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Full Length Research Paper

Blood glucose lowering effect of aqueous extract of *Graptophyllum pictum* (Linn) Griff. on alloxan-induced diabetic rats and its acute toxicity in mice

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This study was aimed at evaluating the claimed anti-diabetic property of the aqueous extract of *Graptophyllum pictum* leaf and to establish an effective dose for the extract. It also attempted to unravel if the extract could be acutely toxic to mice. The anti-diabetic study was carried out on alloxan-induced diabetic Wistar rats. After diabetic induction, the rats were divided into 5 groups. Groups 1 to 3 were orally administered 100, 150 and 200 mg/kg body weight extract by gastric probe for four weeks; group 4 was administered 10 mg/kg body weight metformin, a well known hypoglycemic drug, while group 5 served as control and received the vehicle of administration (distilled water). The fasting blood glucose level (FBGL) of the rats was checked before commencement of treatment and weekly during the drug administration period using Roche Accu-chek Active Glucometer. The percentage change in FBGL before commencement of treatment and during the treatment period was calculated and expressed graphically. The acute toxicity of the extract was studied in 4 groups of Swiss albino mice which were orally administered high doses (1 to 4 g/kg) of the extract. The results obtained from the anti-diabetic study showed a significant reduction ($P < 0.05$) in the mean fasting blood glucose level in all the three groups of animals treated with the plant extract when compared to the control; and it exhibited effective anti-diabetic potency when compared with metformin (a standard anti-diabetic drug); the effective dose was established at 100 mg/kg. The LD_{50} could not be determined as none of the treated mice died during the acute toxicity study. These findings suggest that the aqueous extract of the leaves of *G. pictum* possess hypoglycemic effect which is comparable to metformin and can be safely administered orally without any immediate unwanted effect. However, this calls for detailed studies to elucidate the therapeutic and long term toxicological profiles of the extract.

Key words: Diabetes, *Graptophyllum pictum*, aqueous extract, hypoglycemic, metformin.

INTRODUCTION

From ancient times, plants have been used as a source of medicine that forms the backbone of human health care. Diabetes mellitus, a chronic and heterogenous disease is one of the world's most devastating human endocrine diseases. WHO (1980) estimate of the people affected by this disease worldwide was put at 230 million and it is projected to become 235 million by 2035 (Chevenne and Fonfrède, 2007); the human population therefore appears to be in the mist of an epidemic of diabetes. Diabetes is defined as a state in which homeostasis of carbohydrate and lipid is improperly regulated by insulin; this results in elevated blood glucose level, a situation referred to as hyperglycemia, if there is no return to normalcy within a period of time, which in due time leads to the syndrome called diabetes mellitus (Tiwari and Rao, 2002). Two main types of diabetes mellitus are known based on their clinical manifestations.

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Abbreviations: GPAE, *Graptophyllum pictum* aqueous extract; **FBGL,** fasting blood glucose level; **CVD,** cardiovascular disease; **NIDDM,** non insulin dependent diabetes mellitus; **IDDM,** insulin dependent diabetes mellitus.

Type 1, known as juvenile onset or insulin dependent diabetes mellitus (IDDM); it manifests before 20 years of age (Cantrill, 1999) and its origin is usually ascribed to autoimmune disorder that destroys the β cells of islet of Langerhans that are responsible for insulin production in the body. Type 2, the non insulin dependent diabetes mellitus (NIDDM) is the most prevalent form and it usually makes its appearance later in life, hence it is referred to as "adult onset diabetes" The underlying metabolic causes result from a combination of defects in insulin secretion and action (insulin resistance). Most patients with type 2 diabetes have insulin resistance and this is predisposing to both diabetes and cardiovascular disease (CVD) (Reaven, 1996) which usually result from high level of glucose in the blood and byproducts of lipid metabolism within the tissues (Ogbonnia et al., 2008). Treatment of diabetes centers on availability of sufficient amount of insulin in the body system.

