Research Article

Anti-diabetic activity of methanolic extract of seed cotyledon of Chrysophyllum albidum in alloxan-induced diabetic rats

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ABSTRACT: The effect of methanolic extract of seed cotyledon of Chrysophyllum albidum was investigated on some selected organs weight and oxidative stress parameters of alloxan-induced diabetic rats. A dose of 100 and 200 mg/kg body weight of methanol extract was given orally for nine (9) days. Preliminary phytochemical screening of extract revealed the presence of flavonoids, tannins, alkaloids and saponins in high concentration. The lethality dose (LD₅₀) result of the plants extract is found to be equal to or less than 5000 mg/kg body weight. Administration of 100 and 200 mg/kg body weight of methanol extract of C. albidum showed a significant (P < 0.05) decrease in blood glucose level compared with induced rats, administered 0.2 ml of normal saline. The animals induced and treated with various doses of methanol seed cotyledon extract of C. albidum showed a non-significant (P > 0.05) decrease in fasting blood sugar compared to the group induced and administered 0.2 ml of normal saline. Administration of extract at 100 and 200 mg/kg body weight showed a non-significant (P > 0.05) increase in glutathione concentration compared with induced rats administered 0.2 ml of normal saline. The rats induced with alloxan and administered 200 mg/kg body weight of extract showed a non-significant (P > 0.05) decrease in catalase activity compared with rats induced and administered 100mg/kg body weight of extract. The animals induced and administered various doses of methanol seed extract of C. albidum showed significant (P < 0.05) reduction in the weight of the liver and kidney compared to the animals induced and treated 0.3 mg/kg body weight of glibenclamide. The results showed that the plant extract has anti-diabetic activity and therefore provides a pharmacological basis for the folkloric uses of seed cotyledon extract of C. albidum in the management of diabetes mellitus.

KEYWORDS: Anti-diabetic properties, Chrysophyllum albidum, alloxan-induced diabetes.
INTRODUCTION

*Chrysophyllum albidum* is a popular forest tree, widely distributed in the low and rain forest zones and frequently found in villages in Nigeria and across the whole of Africa (Bello and Henry, 2015). The roots, barks and leaves of *Chrysophyllum albidum* have been employed in folk medicine for the treatment of diseases. The back is used for the treatment of yellow fever and malaria, while the leaf is used as an emollient for the treatment of skin eruption, stomach ache and diarrhea (Mfotabong et al., 2011).

Diabetes is a chronic disease characterized by high blood glucose level and abnormal metabolism of carbohydrates, protein and fat associated with a relative or absolute insufficiency of insulin secretion and with various degrees of insulin resistance, accompanied by glycosuria, polydipsia, and polyuria (Salaja et al., 2003). This is either as a result of insufficient endogenous insulin production by the pancreatic beta cells (type-1 diabetes); or impaired insulin secretion (type-2 diabetes) (Hussian and Theise, 2004). During diabetes, persistent hyperglycemia causes increased production of free radicals for all tissues from glucose autooxidation and protein glycosylation (Robertson, 2004). Oxidative stress is more obvious in type 2 diabetes and this appears to underlie the development of diabetes complications (Choi et al., 2008). Antioxidants are those substances which possess free radical chain reaction breaking properties. Recently there has been an upsurge of interest in the therapeutic potential of medicinal plants as antioxidants in reducing oxidative stress-induced tissue injury (Pourmorad et al., 2006). Among the numerous naturally occurring antioxidants; ascorbic acid, carotenoids and phenolic compounds are more effective (Duh et al., 1999). They are known to inhibit lipid peroxidation (by inactivating lipoxygenase), to scavenge free radicals and active oxygen species by propagating a reaction cycle and to chelate heavy metal ions (Sundararajan et al., 2006). The use of oral anti-diabetic drugs is limited due to their adverse side effects including the haematological, cutaneous and gastrointestinal reactions, hypoglycaemic coma and impairment of liver and kidney functions. In addition, they are not suitable for use during pregnancy (Alarcon-Aguilara et al., 2000). Besides, they are not affordable by low income earners. All these factors have led to the need for plants with hypoglycaemic properties and their uses in the management of diabetes (Calisto, 2000). The ready availability of *C. albidum* has made it a candidate of choice for new anti-diabetes drug. The aim of this study is to evaluate the anti-diabetic and antioxidant activities of oral administration of methanol seed cotyledon extract of *C. albidum* of alloxan-induced diabetic rats.

