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Anti-diabetic activity and phytochemical composition of ethanol leaf extract of *Momordica charantia* (Linn.) on alloxan- induced diabetic rats

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Abstract

Diabetes is a metabolic disease in which there is high blood sugar levels over a prolonged period, that is affecting major population of developing countries causing death. The ethanol leaf extract of *Momordica charantia* was evaluated for anti-diabetic activity in alloxan-induced diabetic rats after single dose (acute study) and prolonged treatment (chronic study). Phytochemical and proximate analysis were carried out on the plant leaves. Diabetes was induced in the rats using alloxan monohydrate 150 mg/kg.

The diabetic rats were treated with various doses of the leaf extract. The blood glucose level (BGL) was measured by using a glucometer. Diabetic rats treated with 69.28, 138.56, and 207.85 mg/kg of the plant leaf extract caused a significant ($P<0.05$-0.01) reduction in fasting blood glucose level (BGL) in the animals both in acute and prolonged treatment study, in a manner comparable to that of the reference standard drug, glibenclamide (10 mg/kg body weight per day). The studies suggest that ethanol leaf extract of *Momordica charantia* posses anti-diabetic properties which can be exploited in the management of diabetes.

Keywords: *Momordica charantia*; Alloxan; Proximate; Phytoconstituents; Anti-Dibetic; Blood Glucose Level (BGL) ; Herbal Medicine

1. Introduction

Momordica charantia, also known as bitter melon has been used in various Asia and Africa herbal medicine system for long time [1,2,3]. In Turkey, it has been used as folk remedy for a variety of ailments particularly stomach complaints [4][5]. In Indian traditional medicine, different part of the plant are used to relieve diabetes, as Stomach Laxation, Antibilious, Emertic, Antihelmetic Agent, to treat Rheumaticsm, Cough etc [5]. *Momordica charantia* when consumed raw or juice form, can be efficious in lowering blood glucose level [6]. The leaf may be made into a tea called "Cerassie", and the juice, extracted from the various plant parts (Fruit Pulp, Seeds, Leaves and Whole Plant), is common folklore remedy for diabetics [7]. *Momordica charantia* has a long history of use as folklore hypoglycaemic agent where the plant has been referred to as vegetable insulin [8]. In this study we probed the effects of Ethanol Leaf Extract of *Momordica charantia* (Linn.) On Alloxan- Induced Diabetic Rats during single and prolonged treatment to observe acute and chronic effects of the extracts on blood glucose levels and weight changes in the diabetic rats in bids to confirm its ethnobotanical uses in the management of diabetes.

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2. Material and methods

2.1. Plants Materials

Fresh leaves of *Momordica charantia* (Linn.), were collected on the 26th April, 2015 from a farmland at Ugep in Yakurr local Government Area of Cross River State, Nigeria.

The plant was identified and authenticated by a taxonomist in the department of botany and ecological studies, university of Uyo, Uyo Nigeria. A voucher specimen of plant species was deposited at Departmental Herbarium with a voucher No: Oyomah, UUH3416 (Yakurr, Cross River). The fresh leaves of the plant were washed with clean water to remove dirt and air dried for 2 weeks on labouratory table and reduced to powder form. The powder 250g was macerated in 900 ml of 95 % ethanol for 72 hours with occasional agitation. The liquid filterate obtained was concentrated in vacuo at 40 ^{0}C. The yield was 10.2 g. The extract was stored in a refrigerator at 4 ^{0}C until used for experiment reported in this study.

2.2. Phytochemical Screening

The standard qualitative and quantitative phytochemical test were carried out with the leaf extract to elucidate the presence or absence of some bioactive compounds in the plant species such as Alkaloids, Cardiac-Glycoside, anhraquinones, Flavonoids, Tannins, Terpennes, Reducing Sugars, Saponins among others [9,10,4,11,12].

2.3. Animal

The animals (Swiss albino mice 20-25 g and rats 120-150 g) of both sexes were used for these experiments. They were obtained from university of Uyo animal house. The animals were housed in the standard cages and were maintained on a standard pelleted feed (Guinea feed) and water ad libitum.