Before the introduction of insulin therapy in 1922, the treatment of diabetes mellitus relied mainly on dietary measures which included the use of traditional therapies based on plants (Gray and Flatt, 1999). Many traditional plant treatment for diabetes exist (Ivorra et al., 1989; Gray and Flatt 1997) out of which few have received scientific or medical scrutiny and WHO (1980), has recommended that traditional plant treatment for diabetes warrant proper evaluation.

Graptophyllum pictum (L.) Griff, family Acanthaceae is commonly known as "Caricature plant" or Joseph's coat" because of the bicolours of its leaf. The plant is ornamental and grows profusely during the raining season in the tropic regions of the world. Lavergne and Vera (1989) claimed that the infusion prepared from the leaf of this plant possess analgesic, anti-inflammatory and anti-diabetic activities. Ozaki et al. (1989) confirmed the analgesic and anti inflammatory activities of the alcoholic extract, while Olagbende-Dada et al. (2009) showed it to be uterotonic and abortifacient after observing its effect on a pregnant goat who fed on it. However, there is no scientific record/evidence to support the use of this plant in the control of diabetes. Hence the present study was designed to test the hypoglycemic effect of the aqueous extract of the leaves of *G. pictum* in alloxan-induced diabetic rats.

MATERIALS AND METHODS

Plant material

Fresh leaves of *G. pictum* were collected from a residential area of Lagos metropolis in Nigeria. The plant was identified by Mr. Odewo of Forestry Research Institute of Nigeria (FRIN), Ibadan, Nigeria. A voucher specimen (PCLACAN01) was deposited in the Institute herbarium.

Extract preparation

The fresh leaves were washed and allowed to drain for 30 min. They were cut into small bits and 1.5 kg of the bits was extracted with distilled water with application of gentle heat at 50 to 60°C for 30 min (guided by the local method of preparation). The yellowish green liquid extract was strained through an absorbent cotton wool, filtered through prepleated filter paper (Schleicher and Schuell USA, No 560) and concentrated under *vacuo* at 40°C and then freeze dried. The dried extract was stored in a refridgerator at -4°C until use. A 20% weight in volume solution of the aqueous extract in water termed GPAE was made and used for oral administration.

pH determination of prepared extract

The pH of the freshly prepared aqueous extract of the leaf and that of GPAE just before administration was determined using Mettler Toledo pH meter.

Animals

Forty mature healthy male Wistar rats (120 to 160 g) bred in the Laboratory Animal Centre of the College of Medicine, University of Lagos were used for the study. They were kept in cages with square mesh at the base and housed at ambient temperature (25 to 30°C) with 12 h light and darkness cycle. They were fed with standard animal chow (Pfizer Nig. Ltd.) and allowed free access to water *ad libitum*.

Induction of diabetes

Diabetes was induced in the morning after fasting the animals overnight by a single intraperitoneal injection of 100 mg/kg of 10% w/v alloxan monohydrate (Sigma) dissolved in distilled water. After 72 h, blood glucose level of the dosed rats was checked and those with blood glucose level more than 200 mg/dl were considered, selected and used for the study.

Evaluation of hypoglycemic activity

Dose selection

Six of the diabetic rats were randomly divided into two equal groups and members of each group were administered 50 and 250 mg/kg of GPAE daily for two weeks, while their fasting blood glucose was checked on alternate days. All the animals in the 250 mg/kg showed progressive reduction in the fasting blood glucose, while none of the 50 mg/kg showed any during this period.

Effective dosing

Twenty five of the diabetic rats were randomly divided into 5 equal groups: Group 1 orally received 100 ml/kg distilled water (vehicle of administration) and served as the control. Groups 2, 3 and 4 received 100, 150 and 200 mg/kg of the aqueous extract (GPAE), respectively, while group 5 received 10 mg/kg metformin (a well known hypoglycemic drug). These doses were given daily for 4 weeks while the fasting blood glucose level (FBGL) of the animals were determined at the end of each week.

Determination of blood glucose

Blood samples were obtained by aseptic prick of the tail tip before the diabetic induction (S_1), 72 h after the induction (S_2) and at the end of each week during the 4 weeks period of drug administration

Table 1. Effect of water, GPAE and metformin on blood glucose level of alloxan-induced diabetic rats.