MATERIALS AND METHODS

All reagents used were of analytical grade. Drug (glibenclamide) used were supplied by Emzor Pharm Ind. Ltd., Nigeria. Normal saline was purchased from New Good Health, Afikpo, Ebonyi State, Nigeria.

Plant materials

The plant material for this study (*Chrysophyllum albidum* seed) was collected from Afikpo, Afikpo North Local Government Area of Ebonyi State, Nigeria between March and April, 2015. The seeds were authenticated at the Botany Unit of Akanu Ibiam Federal Polytechnic Unwana, Afikpo, Ebonyi State. It was air dried to constant mass, ground to powder, extracted with methanol and air dried.

Extraction procedure

The ground *Chrysophyllum albidum* seed cotyledon (97.9 g) was macerated in 400 ml of methanol for 48 hours. The macerate was filtered using Whatman No. 4 filter paper, the filtrate obtained was allowed to dry under room temperature. The weight of the plant was taken after drying and extract yield was calculated.

Method of induction of diabetes

The rats were injected with alloxan monohydrate, dissolved in normal saline at a dose of 130 mg/kg body weight intraperitoneally. On day 3, elevated blood glucose was observed; the animals were monitored for another day before diabetes was confirmed.

Experimental design

A total of twenty-five male albino mice weighing between 180-220 g were used for the study, the rats were obtained from the faculty of Veterinary Medicine, University of Nigeria, Nsukka, Nigeria. They were acclimatized for seven days in the Department of Science Laboratory Technology animal house given regular feed and water *ad libitum*. The rats were divided into seven different groups with five animals per group (n=5).

Group A: Normal control.

Group B: Alloxan-induced diabetic rats administered 0.2 ml of normal saline.

Group C: Alloxan-induced diabetic rats treated with 0.3 mg/kg body weight of glibenclamide.

Group D: Alloxan-induced diabetic rats treated with 100 mg/kg body weight of methanol extract.

Group E: Alloxan-induced diabetic rats treated with 200 mg/kg body weight of methanol extract.
Table 1: Phytochemical Screening Result of Methanol Extract of Chrysophyllum albidum

<table>
<thead>
<tr>
<th>Phytochemical</th>
<th>Mean ± SEM (mg/100g)</th>
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<tbody>
<tr>
<td>Saponin</td>
<td>0.028 ± 0.0025</td>
</tr>
<tr>
<td>Tannins</td>
<td>135.55 ± 0.0025</td>
</tr>
<tr>
<td>Phenol</td>
<td>222.56 ± 0.0025</td>
</tr>
<tr>
<td>Alkaloid</td>
<td>513.50 ± 0.0015</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>112.59 ± 0.0015</td>
</tr>
</tbody>
</table>

Acute toxicity and lethality (LD₅₀) test

The acute toxicity and lethality of ethanol extract of Chrysophyllum albidum seed was determined using the modified method of Lorke (1983). The test was divided into two phases. In phase one, nine (9) randomly selected adult mice were divided into three groups. Three per group (n = 3) and receive 10, 100 and 1000 mg/kg body weight of methanol extract and the signs of toxicity and number of death for a period of 24 hours were recorded. After 24 hours of observation, the doses for the second phase were determined based on the outcome of the first phase. Since there was no death, a fresh batch of animals was used following the same procedure in phase 1 but with higher dose ranges of 1900, 2600 and 5,000 mg/kg body weight of extract. The animals were also observed for 24 hours for signs of toxicity and possible number of death. The LD₅₀ was calculated as the geometric mean of the high non-lethal dose and lowest lethal dose (Lorke, 1983).

Phytochemical analysis

The quantitative phytochemical analysis of the sample was carried out using procedures outlined by Harborne (1984) and Pearson (1976).