2.4. Determination of Median Lethal Dose (LD50)

The median lethal dose (LD50) of the extract was estimated using albino mice by intraperitoneal (i.p) route using the method of Lorke [13]. This involved the administration of different doses of the extract to groups of three mice each. Observation of the animal for manifestation of physical sign of toxicity such as Writhing, Palpation, Decreased motor activity, body/limb tone breathing and Death. The number of deaths in each group within 24 hours was recorded. The LD50 was calculated as geometrical means of the maximum dose producing 0 % as (a) and the minimum dose producing 100% mortality as (b)

$$LD50 = \sqrt{ab}$$

2.5. Proximate Analysis

The standard recommended method of the association of official analytical chemists were used for the determination of moisture content, Crude Protein, Crude Fat, Carbohydrate, Crude Fibre and Ash [14,15, 16].

2.6. Induction of Diabetes

The animals (male rats) were fasted overnight and diabetes was induced by a single intraperitoneal injection of a freshly prepared solution of Alloan monohydrate (150 mg/kg) in ice cold 0.9 % saline (Nacl) solution. The animals were then given 5 % dextrose solution to drink immediately after induction of diabetes to overcome the drug induced hypoglycemia. Control rats were injected with normal saline alone. After 72 hours, rats with blood glucose level (BGL) above 200 mg/dl were considered diabetic and selected for the experiment.

The animals were divided into five groups of 6 rats each and treated as follows:

- Group 1; Diabetic rats, treated with 10 ml/kg body weight per day of distilled water orally for 14 days (negative Control Rats).
- Group 2; Diabetic rats treated with 10 mg/kg body weight/day of glibenclamide (Standard Drug) orally for 14 days (Positive Control Experiment).

- Group 3; Diabetic rats treated with 69.28 mg/kg body weight/per day of *Momordica charantia* extract solution orally for 14 days.
- Group 4; Diabetic rats, treated with 138.56 mg/kg body weight/per day with *Momordica charantia* ethanol leaf extract solution orally for14 days.
- Group 5; Diabetic rats, treated with 207.85 mg/kg body weight/ day with *Momordica charantia* ethanol leaf extract solution orally for14 days.

The body weight gain and fasting BGL of all the rats were recorded at regular intervals during the experimental period. For the acute study, the BGL was monitored for 1,3,5,7 and 24 hours of administration of single dose of the extract and at the end of 1, 3, 5,7,24 and 7,14 days for prolonged treatments. The BGL was monitored in the diabetic rats by tail tipping method.

The blood was dropped on reagent pad of dextrostix, which was inserted into microprocessor digital blood glucometer and the reading recorded [17].

2.7. Statistical Analysis

Data obtained from this study were analysed as mean + standard error of the mean (SEM) and were analyzed statistically using one-way analysis of variance followed by turkey-krammer multiple comparison test and values of $P < 0.01$ and 0.05 significant at 1 % and 5 % levels of significance [18].

3. Results

3.1. Phytochemical Screening

Table 1 Qualitative Screening of the Ethanol Leaf Extract of *Momordica charantia*

	Bioactive compounds	**Test Done**		**Inference**
1	Tannins	i	Ferric Chloride	ND
		ii	Lead Acetate	ND
2	Saponnins	i	Frothing	+++
		ii	5%NaCo3	+++
		iii	Fehling Solution	+++
3	Alkaloids	i	Dragendoff's	++
		ii	Hagger's	++
4	Phlobatannins	i	Dilute Hcl	ND
		ii	Formaldehyde	ND
5	Anthraquinones	i	Free Borntrager's	ND
6	Cardiac-Glycoside	i	Sakowski	+++
		ii	Liberman's	+++
		iii	Keller-Killiani	+++
7	Flavonoids	i	Shinoda	+
		ii	Sodium Hydroxide (NAOH)	+
8	Deoxy-Sugar	i	Glacial Acetic Acid	+++

KEY: ND = Not detected; + = Present in low concentration; ++ = Present in moderate concentration; +++ = Present in high concentration

Phytochemical screening of the ethanol leaf extract of *Momordica charantia* reeals the presence of compounds like Saponins, Alkaloids, Cardiac-glycoside, Flavonoid and Deoxy-Sugar

The prelimary qualitative phytochemical screening of the ethanol leaf extract of *Momordica charantia* in table 1 above showed that Saponnins, Cardiac glycosides and deoxy-sugar were the most prominent secondary metabolites detected in the plant.