Treatment	Fasting blood glucose level (mg/dl)					
	S_1	S_2	W_1	W_2	W_3	W_4
Diabetic + distilled water	69 ± 3.7[a]	220 ± 5.0[b]	225 ± 2.7[b]	236 ± 4.4[b]	245± 3.0[b]	265 ±3.5[b]
Diabetic +100 mg/kg GPAE	67 ± 2.4[a]	258 ± 7.5[b]	143 ± 3.9[c]	112 ± 7.1[c]	99 ± 7.8[a]	92 ± 8.3[a]
Diabetic + 150 mg/kg GPAE	73 ± 2.9[a]	262 ± 4.4[b]	140 ± 6.1[c]	106 ± 5.8[c]	96 ± 1.2[a]	88 ± 2.0[a]
Diabetic + 200 mg/kg GPAE	70 ± 5.1[a]	256 ± 6.0[b]	137± 4.2[c]	98 ± 3.5[a]	88 ± 2.8[a]	83 ± 4.0[a]
Diabetic + 10 mg/kg metformin	72 ± 4.6[a]	261 ± 5.2[b]	135 ± 3.1[c]	97 ± 4.1[a]	85 ± 1.5[a]	78 ± 1.5[a]

Values with the same superscripts are not significantly different at $P < 0.05$

Table 2. Calculated percentage of glycemic change (GC) produced in alloxan-induced diabetic rats by distilled water, GPAE and metformin.

Treatment	Calculated percentage change in glycemic level			
	Week 1	Week 2	Week 3	Week 4
Control (distilled water)	2.3	7.3	11.4	20.4
100 mg/kg extract	- 44.6	- 56.6	- 61.6	- 64.3
150 mg/kg extract	- 46.6	- 59.5	- 63.4	- 66.4
200 mg/kg extract	- 46.5	- 61.7	- 65.6	- 67.6
10 mg/kg metformin	- 48.3	- 62.8	-67.4	- 70.1

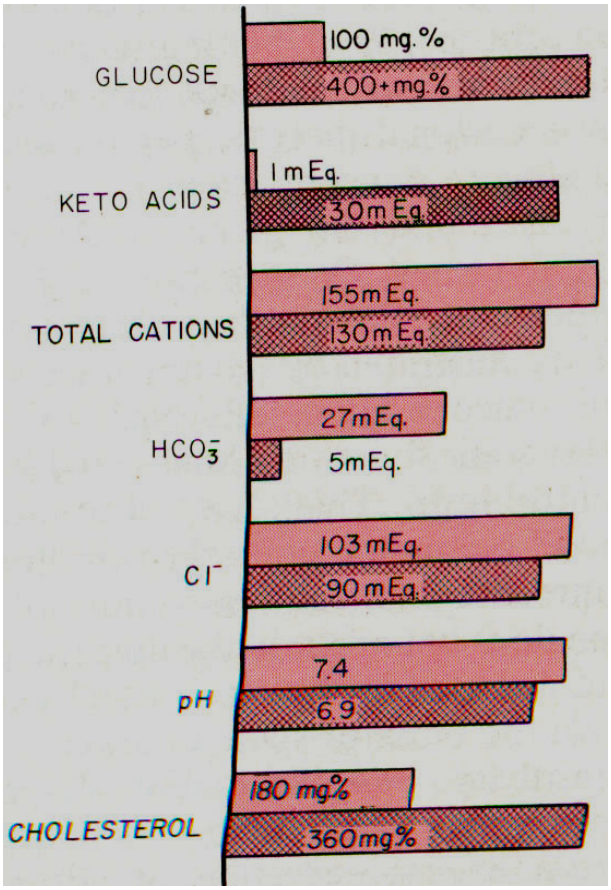

Figure 1. Change in blood constituents in diabetic coma, showing normal (light bar) and diabetic (dark bar) values (Guyton, 1981).

