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Effect of Methanolic Extract of *Momordica charantia* L. Leaves on Alloxan Treated Wistar Rats

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Phytochemistry of *Momordica charantia* L. leaves revealed the presence of flavonoids and tannins. The hypoglycaemic effect of *M. charantia* leaves as well as the plant's effect on rats' weight under different treatment patterns was assessed. Forty wistar rats weighing between 140-250 g were categorized into eight experimental groups of five wistar rats per group. The efficacy of 250 mg kg^{-1} methanolic extract of the leaves on alloxan-induced diabetic rats showed mild hypoglycaemic effect within 24 h. There was no evidence to establish that parenteral route is more efficacious than the oral route of administration of the treatment plant. The experimental rats that was alloxan-treated to induce hyperglycaemia and treated with 500 mg kg^{-1} of methanolic extract of the treatment plant as well as those induced with alloxan without treatment with methanolic extract of *M. charantia* showed significant loss in weight ($p < 0.05$) after twelve weeks. The controls as well as those treated exclusively with methanolic extract of treatment plant without alloxan treatment had significant weight gain ($p < 0.05$). The results generally indicate that methanolic extract of the leaves of *M. charantia* has hypoglycaemic potential especially on long-term use.

Key words: *Momordica charantia*, leaves, alloxan, hyperglycaemia, weight, wistar rats

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INTRODUCTION

Plant Extracts have been very useful sources of medication for various disease conditions (Ainslie, 1937; Ayensu, 1978). About 75% of the population of developing countries depend on medicals of plant origin (Ampofo and Johnson-Romand, 1978). Traditional healers have alluded healing powers to many plants, some of which have been substantiated by scientific investigation. For example, Sofowora (1982) reported the growth inhibitory effect of the leaf extract of *Azadirachta indica* on *Plasmodium falciparum* culture. Reports from Nigeria and Sierra Leone (Ainslie, 1937; Ayensu, 1978) indicate that leaves of *Spondias mombin* are used to treat tumors. Recent phytochemical examination of plants with a suitable history of efficacy against cancer in folklore medicine has resulted in the isolation of bioactive compounds with anti-tumor properties. Some of such plants include *Laurus nobilis, Juglaris regia, Musa sapientum, Momordica charantia* and *S. mombin* (Chin Hee kim *et al*., 1999). Antoine (2005) reported that many patients use herbal products concomitantly with (orthodox) anti-diabetic medications, even though some herbs can have undesirable and possibly hazardous effects on blood glucose levels. The use of complementary and alternative medicine modalities by the public in Western countries is on the rise and studies by Ryan *et al.* (2001) and Dunning (2003) revealed that patients with diabetes do not differ from the general public in their willingness to use complementary and alternative medicine. Herbal products, a major component of complementary and alternative medicine, are readily available to the public through pharmacies, nutritional health stores and the Internet. Dunning (2003) claimed that many of the herbal products are used concomitantly with anti-diabetic medications, with or without the knowledge or consent of the patient's health care practitioners. Diabetes mellitus is a systemic disease characterized by excess sugar level in the blood. It exists in two forms-the type 1 diabetes mellitus and the type II diabetes mellitus. Regardless of the type of diabetes, patients are required to control their blood glucose with medications and/or by adhering to an exercise programme and a dietary plan. Insulin therapy by injection is given to those with type 1 diabetes mellitus and to some patients with type II diabetes mellitus.

Patients with type II diabetes mellitus are controlled on restricted diet and instructed to exercise to help control the weight. Where diet and exercise fail to control the blood glucose at the desired level, oral anti-diabetic medication is prescribed; while in some cases, insulin injections are necessary.

M. charantia has insulin-like molecules and it helps to remove glucose from the bloodstream (Mahomoodally *et al*., 2004). Cummings *et al.* (2004) also claimed that *M. charantia* act like insulin by forcing amino acid uptake into skeletal muscle tissue. Earlier animal studies have supported the efficacy of this plant as a diabetes mellitus treatment (Ahmed *et al*., 2004) and (Miura *et al*., 2004).

Srivastava *et al.* (1993) and Akhtar *et al.* (1981) had demonstrated the hypoglycaemic potential of *M. charantia* in normal and diabetic rats while Leatherdale *et al.* (1981) did the same in patients with type II diabetes.

M. charantia belongs to the family *Cucurbitaceae.* It is a prostrate hairy perennial herb with long simple hairs, climbing by means of tendrils and reproducing from seeds. The stem is angled, more or less hollow and minutely hairy. The leaves are alternate up to 5 cm long with spiral tendrils at the opposite side unevenly lobed; and the lobes being more or less with notched margins. The petioles are 4-5 cm long and produce offensive smell when crushed. The inflorescence consists of solitary flowers, which exist, in male and female forms on the same plant. The fruit is warty and the plant grows in tropical climates and has been used as a traditional medicine for thousands of years. The leaves and vines of the *M. charantia* plant are frequently used in traditional medicine, but the fruit is used most often due to its effectiveness and safety.

