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Phytochemical Analysis and Hypoglycaemic Activity of the Methanol Stem Bark Extract of *Tabernaemontana pachysiphon* Stapf (Apocynaceae)

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ABSTRACT

Tabernaemontana pachysiphon Staph. (Apocynaceae) stem bark is used ethnomedicinally for the treatment of diabetes. This study determined the phytochemical constituents and evaluate the methanol stem bark extract for acute toxicity and hypoglycaemic activity in Wistar albino rats. Phytochemical analysis was done using standard methods. Acute toxicity was evaluated by administering 0.5, 1, 2 and 4 g/kg body weight of extract to rats. Diabetes was induced by administering a single dose of 50 mg/kg streptozotocin (i.p) and the hypoglycaemic effects of daily doses of 125, 500 and 1000 mg/kg extract, orally administered to fasted normal and diabetic rats for 14 days, were monitored at 1, 2, 4, 8 and 12 h, as well as on days 7 and 14. Phytochemical screening revealed the presence of glycosides, saponins, terpenoids, flavonoids and alkaloids. The extract at a dose of 4 g/kg, caused neither death nor any observable symptoms of toxicity after 24 h and then, for 14 days. In non-diabetic rats, doses of 500 and 1000 mg/kg extract produced significant reductions ($p < 0.05$) in blood glucose at 8 h and 2 h, respectively. In diabetic rats, doses of 500 and 1000 mg/kg extract caused significant reductions ($p < 0.05$) at 4 h and 2 h, respectively, with maximum effect at 12 h. In both, the effect of the extract was found to be dose-dependent. In conclusion, this study revealed that the methanol stem bark extract of *T. pachysiphon* possessed hypoglycaemic effects, thus validating the ethnomedicinal claims of the plant.

Keywords: *Tabernaemontana pachysiphon, acute toxicity, hypoglycaemic effect, phytochemicals.*

Introduction

Diabetes mellitus (DM), one of the largest global health emergencies of the 21st century is a chronic condition that occurs when there is an inadequate amount of insulin in the body or when insulin in the body cannot be used due to insulin resistance. This results in increased levels of glucose in the blood, with symptoms of polyuria, polydipsia and polyphagia.[1, 2] Diabetes and its complications if left untreated for a long time, are major causes of death.[2] Worldwide, WHO reported 415 million people had diabetes and estimated that this would increase to 642 million people in the year 2040.[2]

As a result of this and due to poverty among the people living in rural areas, accessibility of drugs available for the treatment of hyperglycaemia and the adverse effects usually associated with the use of such drugs, there is thus an urgent need to search for cheaper hypoglycaemic alternatives that are efficacious, affordable, available, accessible and acceptable to peoples of such cultures or communities, since various clinical trials and epidemiological studies by different groups show hyperglycaemia to be the main cause of the complications observed in DM.[3-5]

One of such alternatives is the use of the plant *Tabernaemontana pachysiphon* Stapf. (Apocynaceae), popularly called "Giant Pin wheel flower" in English; "Ibu" in Edo; "pete – pete" in Igbo and "abododo" in Yoruba.[6, 7] *T. pachysiphon* is a shrub or small tree that thrives in the under-storey of light forest, from sea level up to 2200 m altitude.[6, 8] It grows up to 15 m in height, with trunk of about 40 cm in diameter. The leaves are simple, opposite, petiolated and broadly to narrowly elliptical. The margin is entire, apex is acuminate to acute and venation is reticulate. The flowers are usually white and sweet-scented. The fruits are pale green in colour and several to many seeded.