(W_1, W_2, W_3 and W_4), and the various glucose concentrations were obtained using Roche Accu-chek Active Glucometer. The concentrations obtained (Table 1) were used to calculate the percentage glycemic change (Table 2) based on the formula:

$$\%\text{Glycemic change (GC)} = \frac{\text{Treated FBGL (W)} - \text{Untreated FBGL (S}_2\text{)}}{\text{Untreated FBGL (S}_2\text{)}} \times 100$$

Statistical analysis

Differences in fasting blood level (FBGL) for all treated and control rats were determined using an analysis of variance. Significant differences were determined using student's t-test and differences were considered significant at $p < 0.05$. All data are expressed as mean ± standard error of the mean.

RESULTS AND DISCUSSION

In the diabetics, insulin deficiency hinders the intake of blood glucose into the body tissues, hence glucose becomes concentrated in the blood at the expense of the tissues. Guyton (1981) declared that when glucose is not available in the tissue cells for energy production, the cells shift to lipid utilization; lipid catabolism generates ketone bodies and acidosis usually results. If the body fluid's pH falls below approximately 7.0, the diabetic person develops coma. Figure 1 shows that any intake that lifts up the pH of the diabetic patient will bring a relief. Guyton (1981) actually recommended that the acidosis that develops during the lipid utilization is often corrected by administering sodium bicarbonate or sodium

