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RESEARCH ARTICLE

EVALUATION OF THE ANTIDIABETIC POTENTIAL OF ETHANOL ROOT EXTRACT OF *MIMOSA PIGRA* LINN (FABACEAE) IN ALLOXAN-INDUCED DIABETIC ALBINO RATS

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ABSTRACT

Mimosa pigra is used in traditional medicine to lower blood glucose in diabetic patients. The objective of this study was to evaluate the antihyperglycaemic activity of the ethanol root extract of *Mimosa pigra* in albino rats. The rats were divided into five groups, each containing five rats. Group 1 served as the normal control (non-diabetic), groups II and III were treated with 250mg/kg and 500mg/kg of the ethanol extract of Mimosa pigra respectively, group IV was treated with glibenclamide and group V, the diabetic control group was untreated. Diabetes was induced through intraperitoneal injection of 160mg/kg alloxan monohydrate. Preliminary phytochemical screening of the ethanol extract revealed the presence of steroids (triterpenes), tannins, flavonoids, phlobatannins, and saponins. The LD50 was found to be greater than 5000mg/kg In the acute study, the 250mg/kg extract showed a reduction in the blood glucose level at the 6th hour which was statistically significant ($P<0.05$) compared to the diabetic control group, while the 500mg/kg extract showed no statistically significant reduction ($P<0.05$) compared to the diabetic untreated animals. In the prolonged study, on days 1, 3, 7, 10, the 250mg/kg extract showed a statistically significant reduction in blood glucose level compared to the diabetic control group, while the 500mg/kg extract showed a reduction in blood glucose levels on days 1, 3, 5 which was statistically significant ($P<0.05$) compared to the diabetic control group. The results suggest that extract of the root of *Mimosa pigra* possess significant anti-diabetic activity.

INTRODUCTION

Diabetes mellitus is one of the major chronic diseases in the world today (Stolar *et al.,* 2008; Kruger *et al.,* 2012). Diabetes is a metabolic disorder of multiple aetiology characterized by chronic hyperglycaemia with disturbances in carbohydrates, fat and protein metabolism resulting from defects in insulin secretion, insulin action or both. There is a significant increase in the number of patients suffering with this disease due to changing lifestyles such as less physical activity (Shaw *et al.,* 2010). Patients with diabetes experience significant morbidity and mortality from microvascular (Retinopathy, neuropathy, nephropathy) and macrovascular complications (heart attack, stroke and peripheral vascular disease). The complications are far less common and less severe in people who have well-controlled blood glucose levels (Andrew, 2000). Presently, there is growing interest in herbal remedies due to the side effects associated with the oral hypoglycaemic agents for the treatment of diabetes mellitus.

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Biological actions of the plant products used as alternative medicines to treat diabetes are related to their chemical composition. Herbal products or plant products that are rich in phenolic compounds, flavonoids, terpenoids, coumarins, and other constituents will usually bring about a reduction in blood glucose levels (He *et al.,* 2005; Jung *et al.,* 2006;Ji *et al.,* 2009). Herbal drugs are being prescribed due to their perceived effectiveness, fewer side-effects and relatively lower costs (Verspohl, 2002). Mimosa pigra is a serious weed in Africa, India, South-East Asia, Australia and some Pacific Islands. *Mimosa pigra* is closely related to *Mimosa pudica* (common sensitive plant). It can be distinguished from *Mimosa pudica* by its large size, large pods (6 to 8 cm long as opposed to 2.5 cm long) and leaves, which have 6 to 16 pairs of pinnae as opposed to 1 to 2 pairs on *Mimosa pudica* leaves (Lonsdale *et al.,* 1995; Miller *et al.,* 2001). There are claims that *Mimosa pigra* roots are sniffed for head colds, a decoction of the leafy stem is used as a mouthwash for toothaches, and that the fruits are used in eye medicines (Anonymous, 1980). Apparently it is also used for the treatment of snakebite in Africa (Irvine, 1961). In Sumatra, roasted and ground mimosa leaves are made into an infusion, which is drunk to treat a weak heart or weak pulse (Grosvenor *et al.*, 1995). A research conducted by

Tanzila *et al.* (2012) on the antihyperglycemic and antinociceptive activity of the methanol extract of *Mimosa pigra L.* leaves at doses of 50, 100, 200 and 400 mg per kg body weight mice showed a significant and dose-dependent reduction in the concentration of blood glucose levels in glucose-loaded mice. Following a study conducted byMbatchou *et al.* (2011), phytochemicals from *Mimosa pigra L.,Acacia nilotica, and Entada africana* on *Salmonella typhi were reported* to have antibacterial activity. The antidiabetic activity of the ethanol root extract of Mimosa pigra has not been reported previously. Thus, this research was aimed at evaluating the antidiabetic activity of *Mimosa pigra* root.