Economically, it is used to make knife sheaths, handles and combs (wood); for fire wood and to make charcoal (branches); to adulterate Hevea rubber, mend broken pots and calabashes (white latex); to make cloth and small ropes (bark); as well as to colour hair brown (leaf pulp).[8] Biologically, various extracts of *T. pachysiphon* have been shown to possess antimicrobial, anti-ulcer and opiate receptor binding activities.[9-12] The aqueous extract of the powdered stem bark at a dose of 5 g/kg, produced no signs of toxicity and death in mice.[13] Although most of the phytochemical work on *T. pachysiphon* has been concerned with the alkaloidal constituents of its various parts, fatty acids such as linoleic, myristic, oleic, palmitic and stearic acids were obtained from the seed oil.[9,14] Uwumarongie and Onwukaeme (2011) as well as Duru *et al.* (2015) reported the presence of alkaloids, cardiac glycosides, flavonoids and saponins in the powdered stem bark of *T. pachysiphon.* [13, 15]

Ethno-medicinally, *T. pachysiphon* is used as a stimulant and for the treatment of sores and ulcers (leaves); as an aphrodisiac, hypnotic, purgative and for the treatment of infections, stomach and headaches (root); as a styptic (latex); as a galactagogue (fruit), as well as for anaemia, ulcer, pain and infections (bark).[8, 16-18] This plant is claimed by herbal practitioners in parts of Ovia North-East Local Government Area of Edo State, Nigeria; to have potent hypoglycaemic effects (personal communication). Hence, due to scanty scientific data confirming the use of the stem bark in the treatment of hyperglycaemia, this study was designed to determine the phytochemical constituents in the methanol

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stem bark extract, as well as evaluate the extract for its acute toxicity and hypoglycaemic activity in non-diabetic and diabetic rats.

Materials and Methods

Materials

All solvents were of the analytical grade and were obtained from BDH Chemicals, Poole, England. Streptozocin (Sigma Chemical Co., USA); Glibenclamide 5 mg (Swiss Pharma, Nigeria) and Tween 80 solution (Guangdong Guanghua Sci-Tech Co. Ltd, China) were also used in this study.

Collection, authentication and preparation of plant material

Fresh parts (fruits, flowers, stem barks and leaves) of *T. pachysiphon* were collected from Ekiadolor forest in Ovia North-East Local Government Area of Edo State, Nigeria; to which Herbarium voucher specimen number FHI 110375 was assigned by Adeniji, K. A., Odewo, S. A. and Adeyemo, A. A (Taxonomists), at the Forest Herbarium, Forest Research Institute of Nigeria, Ibadan.

The stem barks were properly rinsed in distilled water and cut into small pieces. These were air dried for twelve (12) days under shade and then transferred to a thermostatically regulated electric heating oven maintained at 40ºC for 30 min. Thereafter, the plant material was powdered and stored in airtight containers until required for use.

Preparation of the plant extract

The powdered stem bark (500 g) was exhaustively extracted with methanol (2.5 L) using Soxhlet apparatus and then concentrated using a rotary evaporator. The extract was reduced to dryness on a thermostatically controlled hot water bath at 40ºC, in an evaporating dish to yield 21.04% extract. The dried extract was stored in a refrigerator at 4ºC.

Phytochemical tests

Preliminary phytochemical tests to evaluate the presence of active secondary plant metabolites were carried out on the methanol stem bark extract using established methods.[19, 20]

Animals

Wistar albino rats (150 – 200 g) of either sex were used for the study. The animals were obtained from the Animal House of the Department of Pharmacology, Ambrose Alli University, Ekpoma, Edo State, Nigeria; and kept in plastic cages. They were transferred to the Animal House of the Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Benin, Benin City, Nigeria; and maintained on a standard diet (Premier Feed Mill Ltd., Edo State) and water *ad libitum.* They were maintained at room temperature and allowed to acclimatize for two (2) weeks. This research was carried out in accordance with internationally accepted laws governing the use of laboratory animals and ethical approval was obtained from the Ethics Committee, Faculty of Pharmacy, University of Benin, Benin City, Nigeria (EC/FP/016/14).

Acute toxicity test

The method of Miller and Tainter was used.[21] Prior to the experiment, the animals were fasted over-night but water was given *ad libitum*. Thirty (30) Wistar albino rats of both sexes were divided into five (5) groups (A - E) of 6 animals per group and given 5 mL/kg Tween-80 solution, 0.5, 1, 2 and 4 g/kg of the methanol stem bark extract, respectively. An observation was made for changes in animal behaviour, mortality and morbidity over a period of 24 h and any signs of delayed toxicity for two weeks.