Table 2 Quantitative Estimate of Some Phytochemical Constituents *Momordica charantia*

Constituents	***Mormordica charantia* (%)**
Saponnins	45.15 + 1.02
Alkaloids	24.02 + 0.08
Flavonoids	16.65 + 1.21
Cardiac-Glycoside	48.49 + 0.1

Table 3 Proximate Analysis of the Leaf of *Momordica charantia*

Parameter	***Momordica charantia* Analysis**
Moisture Content	85.9 %
Crude Protein	0.87 %
Crude Lipid (Fat)	0.5 %
Ash	7.33 %
Crude Fibre	0.05 %
Carbohydrate	5.35 %
Calorie Value (Kcal)	61.1 Kcal

3.2. Anti-diabetic Activity of Ethanol Leaf Extract of *Momordica charantia*.

Table 4 Effects of Ethanol Leaf Extract of *Momordica charantia* on the Body Weight of Alloxcan-Induced Diabetic Rats

Group	**Treatment**	**Dose mg/kg**	**Day 0**	**Day 7**	**Day 14**	**Initial Average Body Weight (g)**	**Final Average Body Weight (g)**	**Body Weight difference (g)**
1	Control	10 ml/kg	254.3±8.41	314.3±13.38	326.0±15.04	151	115	36
2	Extract	69.28	258.3±7.31	231.2±10.11	226.4±9.48*	146	131	15
3	Extract	138.56	247.7±9.06	170.3±12.41	148.0±12.7*	153	143	10
4	Extract	207.85	249.7±6.94	149.0±8.54	124.0±9.17	143	125	18
5	Glibenclamide	10	270.7±6.38	112.0±7.02*	99.0±9.48*	148	135	13

Data are expressed as means (±SEM) of five replicate sampling at P = 0.05

The treatment of diabetic rats with the ethanol leaf extract of the plant and glibenclamide standard drug brought improvement in the body gain compared to untreated rats with remarkable Degredation in the body tissue. A dose-dependent reduction in BGL was observed in alloxan-induced diabetic rats treated with ethanol leaf extract of

Momordica charantia. Both in acute and prolonged study there was a significant (P < 0.001, 0.05, 0.001) reduction in BGL of the diabetic rats within the period of study compared to the control.

Table 5 Anti-diabetic Effects of Ethanol Leaf Extract of *Momordica charantia* on the Blood Glucose Level of Alloxcan-Induced Diabetic Rats during Acute Study

Group	Treatment	Dose mg/kg	Blood Glucose Level (mg/d/) Hours					
			0 Hour	1 Hour	3 Hours	5 Hours	7 Hours	24 Hours
1	Control	10 ml/kg	254.3±8.41	260.3±8.41	265.3±7.84	265.7±8.19	264.0±8.25	281.3±5.84
2	Glibenclamide	10	270.7±6.39	265.7±6.94	245.0±8.08	238.0±8.15	212.3±9.21*	147.7±8.25
3	Crude extract	69.28	258.3±7.31	263.8±7.25	250.2±7.41	248.0±8.25	245.8±8.09	236.7±9.25*
4	Crude extract	138.56	247.7±9.06	244.7±8.65	233.3±9.56	244.0±10.44	199.0±9.85	189.3±9.68*
5	Crude extract	207.85	249.7±6.94	240.0±5.20	224.0±8.19	201.7±8.69	180.7±6.69*	167.3±2.96*

The value shown are means (±SEM) of five replica at P≤ 0.05.

