IJP (2016), Vol. 3, Issue 2 **(Research Article)**

Received on 12 December 2015; received in revised form, 29 January 2016; accepted, 30 January 2016; published 29 February 2016

PHYTOCHEMICAL SCREENING AND FRACTIONATION OF *MOMORDICA CHARANTIA* LINN. FRUIT TO SHOW ANTIHYPERGLYCAEMIC ACTIVITY

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Keywords:

Momordica charantia, Phytochemical screening, Antidiabetic activity

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ABSTRACT: Diabetes mellitus is a metabolic disorder in the endocrine system. This dreadful disease is found in all parts of the world and is becoming a serious threat to mankind health. There are lots of chemical agents available to control and to treat diabetic patients, but total recovery from diabetes has not been reported up to this date. An alternative to these synthetic agents, plants provide a potential source of hypoglycemic drugs and are widely used in several traditional systems of medicine to prevent diabetes. Several medicinal plants have been investigated for their beneficial use in different types of diabetes. The effects of these plants may delay the development of diabetic complications and correct the metabolic abnormalities using a variety of mechanisms. whole fruit of *Momordica charantia* was selected in this study to find out the phytochemicals & antidiabetic property. Phytochemicals were extracted using water. For the current article screening of alkaloid, carbohydrates, glycosides, flavonoids, proteins, tannins & phenolic compounds and also done single dose antidiabetic activity. Screening of phytochemicals showed a positive result for the presence of Alkaloid, carbohydrates, glycosides, flavonoids, amino acids, saponins, and steroid. After preliminary phytochemical investigations, aqueous extract & its fractions were evaluated for activity employing a single dose in normal and alloxan induced diabetic albino rats. All the extract and fractions were given orally at a dose of 400 mg/kg b.w. The present study shows that the extract of Momordica charantia at 400 mg/kg b.w has significant antidiabetic activity. Among all fractions, a non-polysaccharide fraction of *Momordica charantia* more significantly reduced blood glucose level & nearly equal to standard glibenclamide after single dose treatment.

INTRODUCTION: Diabetes is a chronic disorder in the metabolism of carbohydrates, proteins, and fat due to an absolute or relative deficiency of insulin secretion with/without a varying degree of insulin resistance [1, 2].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.IJP.3(2).92-96

Article can be accessed online on: www.ijpjournal.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.IJP.3(2).92-96

It has now become an epidemic with a worldwide incidence of 5% in the general population.

The number of adults with diabetes in the world will rise from 135 million in 1995 to 300 million in the year 2025 [3]. The countries with the most significant number of diabetic people in the year 2025 will be India, China, and the United States [4]. There are more than 30 million people with diabetes mellitus in India, and the Incidence is increasing [5]. Insulin therapy is not enough to cure such disorders. The present-day insulin treatments, when taken orally, pose problems of specific side

effects, broken up and digested by the gut enzymes & insulin resistance are still impervious to treatment. Compared with synthetic drugs, drugs derived from plants are more frequently considered to be less toxic with fewer side effects. There is an increasing demand by patients to use natural products with antidiabetic activity [6]. Therefore, the search for more effective and safer natural antidiabetic agent devoid of adverse effect originating from plants. The fruits of *Momordica charantia* were chosen for investigation.

The plant *Momordica charantia* or bitter melon, a member of the Cucurbitaceae family [7]. Momordica charantia (bitter melon) is a favorite fruit used for the treatment of diabetes and related conditions amongst the indigenous populations of Asia, South America, India and East Africa [8]. It contains Gurmarin, a polypeptide considered to be similar to bovine insulin, which has been shown in experimental studies to achieve a positive sugar regulating effect by suppressing the neural response to sweet taste stimuli. Karela's principle constituents are lectins, charantin and momordicine. The fruits have long been used in India as a folk remedy for diabetes mellitus.

Momordica charantia have shown to possess various biological and pharmacological activities including anthelmintic, antibacterial, antibiotic, antidiabetic, anti-inflammatory, antileukemic, antimicrobial, antimutagenic, antimycobacterial, antioxidant, antitumor, antiulcer, antiviral, aperitive, aphrodisiac, astringent, carminative, cytostatic, cytotoxic, depurative, hormonal, hypocholesterolemic, hypotensive, hypotriglyceridemic, hypoglycemic, immunostimulant, insecticidal, lactagogue, laxative, purgative, refrigerant, stomachic, tonic, vermifuge [9].

