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PHYTO CHEMICAL SCREENING AND ANTI DIABETIC ACTIVITY OF *MOMORDICA CHARNTIA* FRUIT EXTRACT

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ABSTRACT

Research aims to investigate the phytoconstitutes and antidiabetic activity of *Momordica charntia.* (Family: Cucurbitaceae) fruit extracts in alloxan induced diabetic albino rats. A comparison was made between the action of *Momordica charntia* extracts and a known antidiabetic drug glibenclamide (600 µg/kg body wt.). An oral glucose tolerance test (OGTT) was also performed in experimental diabetic rats. The petroleum ether, chloroform, methanolic extracts of *Momordica charntia* were obtaine by soxhlet extraction method and subjected to standardization by following pharmacognostical and phytochemical screening methods. Dose selection was made on the basis of acute oral toxicity study in mice (50mg to 5000 mg/kg body weight) as per OECD guidelines. *Momordica charntia* chloroform extract and methanolic extract showed significant ($P<0.01$) antidiabetic activity. In alloxan induced model, blood glucose level of these extracts on the seventh day of study were (ME:96.33±9.75) and (CE: 83.33±6.62) in comparison of diabetic control (224.50±4.72). In OGGT model (glucose loaded rats), exhibited glucose level after 30 min. for ME (127.66±3.21) and 90 min. (99.66±4.79) whereas after 30 min. for CE (122.33±3.32) and 90 min. (96.12±3.25). These extracts also prevented body weight loss in diabetic rats. The anti hyperglycemic action of the extracts may be due to improving the glycemic control mechanisms. The drug has the potential to act as antidiabetic drug.

KEYWORDS: *Momordica charntia;* Diabetes mellitus; Alloxan monohydrate; Oral glucose tolerance test.

INTRODUCTION

Diabetes mellitus (DM), one of the most common endocrine metabolic disorders has caused significant morbidity and mortality due to microvascular (retinopathy, neuropathy, and nephropathy) and macrovascular (heart attack, stroke and peripheral vascular disease) complications.[1] DM occurs when the body does not produce enough insulin or is unable to use insulin effectively. Insulin is a hormone secreted by the beta cells of islet of lengerhans in the pancreas that helps cells to absorb and use glucose for energy throughout the body. If the body does not produce enough insulin or is incapable to use insulin efficiently, glucose concentration builds up in the blood instead of being absorbed by cells in the body and hence the body is starved of energy. Type 2 diabetes are the most common form of diabetes which is caused by a combination of factors, including insulin resistance, a condition in which the body's muscle, fat and liver cells do not respond to insulin effectively. On the other hand Type 1 diabetes develops when the body can no longer produce enough insulin to compensate for the impaired ability to use insulin.[2] The number of people suffering with diabetes worldwide is increasing at an alarming rate.[3]

According to World Health Organization (WHO) projections, the prevalence of diabetes is likely to increase by 35%. Currently there are over 150 million diabetics worldwide and this is likely to increase to 300 million or more by the year 2025. Statistical projection about India suggests that the number of diabetics will rise from 15 million in 1995 to 57 million in the year 2025, the highest number of diabetics in the world.[4]

Since ancient times, plants and plant extracts were used to combat diabetes. Many traditional medicines in use are derived from medicinal plants, minerals and organic matter. The World Health Organization (WHO) has listed 21,000 plants, which are used for medicinal purposes around the world. Among them, 150 species are used commercially on a fairly large scale.[5,6] Recently, some medicinal plants have been reported to be useful in diabetes worldwide and have been used empirically in antidiabetic and antihyperlipidemic remedies. Antihyperglycemic activity of the plants is mainly due to their ability to restore the function of pancreatic tissues by causing an increase in insulin output or inhibit the intestinal absorption of glucose or to the facilitation of metabolites in insulin dependent processes. More than 400 plant species having hypoglycemic activity have

been available in literature, however, searching for new antidiabetic drugs from natural plants is still attractive because they contain substances which demonstrate alternative and safe effects on diabetes mellitus. Most of plants contain glycosides, alkaloids, terpenoids, flavonoids, carotenoids, *etc.*, that are frequently implicated as having antidiabetic effect.[7]