MATERIALS AND METHODS

Plant collection and Identification

The plant material (root) was collected from Chaza village in Suleja, Niger State by Mallam Mu'azam of The National Institute for Pharmaceutical Research and Development (NIPRD) Abuja. The plant was identified and authenticated in the herbarium of the same institute by Jemilat Ibrahim. A voucher specimen (NIPRD/H/6405) is deposited in the herbarium of NIPRD Abuja, Nigeria.

Preparation of plant material

The roots of *Mimosa pigra* Linn were washed under a running tap to remove debris, then sliced into small pieces and air-dried for about a month. The air-dried root was pulverized by grinding using a mechanical grinder and stored in an air tight container. Thereafter, 507g (0.57kg) of the coarse powder of air-dried root was subjected to 96%v/vethanol (Sigma-Aldrich, Germany) extraction for 72 hours. The filtrate was concentrated using a rotary evaporator and evaporated to dryness over a water bath at 40ºC. The percentage yield was then determined and the extract was stored in a refrigerator.

Phytochemical screening of plant material

Phytochemical screening was carried out by established protocols (Harborne, 1998).

Animals used

Albino rats (150-200g) of both sexes were obtained from the animal House of the Department of Pharmacology, Faculty of Pharmaceutical Sciences, University of Port Harcourt. All animals were housed in cages (5 in each cage) in the animal house of the Department of Pharmacology, Faculty of Pharmaceutical Sciences, University of Port-Harcourt. The animals were fed with standard diet and water *ad libitum* and were deprived of food and water overnight prior to the experiment. All the standard ethical requirements for experimental animals were complied with.

Experimental Protocol

Induction of Diabetes Mellitus

The method of Okokon *et al.* 2012 was modified and adopted. Diabetes mellitus was induced in a batch of normoglycaemic albino rats by the intraperitoneal injection of 160 mg/kg body weight of alloxan monohydrate (Sigma-Aldrich) after being fasted overnight. After 72 hours, the animals showing blood glucose level above 200 mg/dl upon testing were considered diabetic and selected for the study (Antia *et al.*, 2010).

Anti-Diabetic Activity Evaluation

The diabetic animals were divided into five groups of five animals each. Group one was the normal control (non-diabetic), group two was administered with 10mg/kg of glibenclamide (Swiss Pharma Nigeria Ltd), group three were the diabetic untreated and were administered with distilled water while groups four and five were treated with 250mg/kg and 500mg/kg of the ethanol extract of Mimosa pigra root extract respectively for 14 days consecutively.Fasting blood glucose level of the animals was monitored at 0hour, 2hours, 4hours and 6hours for acute study. Fasting blood glucose levels of the rats were monitored on days 0, 1, 3, 5, 7, 10 and 14 before administration of food for prolonged study. Blood glucose samples were collected by tail snipping method. The blood glucose level was determined by the use of Accu-check active glucometer (GmbH, Mannheim, Germany) test strips and readings were recorded daily in mg/dl (Okokon *et al.,* 2012).

Statistical Analysis

All the results obtained were analyzed using ANOVA and Student's t-test. Blood glucose levels of extract treated groups were compared to that of glibenclamide and control groups. P-values <0.05 were considered to be statistically significant.

RESULTS

Preliminary phytochemical screening of the ethanol extract of the roots of *Mimosa pigra L* showed the presence of tannins, phlobatannins, flavonoids, triterpenes and saponins (Table 1).

