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Anti-diabetic activity of *Mimosa pigra* Linn (Fabaceae) methanol leaf extract on alloxan-induced diabetic rats

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Abstract

The leaves of *Mimosa pigra* have been reported in ethnobotany to be used for the management of the symptoms of diabetes. This study is to investigate the anti-diabetic activity of the methanol extract of the leaves of this plant. The leaves were dried, pulverized and macerated successively in n-hexane, acetone and methanol. The methanol extract (1 g/kg body weight) was evaluated *in vivo* for anti-diabetic activity using adult male Wistar albino rats. Alloxan (150 mg/kg b.wt.) was used for induction of hyperglycemia, and Metformin (2.5 mg/kg) was used as the standard oral hypoglycemic agent. Blood glucose levels were monitored with a glucometer. The data is presented as Standard deviation (±) of the mean of blood glucose levels (mg/dl), and analyzed using one way analysis of variance (ANOVA) at ($p<0.05$). The methanol extract of the leaves showed significant percent decrease ($p<0.05$) in blood glucose levels of 3.80, 7.15 and 10.35 %, at 60, 120 and 180 min, respectively, on Day 1. And from Day 2 to Day 7 there was a continued decrease of 10.75, 16.36, 22.09, 29.06, 33.4 and 36.67 %, respectively, compared to the control. For the same period, the standard drug Metformin demonstrated greater decreases of 24.45, 30.38, 37.37, 40.93, 46.18 and 52.30 %, respectively. Phytochemical screening revealed the presence of flavonoids and tannins in the methanol and acetone extracts, while saponins and triterpenes were present in both the hexane and methanol extracts. The presence of these metabolites could be responsible for the observed anti-diabetic activity of this plant.

Keywords: *Mimosa pigra*; Fabaceae; Antidiabetic; Alloxan; Wistar rats

1. Introduction

The World Health Organisation (WHO) notes that inappropriate use of traditional medicines or practices can have negative or dangerous effects and that further research is needed to ascertain the efficacy and safety of the medicinal plants used by traditional medicine systems [1]. In the Southern parts of Nigeria, the plant *Mimosa pigra* has been in use in ethnobotany for the management of symptoms of diabetes mellitus. There have been claims that the roots of this plant are sniffed for head colds, the decoction of the stem used as mouthwash, and the fruits used as eye medicine. In some parts of the Philippines, it is used for the treatment of snake bite. In Sumatra, roasted and ground *M. pigra* leaves are made into an infusion which is drunk to treat a weak heart, and also used in the treatment of diabetes [2]. This plant is also used as green manure, animal feed, timber, and for erosion control [3].

Mimosa pigra (Fabaceae), also called Catclaw Mimosa, is a flowering upright prickly shrub or small tree. Pairs of leaflets are borne on the leaf stalk. These leaves are sensitive and they fold together when touched, also during the night. The flowers are pink or cream coloured and arranged in globular clusters. The fruit is an elongated flattened pod containing brown or greenish coloured seeds.

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Mimosa pigra was reported to have anti-microbial activity [3]. Another member of the genus Mimosa, *M. pudica,* has been reported to have wound healing potentials [4], hypolipidemic activity [5], anti-oxidant activity [6] and anti-diabetic activity [7]. The present study is designed to assess the anti-diabetic properties of *Mimosa pigra* in order to assess justification for its use in the management of symptoms of diabetes mellitus in ethnomedicine.

2. Material and methods

The plant was collected from Niger State in Nigeria, and was authenticated at the herbarium unit of the National Institute of Pharmaceutical Research and Development (NIPRD), Abuja with Voucher number NIPRD H/6405. The fresh leaves were collected, washed, shade dried, pulverized and extracted successively with n-hexane, acetone and methanol by maceration. Male Wistar Albino rats, Alloxan monohydrate (Sigma-Aldrich, Germany), Metformin (Glucophage®) and Glucometer with the test strips were used for the study.