MATERIALS AND METHODS

Collection, harvesting and preparation of *Momordica charantia*: The leaves of *Momordica charantia* were collected from the premises of the University of Benin, Benin City, Edo State, Nigeria in December, 2004. It was identified botanically using a handbook on West African weeds (Akobundu and Agyakwa, 1988). Identification was authenticated by Professor MacDonald Idu of Botany Department, University of Benin, Benin City, Nigeria with herbarium number BTN 142 assigned. The leaves were washed and air-dried for five days, cleaned of debris and kept in the oven to dry at 40°C for 3 h. The dried leaves were ground to a powdered form at the department of Pharmacognosy, University of Benin, Benin City. Three hundred milligram of powdered sample was subjected to methanolic extraction and 16.3% yield was obtained for use.

Phytochemical screening of *Momordica charantia* leaves: Ten kilogram of powdered sample was weighed and stored in a moisture free airtight container for use. Phytochemical screening for the presence of tannins, flavonoids, saponins, alkaloids and anthraquinones in the plant extract was done following the procedures of Odebiyi and Sofowora (1973) and Sofowora (1993) in the department of Pharmacognosy, university of Benin, Benin- City.

Experimental animals: Forty wistar rats weighing between 140-250 g were kept in cages in animal house of Faculty of Pharmacy and Anatomy Department of the University of Benin. The experimental animals were kept in separate cages. They were all allowed to acclimatize for three weeks before treatment was commenced during which period they were fed on standard mouse cubes obtained from Pfizer Livestock feeds (Nig) Ltd. Benin City. They were supplied with water ad libitum in standard drinking bottles. The rats were randomly categorized for experimental purpose into different treatment groups of five rats per group. One main control group (M) and seven treatment groups A1, A2, B1, B2, C1, C2 and D with each group consisting of five rats ascribed the suffix Ma, Mb, Mc, Md and Me for the control group M and A1a, A1b, A1c, A1d and A1e for the treatment group A1. The same categorization thus applies to all the groups.

The duration of experiment was twelve weeks to ascertain treatments' effect on rat's weight, conducted from May 12 to August 11; 2005. The main control group M was given Pfizer feeds and water *ad libitum* throughout the period. The A1 group was alloxan - treated to induce hyperglycaemia and after overnight fasting were given intraperitoneal administration of 250 mg kg^{-1} of alcoholic (methanol) extract of the treatment plant for 48 h to test glucose reducing potential of the treatment plant before increasing the dose to 500 mg kg^{-1} for the remaining treatment period. A2 group was also alloxan-treated and received the same volumes as in A1 but of methanol without extract treatment. The B1 group was induced with alloxan but not treated with extract before sacrificing. B2 was alloxan-treated but was sacrificed before any evidence of induced diabetes. The C1 group were normoglycaemic rats, i.e., were not alloxan-treated to induce hyperglycaemia but received oral administration of 250 mg kg^{-1} of treatment plant for the first 48 h before increase to 500 mg kg^{-1} of treatment plant while the C2 group, also normoglycaemic without alloxan treatment, were given the same volumes as in C1 but of methanol as a negative control. The D group was given 500 mg kg^{-1} of extract intra peritoneally but were not given alloxan treatment.

2.5 gram of extract were diluted in 10 mL of alcohol to give 250 mg mL^{-1}. Alloxan was prepared as 50 mg mL^{-1} in distilled H_2O and administered at a dose of 150 mg kg^{-1} to get the volume equivalent thus.

$$\frac{X \times 150}{50}$$

where X = weight in kg

Time course of the mean hypoglycaemic effect of extract on treatment groups was recorded at time in h (0, 2, 6, 12 and 24). Each determination was carried out in duplicate and the mean reported (WHO Expert Committee on diabetes mellitus, 1980).

Chemicals and reagents: All Chemicals were of analytical grade. Potassium oxalate, sodium fluoride, benzene, hydrochloric acid and lead acetate were obtained from BDH chemicals Ltd., (Poole UK). Alloxan monohydrate was obtained from sigma Aldrich Inc. USA. Ammonia was from Merck (Germany), Glucose oxidase kit and Glucose strips (Accu Chek) were from Roche Diagnostics Gmbh, Mannheim, Germany. Methanol, Acetone, Potassium chloride and Haematoxylin and Eosin were from BDH Chemicals Ltd., Poole, England. Chloroform was from May and Baker Ltd; Dagenham, England and sodium hydroxide was from Avondale Laboratories, Banbury, Oxon, England. The confirmation of hyperglycaemia was made after 48 h with blood glucose values of ≥ 120 mg dL^{-1}.

RESULTS

Result of phytochemical analysis of methanolic extract of *Momordica charantia* used, revealed flavonoids and tannins as the main constituents.