Since the initial findings in 1943 of alloxan induced β-cell necrosis in rabbits, it has been used for inducing experimental diabetes till so far. Alloxan is a uric acid derivative act by selectively destroying the pancreatic beta islets leading to insulin deficiency, hyperglycaemia and ketosis. Because of its low stability, relatively very shorter half-life and acidic nature of solution, intravenous route of administration of alloxan is preferred.[8]

Momordica charntia (commonly known as bittermelon, karela, balsam pear or bittergourd) is a tropical vine climber belonging to the family Cucurbitaceae. It is a climbing perennial that usually grows up to 5 m and bears elongated fruits with a knobbly surface. It is a useful medicinal and vegetable plant for human health in natural remedies and one of the most promising plants for diabetes used traditionally.[9] The fruits, stems, leaves and roots of bitter melon have all been used in traditional medicine to treat ailments such as hyperlipidemia, digestive disorders, microbial infections, helminthiasis and menstrual problems. Bitter melon has been shown to possess powerful antiviral properties and broad-spectrum bactericidal activity. Bitter melon has anti-carcinogenic properties. Extract of bitter melon modulates signal transduction pathways for inhibition of breast cancer cell growth and can be used as a dietary supplement for prevention of breast cancer. Traditionally, bitter melon has also been used as an abortifacient agent used to induce abortions. Therefore, pregnant women are advised to avoid consumption of the plant. The extract of the seed also have antispermatogenic effect.[10] Chemical constituents responsible for blood sugar lowering action of bittergourd is mainly saponins, tannins, momordicine and 3-hydroxycucurbita-5 and also contains high dosage of polypeptide P (plant insulin).

MATERIALS AND METHODS

Plant material

Fruits of *Momordica charntia* were collected in and around the local garden area of Bangalore, Karnataka and authenticated by the regional research institute, Bangalore. The herbarium specimen has also preserved in the same college. The collected fruits were dried under shade and powdered to coarse consistency in a grinder. The powder passed through 40 # mesh particle size and stored in an airtight container at room temperature.

Preparation of plant extract

About 3 kg of the fresh air-dried, powered crude drug was extracted with petroleum ether, chloroform, methanol by adopting a soxhlet extraction procedure. The extract was filtered and concentrated to dryness at room temperature to avoid the decomposition of the natural metabolites. The yield of the extracts of petroleum ether, chloroform and methanol was found to be 1.40%, 5.20%, 22.56% w/w respectively. All the extracts were preserved in a refrigerator till further use. Study of petroleum ether extract was exempted due to its very low yield. Physical and chemical analysis was carried out in chloroform and methanol extracts only. A known volume of extract was suspended in distilled water and was orally administered to the animals by gastric intubation using a force feeding needle during the experimental period.

Detection of physical constant[11]

Extractive values, loss on drying (LOD), determination of ash value, acid-insoluble and water-soluble ash were evaluated. Presences of phytochemical constituents were determined for freshly prepared methanolic and chloroform extract of fruits by using preliminary phytochemical screening.

Animals

Adult Albino rats of wistar strain (150-200g) of either sex were procured from the animal house of T John college of Pharmacy, Bangalore, with 12 hour light and 12 hour dark cycles. Standard pellets, were used as a basal diet during the experimental period. The control and experimental animals were provided food and drinking water ad libitum. All the experiments on animals were conducted according to the ethical norms approved by CPCSEA, and ethical clearance was granted by institutional ethical committee in resolution of T John College of Pharmacy, Bangalore (Ethical committee IAEC reg. no.: 1740/PO/a/14/ CPCSEA).