2.1. Extraction of plant material

The pulverized plant material was macerated in n-hexane for 72 hours and filtered. The mac was then macerated with more portions of n-hexane to achieve exhaustive extraction. The filtrates were pooled together to obtain the n-hexane extract. The mac was then air dried for one hour and then macerated with acetone for 72 hours as described for n-hexane. The acetone filtrates were pooled together and concentrated using a rotary evaporator to obtain the acetone extract. The mac from the acetone extraction was macerated with methanol for 72 hours, as described above. The methanol filtrates were pooled together and concentrated to obtain the methanol extract.

2.2. Phytochemical Screening

Phytochemical tests were carried out on the extracts using standard procedures [8], [9].

2.2.1. Anti-diabetic assay

Adult male Wistar albino rats with an average weight of 150 g, were obtained from the department of Pharmacology of the Faculty of Pharmaceutical Science, University of Port Harcourt, Nigeria. They were allowed to acclimatize in the animal house for one week before induction of hyperglycemia. The rats were fed throughout the research period with animal feed and water.

All the rats were fasted overnight before the induction of hyperglycemia [10]. 5 rats were then separated into a group marked A, as the normal untreated. The rest of the rats were injected with Alloxan in a single intraperitoneal dose of 150 mg/kg body weight of the rats. The alloxan was prepared in normal saline. The blood glucose levels of all the animals were taken just before the administration of the alloxan. After the induction, all the rats were allowed free access to food and water. The animals were allowed to rest for 72 hours when sustained hyperglycemia is expected [11]. At this point, rats showing a glucose blood level of about 200 mg/dl were considered diabetic and were selected for the study [12]. These diabetic rats were placed in three more cages marked B, C, and D, with each group containing 5 rats.

In the treatment schedule, the rats in group A are to remain normal, untreated control; group B, diabetic untreated control; group C, to be treated with the methanol extract; and group D, to be treated with the standard drug, metformin. On Day 1, all the animals were fasted overnight before the treatment commenced. Basal blood glucose levels at 0 time were determined before the oral treatments were started. This was done by obtaining blood from the tails of the rats and checking the glucose levels with the glucometer strips after this, the methanol extract (1 g/kg body weight) and Metformin (2.5 mg/kg) were administered immediately to each rat in cages C and D, respectively. Subsequently, blood glucose levels were taken at 60 min, 120 min, and 180 min on this Day1 from all the rats. From D 2-7, the rats in cages A and B received no treatment except normal saline. Those in cage C received the methanol extract 3 times a day , at 10 am, 12 noon, and 2 pm; rats in cage D received Metformin at the same times. 2 hours later, at 4 pm, blood glucose levels of animals in all the cages were measured and recorded [13].

2.3. Statistical Analysis

Data were presented as ± Standard Deviation of blood glucose level (mg/dl) and analysed using one way analysis of variance (ANOVA) at ($p<0.05$) on graph pad prism version 6.01.

3. Results and discussion

Table 1 Percentage yield of the extracts from 250 g of the leaves

Extract	Weight (g)	Percent yield (%)
n-hexane	4.296	1.72
Acetone	18.535	7.41
methanol	21.594	8.64

Table 2 Phytochemical Screening Results of the Extracts

TESTS	N-HEXANE extract	ACETONE extract	METHANOL extract
Saponins	+	-	+
Tannins	-	+	+
Flavonoids	-	+	+
Alkaloids	-	-	-
Anthraquinones	-	+	-
Terpenes	+	+	+
Steroids	+	+	-

Key: + present; - absent.

Table 3 Day 1 mean blood glucose levels (mg/dl) of diabetic rats treated with methanol extract of *Mimosa pigra* linn (Fabaceae), measured at intervals

Group/Treatment	0 (min)	60 (min)	120 (min)	180 (min)
A (normal rats)/ normal saline	73.6 ±3.78	72.2± 3.90	71.6± 4.72	69.8 ±4.03
B (diabetic rats)/ normal saline	244.8± 14.48	244.6 ±13.13	241.8 ±13.95	239.6 ±15.57
C (diabetic rats)/ methanol extract	250.5 ±20.09	235.3 ±26.4 (3.80 %)	224.5 ±25.72 (7.15 %)	214.8 ±17.88 (10.35 %)
D (diabetic rats)/ metformin	233.0± 26.0	222.6 ±16.21 (8.99 %)	211.2 ±15.99 (12.65 %)	181.0 ±12.49 (24.45 %)^0

Each value represents the mean ±S.D. of blood glucose levels of the rats; n = 5; Figures in parenthesis represent % decrease in blood glucose levels;
^{0} = values considered statistically significant against the diabetic control at $p<0.05$.