Hypoglycaemic activity

Non-diabetic rats

Overnight fasted non-diabetic male Wistar albino rats were divided into five groups (I to V) of five rats each. After determination of basal blood glucose levels, daily doses of the extract at 125, 500 and 1000 mg/kg were administered to groups I, II and III, respectively. The control group (5 mL/kg of 5% Tween-80 solution) and glibenclamide (5 mg/kg) were administered orally to groups IV and V, respectively. Animals were treated continuously for 14 consecutive days. The blood glucose levels were measured at 0, 1, 2, 4, 8 and 12 h; and on day 7 and day 14.[22, 23]

Diabetic Rats

Diabetes was induced in male albino rats by administering a single dose of streptozotocin, 50 mg/kg in citrate buffer (0.1 M, pH 4.5) intraperitoneally, to the overnight fasted rats. Blood glucose levels of the rats were measured after 48 h and animals with fasting blood glucose level above 250 mg/dL were considered diabetic and used for the experiment. Overnight fasted diabetic rats were divided into five (5) groups of five rats each. Basal blood glucose levels were determined, thereafter, daily doses of the extract at 125, 500 and 1000 mg/kg were administered to groups I to III respectively. The control group (5 mL/kg of 5% Tween-80 solution) and glibenclamide (5 mg/kg) were administered orally to groups IV and V respectively. Animals were treated and blood glucose levels determined as same intervals as the non-diabetic group. [22, 23]

Measurement of blood glucose and body weights

Blood glucose levels were determined by blood samples obtained from the tail tips of the rats using a glucometer ((ACCU–CHEK®, Germany). The percentage glycaemic change was then calculated using the formula:

$$\%\ Glycaemic\ change = \frac{\text{Glucose conc. at time T} - \text{Basal blood glucose level at time 0} \times 100}{\text{Basal blood glucose level at time 0}}$$

where T = 1, 2, 4, 8, 12 h, 7th or 14th day. [22]

Body weights of diabetic and non-diabetic animals were determined on days 0, 7 and 14.

Statistical analysis

Blood glucose levels were expressed as Mean ± Standard Error of Mean (SEM). Statistical analysis was done by ANOVA (one-way Analysis of variance) followed by Dunnett's multiple comparison tests. Level of significance was considered at $p < 0.05$. Graph Pad Instat Version 2.0.5 software (UK) was used.

Results and Discussion

The result of the phytochemical screening of the methanol extract of the stem bark of *T. pachysiphon* revealed the presence of glycosides, saponins, triterpenoids, flavonoids and alkaloids (Table 1). Tannins, cyanogenetic glycosides and anthracene derivatives were absent, unlike in the report of Duru *et al*., [15] where anthraquinone and tannins were present in the ethanol extract of the stem bark of *T. pachysiphon*. The absence of the above-named phytochemicals in the methanol extract may be due to the effect of environmental differences such as soil type, temperature, humidity, altitude and rainfall on the plant material collected.[24]

The acute toxicity tests on the plant extract showed that there was no mortality at a dose of 4 g/kg after 24 h and the absence of any toxicity symptoms after a further 14 day period suggests that the extract when administered orally maybe relatively non-toxic.[26]

Streptozotocin, a nitrosourea derivative, is one of the most commonly used chemicals for the induction of diabetes in rats due to its convenience and ease of use. It is a naturally occurring broad-spectrum antibiotic that is cytotoxic, especially toxic to the insulin-producing pancreatic beta cells in mammals, resulting in the degeneration of the Langerhans islets beta cells and then, hyperglycaemia. [27, 28] Streptozotocin when administered in a large dose (100 mg/kg) or in repeated low doses (30 - 40 mg/kg) for several days usually produce Type 1 diabetes.[29] However, administration of streptozotocin at 50 mg/kg produced type 2 diabetes, as the rats developed blood glucose levels 3 - 4 times higher than normal, failed to gain weight, survived without insulin supplementation and failed to develop ketosis.[23, 30]