Phytochemical screening results

Table 1.Phytochemical Screening Results

Chemical Constituent	Observation
Alkaloids	-
Tannins	+
Anthraquinones	-
Phlobatannins	+
Flavonoids	+
Triterpenes	+
Saponins	+
Phenolic compounds	-

+ shows presence of chemical constituent
- shows absence of chemical constituent

The study showed a fluctuation in the blood glucose levels of the diabetic treated rats using the extract (250mg/kg and 500 mg/kg) and standard drug (glibenclamide) within the acute study period. The 250mg/kg extract showed a reduction in the blood glucose level at the 6^{th} hour which was statistically significant compared to the diabetic control group, while the 500mg/kg extract showed no statistical significance compared to the diabetic untreated animals. However, the 250mg/kg and 500mg/kg extracts showed a significant hypoglycaemic effect, while the standard drug showed no significant reduction in blood glucose (Table 2).

Table 2. Effects of the ethanol extracts of *Mimosa pigra* roots on the blood glucose level during acute study (Mean± SEM)

Treatments	Dose (mg/kg)	Blood Glucose Level (mg/dl) in Hours			
		0hr	2hrs	4hrs	6hrs
Ethanol Extract of *Mimosa pigra*	250	463.60±48.68	357.60±51.81	313.80±57.14	360.00±63.67*
	500	537.80±27.79	424.60±30.57	389.20±99.94	391.80±108.03
Glibenclamide	10	531.20±36.21	447.00±54.62	478.00±53.96	485.8±39.33
Diabetic Control	(10ml/kg of Water)	558.60±24.24	485.60±50.19	465.60±57.78	511.4±13.67
Normal Control	(10ml/kg of Water)	86.60±6.02	99.20±3.02	106.00±2.82	107.2±4.99

*= P<0.05 compared to the diabetic control group, n=5

Table 3. Effects of the ethanol extracts of *Mimosa pigra* roots on the blood glucose level during prolonged study. (Mean± SEM)

Treatments		Ethanol Extracts of *Mimosa pigra*		Glibenclamide	Diabetic Control (10ml/kg of Water)	Normal Control (10ml/kg of Water)
		250(mg/kg)	500(mg/kg)	10mg/kg		
Blood glucose level (mg/dl) in days	Day 0	463.60±48.68	537.80±27.79	531.20±36.21	558.60±24.24	86.60±6.02
	Day 1	336.60+64.90*	322.60+75.13*	389.60±58.29*	591.60±1.86	121.40±3.12
	Day 3	279.20+61.60*	231.80+69.11*	317.00±70.90*	594.40±1.07	137.40±15.01
	Day 5	110.60+25.54	252.80+65.95*	269.80±74.17*	580.60±3.47	119.00±8.93
	Day 7	324.20+78.66*	198.20+19.13	346.60±47.90*	590.40±2.99	125.20±10.36
	Day 10	222.80+54.91*	72.00	214.80±83.91*	585.60±3.58	116.40±11.70
	Day 14	140.00	125.00	273.60±55.16	363.20±14.94	109.40±10.97

*= P<0.05 compared to the diabetic control group, n=5

The 250mg/kg extract reduced the blood glucose level on days 1, 3, 7, 10 which was statisticallysignificant compared to the diabetic control group, while the 500mg/kg extract showed a reduction in blood glucose levels on days 1, 3, 5 which was statistically significant compared to the diabetic control group (Table 3).

DISCUSSION

Antidiabetic agents currently in use include insulin and various oral antidiabetic agents such as sulfonylureas, biguanides, α-glucosidase inhibitors and meglitinides. These treatments have their own drawbacks, ranging from resistance and adverse effects to lack of responsiveness in large segment of patients population. Also, with increasing incidence of diabetes mellitus in rural populations throughout the world and due to adverse effects of synthetic medicine, there is a clear need for development of indigenous, inexpensive botanical sources for anti-diabetic crude or purified drugs (Venkatesh *et al.,* 2003) Preliminary phytochemical screening of the ethanol extract of the roots of *Mimosa pigra L* showed the presence of tannins, phlobatannins, flavonoids, triterpenes and saponins.