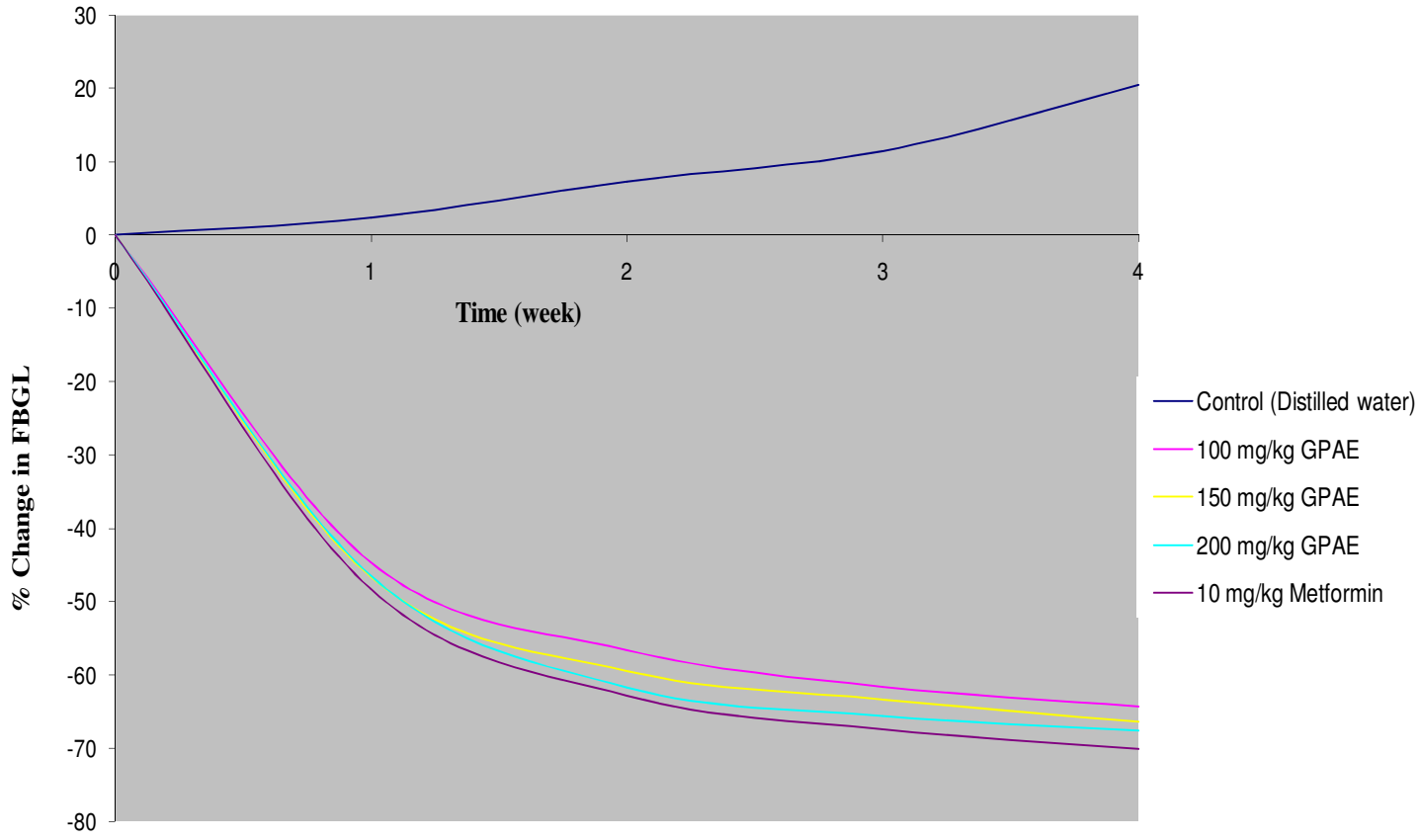

Figure 2. Percentage change in FBGL of diabetic rats in 4 weeks oral administration of water, GPAE and metformin.

lactate solution. According to Lavergne and Vera (1989), three or four fresh leaves of *G. pictum* are boiled in a liter of water and a cup of the aqueous extract is taken in the mornings to control diabetes. The pH of *G. pictum* aqueous extract immediately after extraction was 9.35 and that of GPAE just before administration was 8.25. This may explain why the folkloric usage recommends extemporaneous preparation of the fresh leaf (to serve as a means of preserving its pH before administration). The result displayed in Figure 2 shows the glucose lowering effect of the aqueous extract of *G. pictum* at the doses used in good comparison with metformin. All the doses administered produced more than 50% reduction in glucose level within 2 weeks of administration. The preliminary phytochemical investigation of the aqueous extract of the leaf by Olagbende-Dada et al. (2009) showed the presence of saponin, tannin and flavonoids in glycosidic forms and a basic constituent (alkaloid not confirmed). It is of strong opinion that the alkaline nature of this aqueous extract contributes to its ability to give the diabetics some relief.

Apart from this, the detection of flavonoids in the extract can also be linked to the blood glucose lowering property. Many reports on herbal remedy for diabetes show that they exhibit anti-oxidant properties due to the presence of flavonoids in them (Ivorra et al., 1989; Baynes and Thorpe, 1999). The free radical scavenging ability of many flavonoids-containing extracts has been postulated as the mechanism which affords relief in many distressful diseased conditions of the body, diabetes inclusive (Melander et al., 1996; Tiwari, 2004). The effective dose is established at 100 mg/kg, increasing this dose, however, produces insignificant change in the observed response (Figure 2). Lesser dose of 50 mg/kg was found to be ineffective. This implies that at the effective dose of 100 mg/kg, the receptor sites are almost fully occupied.

The results of the acute toxicity study shown in Table 3 suggest that the aqueous extract when taken within a short period of time, is without any harmful effect since half maximal lethal dose (LD_{50}) could not be determined for lack of fatality; however, toxicity study based on long period of administration needs to be embarked upon especially in females of reproductive age since an earlier report by Olagbende-Dada et al. (2009) on the aqueous ethanolic extract confirmed the plant as uterotonic.

Conclusion and recommendation

The present study has justified the safe use of the aqueous extract of the fresh leaf of *G. pictum* within a

Table 3. Acute toxicity of GPAE in mice.

Group	Dose (g/kg)	Log Dose	Number of deaths	Death (%)	Probit value
1	1.0	0.00	0/5 [a]	0.0	-
2	2.0	0.30	0/5 [a]	0.0	-
3	3.0	0.48	0/5 [a]	0.0	-
4	4.0	0.60	1/5 [a]	20.0	4.16
5	Control	-	0/5 [a]	0.0	-

Values with the same superscripts are not significantly different at $P < 0.05$.

short period of oral administration in diabetic condition. Long term toxicity study is recommended.

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