In the present study, the effects of the extract in non-diabetic rats at specific time points are shown in Figure 1. Doses of 500 and 1000 mg/kg produced significant reductions ($p < 0.05$) in blood glucose levels from the 8 h and 2 h respectively, with maximum effect at 12 h. The effect was sustained up to the 7th day. Glibenclamide produced a significant reduction in blood glucose ($p < 0.01$), which was sustained throughout the period of the experiment. In diabetic rats, the effects of the extract at specific time points are shown in Figure 2. The extract at 500 and 1000 mg/kg produced significant reductions ($p < 0.05$) in blood glucose levels from 4 h and 2 h respectively; with maximum effect at 12 h. Like in the normal rats, the effect was sustained up to the 7th day. The hypoglycaemic effect of the extract in the diabetic rats further indicates that the type 2 diabetes was induced in this study. However, in both the normal and diabetic rats, the hypoglycaemic effect of the extract was found to be dose-dependent, as an increase in dose resulted in better hypoglycaemic activity. The above results which show the hypoglycaemic effect of the extract both in non-

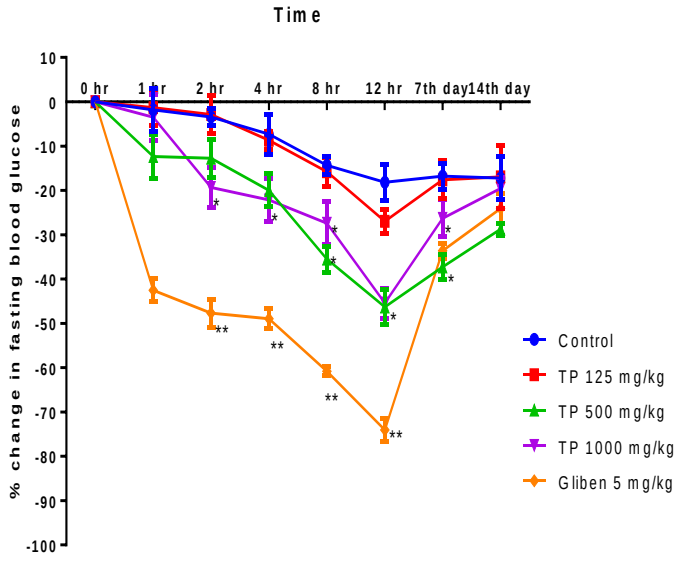

Figure 1: Percentage glycaemic change after administration of *T. pachysiphon* in non-diabetic rats Data are Mean ± SEM. n = 5. $^{*}p < 0.05$, $^{**}p < 0.01$; as compared to control. Control group received 5 mL/kg of Tween 80; TP: *T. pachysiphon*; Gliben: glibenclamide.

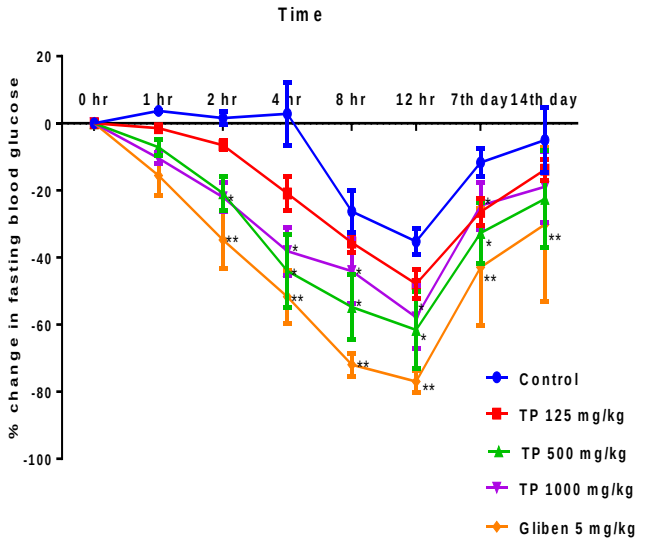

Figure 2: Percentage glycaemic change after administration of *T. pachysiphon* in diabetic rats. Data are Mean ± SEM. n = 5. *p < 0.05, **p < 0.01; as compared to control. Control group received 5 mL/kg of Tween 80; TP: *T. pachysiphon*; Gliben: glibenclamide.