This can be related to a study conducted by 20, Bilal *et al.,* 2013 which revealed with the help of phytochemical analysis, that the chloroform extract of the root of *Mimosa pudica* contains steroids, alkaloids, glycosides, flavonoids and phenolic compounds. This corresponds to the study done by Mirsha *et al.,* 2010 which reported plant constituents under the category of polysaccharides, peptides, alkaloids, glycopeptides, triterpenoids, amino acids, steroids, xanthones, flavonoids, lipids, phenolics, coumarins, iridoids, alkyl disulphides, inorganic ions and guanidines to have antidiabetic activity. The acute toxicity of the ethanol extract of the root of *Mimosa pigra* shows no adverse effect or mortality even at 5000mg/kg which shows it is relatively safe (Lorke, 1983). From the results obtained and the analysis carried out (P<0.05), the ethanol extracts (250mg/kg and 500mg/kg) of *Mimosa pigra* exhibited a dose dependent significant hypoglycaemic activity within the acute study period and the prolonged treatment period when compared with the diabetic control group.During the acute study period, the 250mg/kg extract showed a reduction in the blood glucose level at the 6th hour which was statistically significant (P<0.05) compared to the diabetic control group.

The 500mg/kg extract of *Mimosa pigra* also showed a reduction in the blood glucose levels of the treated diabetic albino rats. However, no statistically significant difference was observed during the acute study when compared to the diabetic untreated animals. During prolonged treatment, a fluctuation was observed in the blood glucose levels of the diabetic treated albino rats using the ethanol extracts (250mg/kg and 500 mg/kg) of *Mimosa pigra* and the standard drug (glibenclamide).The 250mg/kg extract showed a reduction in blood glucose levels on days 1, 3, 7, 10 which was statistically significant (P<0.05) compared to the diabetic control group. The 500 mg/kg extract of *Mimosa pigra* showed a reduction in blood glucose level on days 1, 3, 5 which was statistically significant (P<0.05) compared to the diabetic control group; however, there was no statistically significant difference on days 7, 10 and 14.

This can be related to a study conducted by Tasnuva *et al.* 2012 which reported that the methanol extract of *Mimosa pigra* stems showed dose-dependent significant reductions in the levels of blood glucose in glucose-loaded mice. The chief constituents responsible for the reduction of blood glucose levels are flavonoids and tannins. Several works have demonstrated that flavonoids may reduce hyperglycaemia and exert protective effect against non-enzymatic glycation of proteins in animals (Ghosh *et al.,* 2007). A study done by Liu *et al.* 2005 showed that tannic acid stimulates glucose transport and inhibits adipocyte differentiation in 3T3-L1cells, thereby reducing blood glucose levels without inducing adipogenesis in patients.Several possible mechanisms or a combination of the mechanisms may be responsible for the observed hypoglycaemic effect of the extract in diabetic rats.

Steroid (triterpenes), tannins, flavonoids, phlobatannins and saponins present in the extract may lower blood glucose either by potentiating the pancreatic secretion of insulin or increasing the glucose uptake, as has been observed in studies with Artemisia extract and extract of *Ageratum conyzoides L.* (Asteraceae), respectively (Farjou *et al.,* 1987; Nyunai *et al.,* 2009). Alternately, a compound or compounds may inhibit glucose absorption in gut, as observed with *Mangifera indica L.* (Anacardiaceae) stem barks (Bhowmik *et al.,* 2009). A further mechanism can possibly be by the increase of peripheral glucose consumption induced by the extract, as was reported by Sahoo *et al.* 2010, about ethanol extract of *Sapindus trifoliatus L.* (Sapindaceae). In either of these mechanisms or a combination of these mechanisms, the resultant effect will be reduction of glucose levels in the blood (Tanzila *et al.,* 2012).

The observed reduction of blood glucose level was more pronounced with the lower dose (250mg/kg) when compared to the higher dose (500mg/kg), this may be as a result of homologous desensitization. Homologous desensitization occurs when a receptor decreases its response to a signaling molecule when that agonist is in high concentration. It is a process whereby after prolonged agonist exposure, the receptor is uncoupled from its signaling cascade, and thus the biological effect of receptor activation is attenuated (Fehmann *et al.,* 1991). The findings of this study showed that the ethanol extract of *Mimosa pigra seems* to possesshypoglycaemic effect on alloxan-induced diabetic rats. This observed hypoglycaemic activity may be attributed to the presence of steroid (triterpenes), tannins, flavonoids, phlobatannins, and saponins in the extract.

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