Chemicals

Alloxan monohydrate (Chemit Lab, Erragaddda, Hyderabad) Glibenclamide (Aventis Pharma Ltd, Verna, Goa), Dextrose, Tween 80, Anesthetic Ether were used. Accu-chek Active Glucometer (Roche Diagnostic Corporation Germany) along with blood glucose-strips was used. All other chemicals and reagents used were of analytical grade.

Acute oral toxicity studies[12]

The acute oral toxicity studies of *Momordica charntia* fruit extracts were carried out as per the OECD guidelines no. 423 (Acute toxic class method). It was observed that the rats were not mortal even at 5000 mg kg^{-1} dose of both chloroform and methanol *Momordica charntia* fruit extracts. Hence, $1/10^{th}$ of the maximum safe dose of both chloroform and methanolic extract was selected for the studies of anti diabetic property.

Experimental models

Oral glucose tolerance test (OGTT)

Fasted rats were divided into four groups. Group I- served as normal control and received distilled water with Tween 80. Group II- received the standard drug

Glibenclamide as an aqueous suspension at a dose of 600µg/kg body weight. Group III and IV received different extracts at a dose of 500mg/kg body weight as a fine Tween 80 suspension. After 30 minutes of extract administration, the rats of all groups were orally treated with 2g/kg of glucose. Blood samples were collected from the rat tail vein just prior to glucose administration and at 30, 60 and 90 minutes after glucose loading. Blood glucose levels were measured immediately by using Gluco-meter.[13]

Alloxan induced diabetic model

The range of the diabetogenic dose of alloxan is quite narrow. Even light overdosing may generally be toxic and may cause the loss of many animals. This loss is likely to stem from kidney tubular cell necrotic toxicity, in particular when too high doses of alloxan are administered. The most frequently used intravenous dose of alloxan in rats is 65 mg/kg, but when it is administered intraperitoneally (i.p.) or subcutaneously its effective dose must be higher. For instance, an intraperitoneal dose below 150 mg/kg may be insufficient for inducing diabetes in this animal species. In mice, doses vary among 100–200 mg/kg by intravenous route (i.v.).

Alloxan monohydrate was first weighed individually for each animal according to the weight and then solubilized with 0.2 ml saline just prior to injection. Diabetes was induced by injecting alloxan at a dose of 65 mg/kg body weight of rat i.v. After one hour of alloxan administration the animals were given feed *ad libitm* and 5% dextrose solution were also given in feeding bottle for a day to overcome the early hypoglycemic Phase. The animals were kept under observation and blood glucose was measured by a gluco-meter. Hyperglycemia was confirmed by the elevated glucose levels in plasma, determined at 48 h and then on day 7 after injection. The rats found with permanent Type 2 were used for the antidiabetic study The diabetic rats (glucose level > 300 mg/dl) were separated and divided into six different groups for experimental study; each group contained six animals.[14,15]

Experimental design

The animals were divided in to five groups (n=6).
Group 1: Normal control (vehicle only)
Group 2: Diabetic control (untreated rats)
Group 3: Diabetic rats treated with Glibenclamide 600µg/kg
Group 4: Diabetic rats treated with *Momordica charntia* Chloroform extract 500mg/kg b.w
Group 5: Diabetic rats treated with *Momordica charntia* methanolic extract 500mg/kg b.w

The effects of administration of the fruit extracts to normal and diabetic rats were determined by measuring fasting plasma glucose levels, serum lipid profiles, liver glycogen levels and initial and final changes in body weight. Day 7 of induction was designated as day 1 for extract administration in diabetic rats.

Fasting plasma glucose was estimated on days 1, 5 and 12 of extract administration. All other biochemical parameters were determined on day 12 after the animals were sacrificed by decapitation.

Statisticsal Analysis

Statistical analysis of the results of the study was subjected to one-way analysis of variance followed by Dunnetts *t-test* for multiple comparisons. Values with $P < 0.05$ were considered significant.[14]

RESULTS

Standardization parameters for *Momordica charntia* fruits were determined and all the parameters were found to be within pharmacopoeial standards limit. Crude powder taken for extraction was of green color with faint odor and bitter taste. Loss on drying, total ash, acid insoluble ash, water soluble ash was found to be 7.02, 5.91, 3.148 and 0.89%w/w respectively. Phytochemical screening of all the extract showed the presence of various phytochemical constituents like glycoside, flavonoids, saponins, tannins, Vitamins, triterpenoids and amino acids.