Determination of blood glucose level

The blood glucose level was determined using one touch glucose monitoring system according to Ezejiofor et al., 2013. Three days after induction of diabetes, which was when the blood glucose levels of the rats were stable, drug treatment commenced, and was continued for 9 days. The blood glucose level was measured every three days for 9 days.

Estimation of plasma glutathione concentration

The reduced glutathione level was determined by the method of Bentler and Kelly (1963). This method was based on the development of yellow colour when 5,5-dithionbis-2-nitroberizoic (DTNB) is added to compound containing sulphydryl groups. The absorbance of the colour developed was read at 412 nm.

Erythrocyte catalase activity

This was determined according to the method of Aebi (1983). Red blood cell lysate was prepared by adding 1.2 ml of distilled water to 0.2 ml of RBC. Then 500 fold dilution of RBC lysate by phosphate buffer was made before the determination of catalase activity. Immediately following the addition of 1 ml phosphate buffer (blank) or hydrogen peroxide solution into 2 ml RBC diluted lysate, the change of absorbance of RBC against blank at 240 nm was recorded every 15 seconds for 1 minute on a UV spectrophotometer. The activity of catalase was calculated using the following equation.

Estimation of plasma malondialdehyde (MDA) concentration

Lipid peroxidation was estimated by measuring spectrophotometrically the level of the lipid peroxidation product, malondialdehyde (MDA) as described by Wallin et al. (1993). Lipid degradation occurs forming such products as malondialdehyde (from fatty acids with two or more double bonds), ethane and pentane (from the n-terminal carbons of 3 and 6 fatty acids, respectively). MDA reacts with thiobarbituric acid to form a red or pink coloured complex which in acid solution absorbs maximally at 532 nm.

Estimation of plasma vitamin C concentration

Plasma vitamin C concentration was determined using the method of Tietz (1983). The principle depends on the oxidation of ascorbic acid to diketoglutronic acid in a strong acid solution and this forms a dinitrophenylhydrazine. The hydrazone dissolves in strong sulphuric acid to produce a red complex which absorbs maximally at 500 nm.

Statistical analysis

The data obtained were analyzed using One Way Analysis of Variance. The data were further subjected to LSD post hoc test for multiple comparisons and differences between Means regarded significant at P < 0.05. The results were expressed as Mean ± SEM.
RESULTS

Yield of the methanol extract of *Chrysophyllum albidum* seed cotyledon

The methanol extract of *Chrysophyllum albidum* seed cotyledon was concentrated in a rotatory evaporator and fixed dried. The extract yield was observed to be 3.20 g (3.26%).

Lethal dose (LD$_{50}$)

In the experiment, there was no lethality or behavioural changes in the three groups of the mice that received 10, 100, 1000 mg/kg body weight of the extract at the end of the first experiment. Based on this result, further increased doses of 5000 mg/kg body weight of the extract showed weakness and one death case was observed within 24 hours of administration. This result showed that the extract was relatively safe at dose below 5000 mg/kg body weight.

Phytochemical screening of methanol extract of *Chrysophyllum albidum*

Table 1 shows phytochemical screening of methanol seed cotyledon extract of *Chrysophyllum albidum*, the concentration of the phytochemicals were observed in the order: Alkaloids > Phenols > Tannins > Flavonoids > Saponins.

Blood glucose levels of treated and non-treated alloxan-induced diabetic rats

The animals induced with 130 mg/kg body weight of alloxan and administered 0.2 ml of normal saline showed significant (P < 0.05) increase in blood glucose level on day 0, 3, 6 and 9 respectively compared to normal control, also the animals induced and administrated 0.3 mg/kg body weight of glibenclamide showed significant (P < 0.05) reduction in blood glucose level on day 0 compared to animals induced and administrated 0.2 ml of normal saline. The animals induced and administrated different doses of methanol seed cotyledon extract of *Chrysophyllum albidum* showed significant (P < 0.05) reduction in blood glucose level on day 3 and 6 compared to the animals induced and administrated 0.3 mg/kg body weight of glibenclamide (Table 2).

