf extract of *Pterocarpus santalinoids* on albino rats.
- To investigate the effect of ethanol leaf extract of *Pterocarpus santalinoids* on alloxan induced diabetic rats.

MATERIALS AND METHODS

STUDY AREA

This research was carried out in Abakaliki, the state capital of Ebonyi State, Nigeria.

MATERIALS AND EQUIPMENTS

CHEMICALS AND REAGENTS

All chemicals and reagents used were of analytical grades.

BIOLOGICAL MATERIALS

The biological materials used were rats and *Pterocarpus santalinoids* leaf. A total of forty four rats were used for this study. The rats aged 5-6 months and weighs 160-180g. The rats used for this research were purchased from the Department of Animal Science, University of Nigeria Nsukka, Enugu State, Nigeria. *Pterocarpus santalinoids* was collected from Mgbabo in Abakaliki, Ebonyi State, Nigeria. It was identified by Dr (Mrs) C.V. Nnamani of Applied Biology Department Ebonyi state University.

Preparation of plant extract of *Pterocarpus santalinoids* leaf
The leaves of *Pterocarpus santalinoids* were washed under running tap water to remove the surface contaminants and were air dried under a shade. The leaves were pulverized using laboratory milling machine and sifted using 0.25 mm sieve. Five hundred milligram of the powdered *Pterocarpus santalinoids* sample was soaked in 1000 ml of ethanol for 48 hours. They were filtered with a clean white cloth and subjected to successive extraction using a water bath at 50°C until the solvents were completely removed. The sample was dried and percentage yield obtained. Extract obtained was stored in a bottle and was subsequently used for various analyses.

**Qualitative Phytochemical Tests of *Pterocarpus santalinus***

Qualitative and quantitative phytochemical analyses were done using the method of [12].

**EXPERIMENTAL DESIGN**

**ACUTE TOXICITY TEST**

The acute toxicity test was carried out by the method described by Lorke (1983).

**INDUCTION OF DIABETES MELLITUS IN ALBINO RATS**

About 16 albino male rats weighing between 120-160 g were divided into four groups of four animals each. Diabetes was induced by intraperitoneally administration of 3 ml/kg body weight of alloxan. Three days after administration of alloxan, blood glucose level of rats were checked and those that have high glucose level (diabetic rats) were placed in group 2, (untreated group) and groups 3 and 4 respectively, received 200 and 400mg/kg body weight of leaf extract of *Pterocarpus santalinoids*. The positive control were given 10 ml of normal saline for twenty one days. Plant extracts were given every morning through oral intubation to all the diabetic rats according to the doses mention above for three weeks.

**Determination of serum glucose**

The method of Ochei and Kolhaktar (2007) was employed in the assessment of plasma glucose level.

**Determination of total serum cholesterol**
The evaluation of total cholesterol was done by the method as detailed by Allain and Roschlain (1979).

**Measurement of serum triacylglycerols concentration**
The method of Tietz (1990) was applied to assay for the triacylglycerol quantities in the blood serum.

**HDL DETERMINATION**
High density lipoprotein cholesterol fraction was separated by the precipitation techniques of Jacob *et al.*, (1990) and the cholesterol content was determined by the method of Allain *et al.*, (1974).

**LDL Cholesterol Determination**
LDL Cholesterol was measured using the calculation below
\[
\text{LDL} = \text{Total Cholesterol} - \text{Triacylglycerides} - \text{HDL Cholesterol}
\]

in mg/dl

**Estimation of albumin levels**
The procedure of Grant *et al.* (1987) was used to measure serum levels of albumin.

**RESULTS**

**Proximate Composition of Ethanol Extract of *Pterocarpus santalinoids***

The result of proximate analysis of *Pterocarpus santalinoids* were shown in Table 1 below. The results showed that the stem bark contained moisture, crude fiber, carbohydrate, fats, protein, and ash contents of 6.5 %, 2.16 %, 68.97 %, 2.81 %, 16.46 %, 2.39 %, respectively
Table 1: Proximate analysis of *Pterocarpus santalinoids*

<table>
<thead>
<tr>
<th>Proximate</th>
<th>Percentage composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>6.5177 ± .00862</td>
</tr>
<tr>
<td>Ash</td>
<td>2.3927 ± .00462</td>
</tr>
<tr>
<td>Fats</td>
<td>2.8153 ± .00404</td>
</tr>
<tr>
<td>Protein</td>
<td>16.4647 ± .00306</td>
</tr>
<tr>
<td>Fiber</td>
<td>2.1650 ± .00400</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>68.9780 ± 1.1700</td>
</tr>
</tbody>
</table>

Phytochemical Analysis of Ethanol Leaf Extracts of *Pterocarpus santalinoids*

The results of the qualitative phytochemical analysis of *Pterocarpus santalinoids* showed that the extract contains alkaloids, glycosides, flavonoids and steroids. The results of the quantitative phytochemical analysis of *Pterocarpus santalinoids* was presented in Table 2. The results also showed that leaf extract of *Pterocarpus santalinoids* contains alkaloids 1487.74 ± 0.001, flavonoids 2439.51 ± 0.007, steroids 3.03 ± 0.002, tannins 1607.28 ± 0.003, saponins 3.9653 ± 0.0037 and phenol 18.01 ± 0.40/100 respectively.