From Table 1, there was significant difference in the body weight change of experimental rats amongst the various groups. M (control) group had the highest weight gain 24.26±4.48, but A1 and B1 treatment groups recorded falls in body weights following treatment (-4.14±1.95 and -5.26±2.56) respectively.

From Table 2, the variation observed in the hypoglycaemic effect amongst the groups is not as a

Table 1: Means of body weight change in (g) of treatment groups and the analysis of variance

Groups	Means±SEM
M	24.26±4.48[a]
A1	-4.14±1.95[d]
A2	8.70±0.45[b]
B1	-5.26±2.56[d]
B2	5.88±0.49[b]
C1	8.30±1.73[b]
C2	8.90±1.42[b]
D	0.56±1.60[c]

*Means with different alphabetic are significantly different at 5% probability level

Table 2: Means of variation in hypoglycaemic effects (mg dL^{-1}) of extract on the treatment groups with time in (h)

Groups	Means±SEM
M	85.64±0.41[b]
A1	156.10±3.71[a]
A2	153.39±2.71[a]
C1	84.87±3.24[b]
C2	87.13±0.95[b]

*Means with different alphabetic remarks are significantly different at 5% probability level

result of the effect of the extract but due to the effect of alloxan, which induced hyperglycaemia in the treatment groups A1 and A2 unlike the normoglycaemic C1 and C2 treatments. There was no significant difference ($p>0.05$) in the effect of extract amongst the groups within the 24 h observation.

In the A1 treatment group, mean hypoglycaemic value dropped slightly from 170 to 150 mg dL^{-1} within the first 6 h before gradually rising to 160 mg dL^{-1} by the end of 24 h indicating that the extract is losing its blood glucose lowering potential. The negative control A2 treatment group showed a mean value which gradually rose from 148 to 162 mg dL^{-1} at the end of 24 h. This was due to the gradual hyperglycaemic inducing effect of alloxan. Similarly, in the C1 treatment group which received no alloxan treatment (normoglycaemic), but had oral administration of extract, showed a gradual diminition in the mean hypoglycaemic value from 97 to 82 mg dL^{-1} in the first 6 h and remained relatively at that level by the end of 24 h. The negative control C2 showed a gradual rise in mean value from 84 to 90 mg dL^{-1} due to the hyperglycaemic inducing effect of alloxan. In both A1 and C1 treatments, no detectable extract's activity was seen in the first 2 h.

DISCUSSION

The control group (M) had remarkable weight gain during the treatment period but there was loss of body weights in the A1 and B1 groups. However since the B1 group received no extract treatment, there is no reason to suggest that the extract is the sole source of the noticed weight loss even though *Momordica charantia* has been shown in animal studies to decrease body fat (Chen and Chan, 2003). The hyperglycaemic condition and alloxan treatment might have been contributory to the loss of weight observed.

The extract of the leaves of *M. charantia* Linn resulted in mild blood sugar levels reduction in both the fasted normoglycaemic and the alloxan treated rats confirming previous studies (Akhtar *et al*., 1981; Srivastava *et al*., 1993; Ahmed *et al*., 2004; Miura *et al*., 2004). This was observed to have occurred mainly within the first 6 h. That there was no detectable extract's effect within the first 2 h suggests that it is not likely as fast acting as insulin in reducing blood sugar. There was a tendency to gradual dimunition in the extract's hypoglycaemic effect after the first 6 h and towards the end of the first 12 h, which almost completely waned by the end of 24 h. There is however no evidence from this study that parenteral route of administration is more efficacious than the oral route as seen in the similarity and statistically non-significant change ($p > 0.05$) observed in the first 24 h treatment of extract in both the A1 and C1 treatment groups. However it can be suggested from these findings that administration of once or twice daily treatment of extract might cause significant hypoglycaemia on long term treatment; thus indicating its possible usefulness in the long term management of hyperglycaemia.

The findings of no significant difference ($p>0.05$) in the mean values of the hypoglycaemic effect of extract between the intra peritoneally administered route A1 group and the orally administered route (C1) suggest need for preference of oral route in the administration of the extract.

Also the extract doesn't seem to have demonstrated marked hypoglycaemic effect as seen in the results of the difference in mean values between 0 and 12 h which was the peak period of action of the extract. This may perhaps be due to the fact that the leaves rather than the whole plant, seed or fruit pulp was used in this study (Ali *et al*., 1993). The presence of flavonoids may have contributed to the observed marginal hypoglycaemic effect observed (Anila and Vijayalakahmi, 2000).

CONCLUSIONS

One may infer from this study that *M. charantia* leaves extract has some degree of hypoglycaemic effect though no significant empirical evidence from this study to establish that the effect is potent with only the leaves extract of the plant. However, use of whole plant is likely to cause more significant hypoglycaemic effect. Further studies will be necessary to confirm this.

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