Fasting blood sugar level of treated and non-treated alloxan-induced diabetic rats

Group B animals, induced and administered 0.2 ml of normal saline showed significant (P < 0.05) increase in fast blood sugar compared to normal control, group C animals, induced and administered 0.3 mg/kg body weight of glibenclamide shows a non-significant (P > 0.05) reduction in fast blood sugar compared to normal control animals.

### Table 2: Blood glucose level of treated and non-treated alloxan-induced diabetic rats

<table>
<thead>
<tr>
<th>Treatment per Day</th>
<th>Day 0 (mg/dl) (%)</th>
<th>Day 3 (mg/dl) (%)</th>
<th>Day 6 (mg/dl) (%)</th>
<th>Day 9 (mg/dl) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Control (Group A)</td>
<td>109.75 ± 9.63$^a$</td>
<td>106.50 ± 8.15$^a$</td>
<td>107.25 ± 5.41$^a$</td>
<td>106.75 ± 3.04$^a$</td>
</tr>
<tr>
<td>Alloxan induced diabetes rats administered 0.2 ml of normal saline (Group B)</td>
<td>462.00 ± 10.17$^b$</td>
<td>455.25 ± 7.59$^b$</td>
<td>452.25 ± 10.05$^b$</td>
<td>455.75 ± 22.63$^b$</td>
</tr>
<tr>
<td>Alloxan induced diabetes rats administered 0.2 mg/kg b.w. of glibenclamide (Group C)</td>
<td>561.00 ± 4.51$^c$</td>
<td>473.25 ± 13.60$^c$</td>
<td>391.25 ± 23.68$^c$</td>
<td>76.25 ± 14.00$^c$</td>
</tr>
<tr>
<td>Alloxan induced diabetes rats administered 100 mg/kg b.w. of <em>C. albidum</em> seed cotyledon extract (Group D)</td>
<td>476.00 ± 8.18$^d$</td>
<td>348.75 ± 18.34$^d$</td>
<td>239.90 ± 12.03$^d$</td>
<td>99.00 ± 14.40$^d$</td>
</tr>
<tr>
<td>Alloxan induced diabetes rats 200 mg/kg b.w. of <em>C. albidum</em> seed cotyledon extract (Group E)</td>
<td>474.25 ± 6.87$^e$</td>
<td>265.80 ± 8.93$^e$</td>
<td>178.50 ± 12.48$^e$</td>
<td>149.75 ± 5.12$^e$</td>
</tr>
</tbody>
</table>

All values were expressed as the Mean ± SD (n = 5) $^a$P < 0.05 vs normal control; $^b$P < 0.05 vs diabetic control group. Figure in parenthesis indicate percentage increase or decrease in blood glucose level compared with the Day 0 of treatment groups. Means decrease.
Table 3: Selected Antioxidant status of Alloxan induced diabetic rats treated with Methanol seed cotyledon extract of *Chrysophyllum albidum*

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Malondialdehyde (mg/ml)</th>
<th>Glutathione (mg/dl)</th>
<th>Vitamin C (mg/dl)</th>
<th>Catalase (μl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Control (Group A)</td>
<td>3.10 ± 0.21</td>
<td>4.45 ± 0.45</td>
<td>0.63 ± 0.04</td>
<td>4.5 ± 0.21</td>
</tr>
<tr>
<td>Alloxan induced diabetes rats administered 0.2 ml of normal saline (Group B)</td>
<td>3.30 ± 0.13</td>
<td>3.70 ± 0.33</td>
<td>0.60 ± 0.04</td>
<td>2.05 ± 0.06*</td>
</tr>
<tr>
<td>Alloxan induced diabetes rats administered 0.3 mg/kg b.w. of glibenclamide (Group C)</td>
<td>3.10 ± 0.13</td>
<td>3.95 ± 0.39</td>
<td>0.64 ± 0.03*</td>
<td>2.10 ± 0.21*</td>
</tr>
<tr>
<td>Alloxan induced diabetes rats administered 100 mg/kg b.w. of <em>C. albidum</em> seed cotyledon extract (Group D)</td>
<td>2.80 ± 0.18</td>
<td>4.00 ± 0.15</td>
<td>0.69 ± 0.04*</td>
<td>2.10 ± 0.25*</td>
</tr>
<tr>
<td>Alloxan induced diabetes rats 200 mg/kg b.w. of <em>C. albidum</em> seed cotyledon extract (Group E)</td>
<td>3.55 ± 0.06*</td>
<td>4.55 ± 0.16</td>
<td>0.64 ± 0.02*</td>
<td>1.45 ± 0.41*</td>
</tr>
</tbody>
</table>