Table 1: Preliminary phytochemical screening of the methanol extract of *T. pachysiphon*

S/No.	Phytochemical	Observation
1.	Glycosides	Present
2.	Saponins	Present
3.	Tannins	Absent
4.	Cyanogenetic glycoside	Absent
5.	Tritrepenoids	Present
6.	Flavonoids	Present
7.	Anthracene derivatives	Absent
8.	Alkaloids	Present

Table 2: Effect of the methanol stem bark extract of *T. pachysiphon* on body weights of non-diabetic rats.

Treatment Groups	Dose (mg/kg)	Day 0 Weight (g)	Day 7 Weight (g)	Day 14 Weight (g)
Control	-	263.33 ± 12.01	265.11 ± 10.88	278.42 ± 11.59
TP	125	267.84 ± 16.71	276.83 ± 17.21	284.46 ± 17.36
	500	273.14 ± 18.71	268.96 ± 14.96	277.97 ± 15.24
	1000	269.80 ± 6.35	261.22 ± 5.77	259.04 ± 8.75
Glibenclamide	5	269.94 ± 13.83	274.21 ± 15.75	284.99 ± 16.47

Control group received 5 mL/kg of Tween 80. Values are Mean ± S.E.M. n = 5. TP: *T. pachysiphon*; control.

Table 3: Effect of the methanol stem bark extract of *T. pachysiphon* on body weights of diabetic rats.

Treatment Groups	**Dose (mg/kg)**	**Day 0 Weight (g)**	**Day 7 Weight (g)**	**Day 14 Weight (g)**
Control	-	187.16 ± 9.55	175.40 ± 10.98	160.55 ± 11.09
TP	125	192.17 ± 10.81	162.20 ± 11.02	169.60 ± 7.89
	500	188.79 ± 6.31	169.37 ± 14.39	166.22 ± 14.60
	1000	179.55 ± 6.43	172.93 ± 7.06	168.85 ± 8.28
Glibenclamide	5	185.96 ± 7.19	153.01 ± 5.67	168.39 ± 7.27

Control group received 5 mL/kg of Tween 80. Values are Mean ± S.E.M. n = 5. TP: *T. pachysiphon*; control

diabetic and diabetic rats suggest that the mechanism of action of the extract maybe due to the stimulation of insulin release from pancreatic *β*-cells, similar to the sulfonylureas.[31]On body weights of non-diabetic and diabetic rats as shown in table 2 and 3 respectively, there were no significant reductions in the weight loss in any of the groups throughout the period of the experiment. Protein-energy wasting accompanying hyperglycaemia has been attributed to altered glucose metabolism with diabetic rats where the failure of body cells to utilize glucose as energy source seems to have ushered proteins as an alternative energy source leading to a metabolic imbalance in protein metabolism with consequent loss of body weight or continuous excretion of glucose from the body. [32, 33, 34] Therefore, improvement in body weights could be due to better control of the hyperglycaemic state in diabetic rats. However, the methanol extract of the stem bark of *T. pachysiphon* did not improve the body weight of diabetic rats.

Phytochemicals such as flavonoids, tannins and alkaloids are some of the documented compounds isolated from plants with a potential to decrease the blood glucose level. [35, 36] Thus, the significant hypoglycaemic effect of the extracts of *T. pachysiphon* could be due to the presence of the abovementioned components in the extracts, which could act synergistically and/or independently to enhance the activity of glycolytic enzymes.

Conclusion

In this study, the methanol extract of the stem bark of *T. pachysiphon* was shown to possess hypoglycaemic activity, which thus validates the ethnomedicinal claims of the plant, for the treatment of diabetes.

Conflict of interest

Authors declare no conflict of interest.

Authors' declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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