In acute toxicity study chloroform and methanolic extracts of *Momordica charntia* fruits at the tested dose level of 5000 mg/kg body weight did not show significant toxicity signs when observed for the parameters during the first four hours and followed by daily observations for 14 days. No mortality was observed and the drug was found to be safe. In order to ascertain a scientific base for the usefulness of this plant in the treatment of diabetes, it was decided to evaluate experimental design of antidiabetic activity by following glucose tolerance test and alloxan-induced model.

In OGTT, both methanolic and chloroform extract showed a significant reduction of blood glucose level from 30 min onwards. The result of OGTT is given in Table 1.

Table 1: Effect of *Momordica charntia* fruit extracts on oral glucose tolerance test in normal rats

Sl No.	Treatment	Blood glucose level mg/dl		
		30 min	60 min	90 min
I	Normal control (vehicle only)	160.83 ±1.72	123.83±1.42	83.83±1.37
II	Glibenclamide 600µg/kg	120.66±3.28**	101.01±2.95**	95.83±2.03*
III	Methanolic extract 500mg	127.66±3.21**	114.23±3.25*	99.66±4.79**
IV	Chloroform extract 500 mg	122.33±3.32**	103.25±2.32**	96.12±3.25*

** Represents statistical significance vs. control (P < 0.05).*

The effect of extract of *Momordica charntia* fruits on fasting plasma glucose levels of normal and Alloxan induced animals are presented in Table 2. The difference between the experimental and control rats in lowering the fasting plasma glucose levels was statistically significant (P < 0.01) when compared with the control and has shown that the onset of action of chloroform extract is as significant as glibenclamide treated group. The data also suggest that the onset of action of methanolic extract is slow but when compared with the basal value it was significant till 3rd day suggesting its long duration of action.

Table 2: Effect of *Momordica charntia* fruit extracts on blood glucose level of alloxan induced diabetic albino rats after sub-acute treatment

Gr No.	Treatment	Blood glucose level mg/dl						
		Basal value (0hr)	1 hr	3 hr	5 hr	3rd day	5th day	7th day
I	Normal control (vehicle only)	80.10 ±1.63	80.83 ±1.71	80.81±1.44	79.83±1.36	81.33±0.98	79.83±0.83	81.16±0.23
II	Diabetic control	212.33 ±7.775	210.50±7.45	211.50±7.38	212.67±6.15	214.05±6.11	219.33±6.59	224.50±4.72
III	Glibenclamide 600µg/kg	210.33 ±7.93	160.66±6.28^{**++}	154.03±7.05^{**++}	124.83±5.04^{**++}	85.33±6.60**	74.33±5.25**	65.66±5.09**
IV	Methanolic extract 500mg	207.65 ±5.77	187.66±6.21 ns	166.46±7.15^{**++}	154.66±4.75^{**++}	124.12±12.94^{**++}	102.54±10.68**	96.33±9.75**
V	Chloroform extract 500 mg	209.66 ±8.51	177.33±7.92^{**++}	155.05±8.10^{**++}	132.03±5.25^{**++}	106.33±3.57**	93.16±9.55**	83.33±6.62**

All values are represented as MEAN± SEM; * Represents *statistical significance vs. control (P < 0.05);* $^{+}$ Represents *statistical significance vs. Basal value (P < 0.05).*

Significant difference was also observed in serum lipid profile, liver glycogen levels and in pancreatic TBARS levels estimated in diabetic rats (Tables 3).