All values were expressed as the Mean ± SD (n = 5)  *P < 0.05* compared with normal control rats

On the other hand, group B animals induced and administered 200 mg/kg body weight of methanol seed extract of *C. albidum* shows a non-significant (P > 0.05) increase in fast blood sugar compared to normal control. Group D animals, induced and administered 100 mg/kg body weight of methanol seed extract of *C. abidum* shows a non-significant (P > 0.05) increase in fast body sugar compared to group C animals. Group D animals, induced and administered 100 mg/kg body weight of methanol seed cotyledon extract of *C. albidum* shows a non-significant (P > 0.05) reduction in fast blood sugar compared to group E animals, induced and administered 200 mg/kg body weight of methanol seed cotyledon extract of *C. albidum* (Figure 1).

**Weight of selected organs of treated and non-treated alloxan-induced diabetic rats.**

Group B animals, induced and administered 0.2 ml of normal saline shows a non-significant (P > 0.05) reduction in the weight of liver and spleen respectively compared to normal control animals, also group C animals, induced and administered 0.3 mg/kg body weight of glibenclamide shows a significant (P < 0.05) increase in the weight of the liver and kidney compared to normal control animals. Group D animals, induced and administered 100 mg/kg body weight of methanol seed cotyledon extract of *C. albidum* shows a non-significant (P < 0.05) decrease in the weight of liver and spleen compared to normal control animals. Group C animals, induced and administered 0.3 mg/kg body weight of glibenclamide showed significant (P < 0.05) increase in the weight of the liver, kidney and spleen compared to group D animals. Also group D animals, induced and administered 100 mg/kg body weight of methanol seed cotyledon extract showed non-significant (P < 0.05) reduction in the weight of liver, kidney and spleen compared to group E animals, induced and administered 200 mg/kg body weight of methanol seed cotyledon extract of *C. albidum* (Figure 2).

**Effect of methanol extract of *Chrysophyllum albidum* on antioxidant parameters of alloxan-induced diabetic rats**

The animals induced with alloxan and administered 0.2 ml of normal saline showed non-significant (P >0.05) reduction in the MDA, glutathione, vitamin C concentration and significant (P < 0.05) reduction in catalase activity compared to the normal control animals. The alloxan induced diabetic rats treated with 0.3 mg/kg body weight of glibenclamide and 100 mg/kg body weight of *C. albidum* seed cotyledon extract showed non-significant (P > 0.05) reduction in plasma malondialdehyde concentration compared to the induced animals administered 0.2 ml of normal saline. The animals induced with alloxan and treated with 0.3 mg/kg body weight of glibenclamide and 100 mg/kg body weight of the extract showed significant (P < 0.05) increase in vitamin C concentration and catalase activity compared to the alloxan induced animals administered with 0.2 ml of normal saline. The glutathione concentration in the diabetic rats treated with 200 mg/kg body weight of the extract was significantly (P < 0.05) higher compared to the diabetic animals treated with 100 mg/kg body weight of the extract (Table 3).
DISCUSSION

Diabetes is a metabolic disease associated with impaired glucose metabolism which adversely alters metabolism of lipids (Nazaruk and Borzym-kluczk, 2015). Diabetes currently is a major problem for the people of the world. It is a chronic metabolic disorder of carbohydrate, fat and protein metabolism characterized by elevation of both fasting and post prandial blood glucose levels (Hussain and Theise, 2004). The synthetic oral anti-diabetic agents can produce serious side effects (Akhtar and Igbal, 1991). The increases in number of diabetic patients have necessitated the need for scientists to find out new methods and plant extracts that can cure diabetes, to overcome the challenges posed by synthetic therapeutics in the management of diabetes.