Table 2: Quantitative phytochemical analysis *Pterocarpus santalinoids*

<table>
<thead>
<tr>
<th>Phytochemical</th>
<th>Quantitative</th>
<th>Qualitative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tanins</td>
<td>1607.2853 ± .00351</td>
<td>++</td>
</tr>
<tr>
<td>Hydrogen cyanide</td>
<td>.8960 ± .00173</td>
<td>+</td>
</tr>
<tr>
<td>Reducing sugar</td>
<td>1731.1840 ± .00400</td>
<td>++</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>2439.5127 ± .00702</td>
<td>+++</td>
</tr>
<tr>
<td>Soluble carbohydrate</td>
<td>106.1203 ± .00666</td>
<td>++</td>
</tr>
<tr>
<td>Phenols</td>
<td>3274.1943 ± .00451</td>
<td>++++</td>
</tr>
<tr>
<td>Steroids</td>
<td>3.0363 ± .00208</td>
<td>++</td>
</tr>
<tr>
<td>Saponins</td>
<td>3.9653 ± .00379</td>
<td>++</td>
</tr>
<tr>
<td>Glycosides</td>
<td>4.9377 ± .00404</td>
<td>+</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>1487.7470 ± .001159</td>
<td>++</td>
</tr>
</tbody>
</table>

Acute Toxicity of Ethanol Extract of *Pterocarpus santalinus* Leaf in Albino Rats.
There was a decrease in physical activities of all the ethanol extract of *Pterocarpus santalinoids* test groups when compared to control that was administered with distilled water. No death was observed. The \( \text{LD}_{50} \) was found to be greater than 5000 mg/kg body weight of the albino Wistar rats.

### Table 3: Acute Toxicity of Ethanol Extract of *Pterocarpus santalinus* Leaf in Albino Rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dose (mg/kg)</th>
<th>No of rats at 24 hours</th>
<th>No of rats at 48 hours</th>
<th>No of rats at 96 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>5 of normal saline</td>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Group 2</td>
<td>100</td>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Group 3</td>
<td>200</td>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Group 4</td>
<td>1000</td>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Group 5</td>
<td>1600</td>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Group 6</td>
<td>2900</td>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Group 7</td>
<td>5000</td>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Effect of Ethanol Extracts of *Pterocarpus santalinoids* on Triglyceride, Cholesterol and HDL levels of alloxan induced diabetes in rats.

The results of effect of ethanol extract of *Pterocarpus santalinoids* on alloxan induced diabetic albino rats were presented in Figures 1, 2, and 3 respectively. The HDL level significantly \( (P<0.05) \) increased in all the diabetic treated rats when compared to the diabetic control. On treatment with graded doses of *Pterocarpus santalinoids* ethanol leaf extract at 200 and 400 mg/kg body weight reduced the LDL level near normal when compared to the diabetic control group, while in cholesterol and triglyceride there was a significant \( (P<0.05) \) increase in triglyceride and total cholesterol level in all the diabetic rats when compared to the non-diabetic control.
However, treatment with *Pterocarpus santalinoids* at 200 and 400 mg/kg body weight reversed the triglyceride and total cholesterol level near normal when compared to the diabetic control rats. This reversal effect of the plant extract was dose dependent.

Figure 1: Effect of ethanol extracts of *Pterocarpus santalinoids* on high density lipoprotein level of alloxan induced diabetic albino rat.

A = 200 mg/kg body weight of *Pterocarpus santalinoids*, B = 400 mg/kg body weight of *Pterocarpus santalinoids*, control (normal rats) and Untreated (diabetic rats)

Bar with the same letters are statistically non significant while those with different letters are statistically significant.
Figure 2: Effect of ethanol extracts of *Pterocarpus santalinoids* on cholesterol level of alloxan induced diabetic albino rat.

A = 200 mg/kg body weight of *Pterocarpus santolinoids*, B = 400 mg/kg body weight of *Pterocarpus santalinoids*, control (normal rats) and Untreated (diabetic rats).

Bar with the same letters are statistically non significant while those with different letters are statistically significantly.
Figure 3: Effect of ethanol extracts of *Pterocarpus santalinoids* on triglyceride level of alloxan induced diabetic albino rat.