Table 3: Effect of *Momordica charntia* fruit extracts on serum lipid profiles, liver glycogen levels of alloxan induced diabetic albino rats

Gr No.	Treatment	Cholesterol (mg/dl)	Triglycerides (mg/dl)
I	Normal control (vehicle only)	60.00 ± 1.89	63.00 ± 3.53
II	Diabetic control	107.66 ± 10.03	99.00 ± 7.35
III	Glibenclamide 600µg/kg	39.33 ± 2.12*	46.16 ± 1.79*
IV	Methanolic extract 500mg	43.00 ± 4.66*	59.00 ± 6.22*
V	Chloroform extract 500 mg	41.00 ± 2.92*	48.66 ± 3.52*

** Represents statistical significance vs. control (P < 0.05).*

Significant changes in body weight were only observed in the glibenclamide treated group (Table IV).

Table IV: Effect of *Momordica charntia* fruit extracts on change of body weight in rats

Gr No.	Treatment	Initial (g)	Final (g)
I	Normal control (vehicle only)	198.00 ± 4.81	204.00 ± 1.54
II	Diabetic control	258.50 ± 17.54	210.83 ± 17.74
III	Glibenclamide 600µg/kg	237.50 ± 18.74	229.50 ± 13.04*
IV	Methanolic extract 500mg	258.33 ± 6.79	243.00 ± 4.46
V	Chloroform extract 500 mg	266.66 ± 10.46	223.66 ± 11.34

DISCUSSION

The basic mechanism of hyperglycemia in DM is excessive production and decrease utilization of glucose by tissues. Excessive production may be due to enhanced hepatic glycogenolysis and gluconeogenesis.[16] In our investigation, the distinction observed between the initial and final fasting plasma glucose levels of different groups under investigation revealed a significant elevation in blood glucose in diabetic control group as compared with normal animals at the end of the 12-day experimental period.

When chloroform extract of *Momordica charntia* fruit was administered to diabetic animals it showed a significant reduction of blood glucose level after 1 hr which is similar to that of the standard drug Glibenclamide. On the other hand methanolic extract of *Momordica charntia* fruit had a delayed action, which showed its action on 3rd hr but the action continued till 3 days. Our investigations point toward the efficiency of both the extract in the maintenance of blood glucose levels in normal and Alloxane induced diabetic rats.

The marked boosting in serum triglycerides and cholesterol observed in untreated diabetic rats is in accord with the findings of Nikkila and Kekki.[17] Under normal circumstances insulin activates enzyme lipoprotein lipase and hydrolyses triglycerides. Insulin deficiency results in failure to activate the enzymes thereby causing hypertriglyceridemia. Our results provide a sufficient data to prove that both the methanolic and chloroform extract reduces the hypertriglyceridemia, which may be due to the enhancement in the release of insulin. Rise of plasma lipid concentration is well documented in patient suffering from diabetis.[18]

In insulin deficient diabetics, the plasma free fatty acid concentration is elevated as a result of increased free fatty acid outflow from fat depots, where the balance of the free fatty acid esterification–triglyceride lipolysis cycle is displaced in favour of lipolysis. Induction of diabetes with alloxan is associated with the characteristic loss of body weight which is due to increased muscle wasting in diabetes.[19] Diabetic rats treated with both the methanolic and chloroform extract showed an increase in body weight as compared to the diabetic control which may be due to its protective effect in controlling muscle wasting i.e. reversal of gluconeogenesis. Glycogen syntheses in the rat liver and skeletal muscles are impaired during diabetes.[20] The decrease seen in hepatic glycogen content in diabetes has been observed in earlier studies[21] and in this study is probably due to lack of insulin in the diabetic state which results in the inactivation of glycogen synthase systems. The significant increase in the glycogen levels of the aqueous extract treated diabetic animals may be because of the reactivation of glycogen synthase system.

CONCLUSION

We conclude that the ME and CE have potent antidiabetic effects in alloxan-induced diabetic rats. The present investigation has also opened avenues for further research especially with reference to the development of potent formulation for diabetes mellitus from *Momordica charntia* fruit. Activity guided fractionation, formulation and its evaluation is in progress and will be available in a short period of time.

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