The present study has shown a decrease in blood glucose levels with the use of different doses of methanol seed cotyledon extract of *Chrysophyllum albidum*. The result suggests that *Chrysophyllum albidum* seed cotyledon extract exhibit anti-diabetic effect which was consistent with the report of Olorunnisola *et al.* (2008) where it was shown that the anti-hyperglycemic and hypolipidemic effects of ethanolic extract of *C. albidum* seed cotyledon in alloxan induced diabetic rats. The pharmacological effects of the phytochemicals detected in this plant have been documented. Flavonoids have been found to be an active principle in herbal medicine and are known to be powerful antioxidants that may protect organs against toxicity and potential damage due to agents such as alloxan. Saponins have been reported to possess hypoglycaemic activity, which may be due to the inhibition of liver glycogenesis and may have contributed to the observed anti-diabetic activity of the plant extract (Laurent *et al.*, 2012).

Oxidative stress result from an imbalance between radical generating and radical scavenging systems that result in increased free radical production or reduced activity of antioxidant defences or both phenomena (Robertson, 2004). The potential of the antioxidant constituent of traditional plant for the maintenance of health has raised interest among patient and scientist. Oxidative stress also appears to be an important factor in the pathogenesis of a number of human infection (Al-Omor *et al.*, 2004). The oxidative effect is mainly due to phenolic compounds, such as flavonoid, phenolic acids that can delay or inhibit the oxidation of lipid or other molecules by inhibiting the initiation or propagation of oxidative chain reactions (Shadidi *et al.*, 1992).

The present study examines the antioxidant potential of methanol seed cotyledon extract of *Chrysophyllum albidum* in alloxan-induced diabetes rats. The results showed a high level of antioxidant potentials which have an important role in...
management of disorders involving oxidative stress. The total phenolic content, which correlated to the total antioxidant capacity, plays a role as free radical scavengers. Scavenging reactive oxygen species and improve the quality of life by preventing macrovascular and microvascular complications. Plants containing natural antioxidants such as tannins, flavonoids, vitamin C and vitamin E can preserve β-cell function and prevent diabetes induced ROS formation leading to inhibition of lipid peroxidation (Hunt et al., 1990).

From the result of this study, the methanolic extract of seed cotyledon of Chrysophyllum albidum was rapidly scavenged by the radicals indicating the ability of the extract to donate hydrogen atoms quickly. The decreased activity of catalase observed in the test animals might be as a result of high level of superoxide radical generation during oxidative stress. There was no significant difference in the concentration of vitamin C across the treated groups. The concentration of GSH reduced in the animals induced and administered 0.2 ml of normal saline which is an indication of reduced liver function. The level of GSH increased in the treated groups, showing the ability of the seed cotyledon extract in increasing the competency of the liver in detoxification of xenobiatics, as GSH is a major detoxifier in the liver.

These findings provide a strong indication that the seed cotyledon extract of C. albidum possesses antioxidative properties that help to stabilize the integrity of the cell membrane.

Conclusion

The plant extract possessed an anti-diabetic activity like the reference drug glibenclamide. This was based on the fact that the Chrysophyllum albidum extract showed a prominent reduction in the blood glucose levels when compared with the untreated groups. Chrysophyllum albidum possess various phytochemical constituents (Flavonoids, tannins, alkaloids, saponins and phenols), which may be responsible for the anti-diabetic activity in alloxan-induced diabetic rats. These compounds that possess this anti-diabetic activity therefore require further investigation.

Malondialdehyde, catalase and glutathione are extracellular antioxidants that convert potential substrates (superoxide anion radicals and hydrogen peroxides) to less reactive form in the body. The present investigation shows that C. albidum has antioxidant properties by scavenging free radicals, decreasing lipid peroxidation and increasing the endogenous blood antioxidant enzyme activity therefore can be used for the treatment of various ailments such as diabetes.

REFERENCES


peroxides and free radicals in LDL modification by glucose. *Diabetes, 39*: 1420-1424.