Table 6 Effects of Ethanol Leaf Extract of *Momordica charantia* on the Blood Glucose Level of Alloxcan-Induced Diabetic Rats during Prolonged Treatment

Group	Treatment Doses	The Blood Glucose Level (Mg/d/) in Days			
		Day 0	Day 1	Day 7	Day 14
1	10 ml/kg distil water	254.3±8.41	281.3± 5.84	341.3±1.3.38	326.0±15.04
2	10 mg/kg glibenclamide	270.7 ±6.39	147.7±8.25*	112.0±7.02*	99.00±9.48*
3	468.89 mg/kg *M. charantia*	258.3 ± 7.31	236.7±9.82*	231.2±10.11	266.4±9.48*
4	939.78 mg/kg *M. charantia*	247.7 ±9.06	189.3±9.68*	170.3±12.41	148.0±12.70*
5	1469.6 mg/kg of *M. charantia*	249.7 ±6.49	167.3±2.96*	149.0±8.54*	124.0±9.17*

Data obtained are expressed as mean + SEM, significant at P = 0.05, when compared to control in five replicates.

4. Discussion

The anti-diabetic properties of *Momordica charantia* were investigated in this study. Diabetic is a killer disease that affects human subjects of different ages according to it's type and the recurrence in both developed and develop countries [19]. The extract which showed moderate toxicity was observed to demonstrate significant antidiabetic activity in alloxan-induced diabetic rats. The leaf/fruits of the plant have previously been reported to possess medicinal properties such as anti-inflammatory, anti-medicrobial, anti-leukemic, anti-tumor, and last and not least the important anti-diabetic property [20].

Phytochemical study of the ethanol leaf extract revealed the presence of Saponnins, Alkaloids, Cardiac-glycoside, Flavonoids and Deoxy-Sugar. The presence of bioactive compounds in this plant species infers a possibility of medicinal efficiency of these preventive compounds present in plants [9]. Alkaloids Saponnins and Cardiac-Glycosides were present in high concentration, these compounds in medicinal plant extracts have been valuable anti-diabetic agents and one or more of these Phytochemical constituents may be responsible for the blood glucose level reduction [21][1] [22]. Flavonoids of different plant origins have shown promising anti-diabetic activity [23, 24, 25]. Diabetes is characterized by a severe loss in body weight due to loss or degradation of structural proteins [26]. The condition was alleviated by the leaf extract of *Momordica charantia* as the treated animal were healthy and agile at the end of the study period of 2 weeks and caused significant decrease in BGL of the treated rats compared to untreated diabetic rats. The sustained but gradual reduction in weight of the untreated diabetic rats during the 14 days clearly indicated that the deterioration in the glucose control mechanism or pathogenic process progresses in stages and would probably climax in the death of the animals if untreated. This observation is the direct reverse of the observed changes in blood glucose which rather

increased over period, hence re-establishing an inverse relationship or negative correlation between blood glucose and weight changes in untreated diabetes condition. The gradual increase in weight up on treatment with the plant extract and glibenclamide indicates that the treatment would have allowed the tissues access to glucose, both to supply energy and build tissue materials needed for growth.

5. Conclusion

The results from this study revealed that the leaves of *Momordica charantia* contain phytochemical constituents Saponnins, Alkaloids, Flavonoids, Cardiac - glycosides. Proximate estimates showed that the plant is good for diabetes patient. The LD_{50} calculated was 692.82 mg/kg. The ethanol leaf extract of this plant obviously reduced the fasting blood glucose level (FBGL) in the treated diabetic rats and gradually reduced the metabolic breakdown of the diabetic rats' tissue. From the result, the plant is recommended therapeutically, for Anti-diabetic treatment.

Compliance with ethical standards

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Disclosure of conflict of interest

The Authors declare that there is no conflict of interests regarding the paper production.

Statement of ethical approval

The present research work was performed using animals for the experimental analysis

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