Phytochemical screening was performed on the three extracts to find the class of constituents present in each of them. From the results of the phytochemical screening in Table 2, alkaloids were absent in all the extracts while flavonoids and tannins were present only in the methanol and acetone extracts. However, alkaloids have been reported to be present in some species of the genus Mimosa [14]. Saponins were present in the methanol and n-hexane extracts. The absence of tannins and flavonoids in the n-hexane extracts could be ascribed to polarity. Flavonoids being poly phenols are polar constituents hence they could have poor solubility in highly non-polar solvents like n-hexane. This is probably the reason for its absence in the n-hexane extract. From the results of the blood glucose levels in tables 3 and 4, the methanol extract of the leaves of this plant *Mimosa pigra* showed a significant reduction in blood glucose levels compared to the control (diabetic untreated) throughout the period of the study. However, there was no significant ($p<0.05$) reduction on day 1of the study. From day 2 to 7, the methanol extract treated group (C) and the metformin treated group (D) demonstrated significant difference in glucose levels compared to the control. This indicates that both treatment groups were able to reduce the glucose levels below that of the diabetic untreated group. From day 2 to 7,

the hypoglycemic activity of the methanol extract was observed to be lower than that of the standard drug, metformin. The flavonoids found present in the methanol extract (Table 2) could be responsible for the observed hypoglycemic activity since flavonoids have been reported to have anti-diabetic activity [15].

Table 4 Day 2-7: Daily mean blood glucose levels (mg/dl) of diabetic rats in the same groups A, B, C and D, treated with the methanol extract of *Mimosa pigra*

GROUP	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6	DAY 7
A	68.2 ±4.03	69.4± 3.65	69.6 ±3.21	69.6 ±2.30	69.6 ±1.14	69.4 ±0.89
B	235.3 ±19	234.7±21.03	234±19.97	233.3 ±20	233 ±18.5	232.3±19.7
C	210±14.58 (10.75 %)	196.3±14.9 (16.36 %)^0	182.3±13.7 (22.09 %)^0	165.5±16.3 (29.09 %)^0	155.0±14.5 (33.48 %)^0	144.8±14.6 (37.67 %)^0
D	179.4±12 (23.75 %)^0	163.4±7.77 (30.38 %)^0	146.6±8.20 (37.35 %)^0	137.8±6.30 (40.93 %)^0	125.4±8.39 (46.18 %)^0	110.8±9.88 (52.30 %)^0

Each value represents the mean ±S.D. of blood glucose levels of the rats; n = 5; Figures in parenthesis represent % decrease in blood glucose levels; ^{0} = values considered statistically significant against the diabetic control at $p<0.05$.

4. Conclusion

This study was aimed at investigating the anti-diabetic activity of the methanol extract of the leaves of *Mimosa pigra* linn (Fabaceae). The leaves of the plant was found to reduce significantly ($p<0.05$) the blood glucose levels of diabetic rats on daily basis using the percentage decrease in level as a parameter. This extract was observed to contain flavonoids, tannins, triterpenoids and saponins. The presence of one or more of these secondary plant metabolites could be responsible for the observed hypoglycemic effect, with the possibility of synergistic activity among them. This result suggests that this plant has the potential of drug development for the treatment of diabetes mellitus.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

We, Elechi, N. and Unamba O.E. affirm that there is no conflict of interest on this work. This is an original research work done by the authors, and that it has not been submitted to any other journal for publication.

Statement of ethical approval

The ethical approval for this work was obtained from the University of Port Harcourt.

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