A = 200 mg/kg body weight of *Pterocarpus santalinoids*, B = 400 mg/kg body weight of *Pterocarpus santalinoids*, control (normal rats) and Untreated (diabetic rats)

Bar with the same letters are statistically non significant while those with different letters are statistically significant.
Figure 4: Effect of ethanol extracts of *Pterocarpus santalinoids* on LDL level of alloxan induced diabetic albino rat.

A = 200 mg/kg body weight of *Pterocarpus santalinoids*, B = 400 mg/kg body weight of *Pterocarpus santolinoids*, control (normal rats) and Untreated (diabetic rats)

Bar with the same letters are statistically non significant while those with different letters are statistically significant.
Figure 5: Effect of ethanol extracts of *pterocarpus santolinus* leaf on Glucose concentration of alloxan induced diabetic albino rat.
A= Control, B= diabetic untreated, C= 200 mg/kg body weight, D= 400 mg/kg body weight of leaf extract.
Bar with the same letters are statistically non significant while those with different letters are statistically significant.

**DISCUSSION AND CONCLUSION**

Induction of diabetes was done using alloxan monohydrate. Interaperitoneally administration of 3 mg/kg body weight of alloxan to albino rats caused elevation of LDL, cholesterol and triglyceride with significant reduction in high density lipoprotein. According to [13], diabetes induction in albino Wistar rats using alloxan resulted in the destruction of the majority of the β-cells of the islets of Langerhans and pancreatic dys-function. Induction of diabetes caused oxidative stress to the liver, which was confirmed by the presence of oxidative marker enzymes in the blood [14]. Diabetes inflamed lipid components in the plasma and electrolytes in the kidney, resulting in dys-lipidemia and electrolyte disruption. The mal-functioning
of the pancreas as a result of the diabetic condition led to an increase in blood glucose sugar and a general lose of weight in untreated diabetic animals [14].

The phytochemical screening of ethanol extracts of *Pterocarpus santalinoids* revealed the presence of alkaloids, flavonoids, glycosides, terpenoids, tannins, phenols, saponins and steroids. The results of the proximate composition showed that *Pterocarpus santalinoids* contained moisture, crude fiber, carbohydrate, fats, protein, and ash contents of 6.5 %, 2.16 %, 68.97 %, 2.81 %, 16.46 %, 2.39 %, respectively.

Our acute toxicity study showed no death at 48 hours of administration of 5000 mg/kg body weight which revealed the non-toxic nature of the ethanol extract of *Pterocarpus santalinoids* from Ebonyi State. Maruthupandian and Mohan (2011) observed that acute toxicity study of *P. marsupium* wood and bark revealed the nontoxic nature of the plant which is similar to our findings.

The alloxan induced diabetic rats elicited significant rise in blood glucose from 85.31 to 428.32 mg/dl (p<0.01). On the contrary, diabetic rats treated with ethanol extracts of *Pterocarpus santalinoids* exhibited decrease blood glucose. According to Ajah et al., (2015) administration of 120 mg/kg body weight of alloxan caused increase in blood glucose level leading to alloxan induced diabetes in albino rats. Our finding is in line with Okoro et al., (2016) who observed significant rise in blood glucose after administration of alloxan monohydrate.

The hypoglycemic ethanol effect of *Pterocarpus santalinoids* was found to be inducing insulin release from pancreatic cells of diabetic rats. The levels of serum lipid profiles, total cholesterol (TC), triglycerides (TG), LDL-C and HDL-C in control and experimental animals were investigated (Figure 1-4). Alloxan induced rats showed significantly increased serum lipid profiles except HDL-C when compared with normal rats. Our result is in agreement with Ajah et al. 2014 and Offor et al. 2014 who observed significant reduction in alloxan induced diabetic rats.

CONCLUSION

The ethanol extract of *Pterocarpus santalinoids* treated rats showed a significant decrease in the content of lipid profiles when compared with diabetic induced rats. Similarly HDL-C level decreased in alloxan induced diabetic rats when compared to normal rats. On administration of ethanol extract of *Pterocarpus santalinoids* to the diabetic rats, HDL-C level was found to be restored close to normal. The level of serum lipid profiles are usually raised in diabetic rats in the present study and such elevation represents risk factor for coronary heart diseases. The hypolipidemic effect may be due to inhibition of fatty acid synthesis (Suba et al., 2004). In normal metabolism insulin activates the enzyme lipoprotein lipase and hydrolyses triglycerides and the deficiency in insulin results in inactivation of these enzymes thereby causing hypertriglyceridemia. The significant reduction of serum lipid levels in diabetic rats after *Pterocarpus santalinus* treatment may be directly attributed to improvements in insulin levels.
REFERENCES