MATERIAL AND METHODS:

Plant Material: The fruits of *Momordica charantia* Linn. collected from local market of Mandsaur (M.P.) in April 2013. The Taxonomical identification of Plant was done by Dr. C. K. Nigwal, Botanist, Government Arts and Science College, Mandsaur, India. The voucher specimen (BRNCP/M/004/2007) was deposited in the Herbarium of Department of Pharmacognosy, B. R. Nahata College of Pharmacy, Mandsaur.

Preparation of Extract: Fresh green whole fruits of *Momordica charantia* Linn. were collected. (500 gm) of whole green fruits of *Momordica charantia* Linn. along with seeds are cut into the pieces. The pieces of fruits were soaked in water in the ratio 10:25 for 1 h at room temperature. It was then filtered through muslin cloth and was evaporated to dryness under reduced pressure [10]. Percentage yield of various extracts is given in **Table 1**.

FIG. 1: EXTRACTION OF KARELA FRUIT

Fractionation of Aqueous Extract: Fractionation of *Momordica charantia* Linn. extract was done using its solubility profile. The aqueous extract obtained through sequential extraction (15 gm) was dissolved in water 250 ml, and excess of ethanol was added to precipitate polysaccharides completely. The precipitate (Polysaccharide fraction) was filtered and dried. The remaining non- polysaccharide fraction was also dried, and their percentage yield concerning aqueous extract was determined **Table 1**.

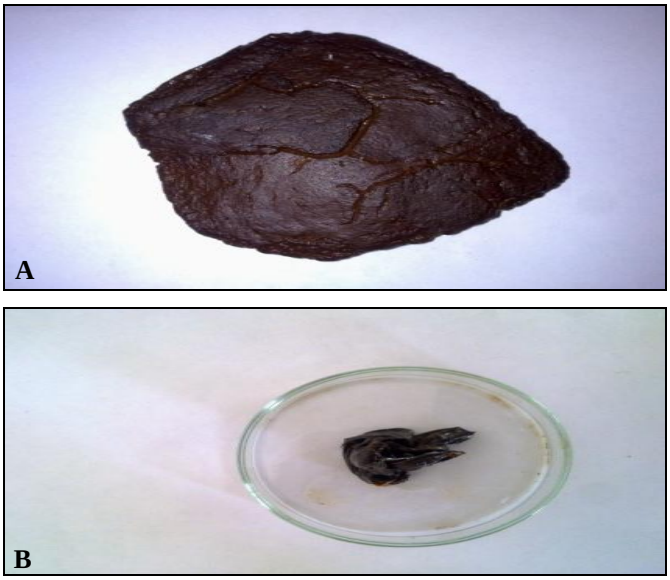


FIG. 2: (a) POLYSACCHARIDE AND (b) NON - POLYSACCHARIDE FRACTION OF AQUEOUS KARELA EXTRACT

Preliminary Phytochemical Screening: To determine the presence of alkaloids, glycosides, flavonoids, Proteins, tannins, terpenes and sugars, a preliminary phytochemical study (color reactions) with various plant extracts and fractions was performed [11].

Experimental Animals & Treatment: Healthy Wistar rats of either sex (150-180 g) with no prior drug treatment were used for the present study. The animals were fed with commercial pellet diet (Kamadenu Agencies, Bangalore, India) and water *ad libitum*. The animals were acclimatized to hygienic laboratory conditions for 10 days before starting the experiment. The animal study was performed in the Division of Pharmacology, B. R. Nahata College of Pharmacy, Mandsaur, with approval from the Institutional Animal Ethics Committee.

Acute Toxicity Studies: The severe toxicity test of the extracts and fractions was determined according to the OECD guidelines No. 420 (Organization for Economic Co-operation and Development). Female Wistar rats (150–180 g) were used for this study. After the sighting study, the starting dose of 2, 000 mg/kg of the test samples were given, to various groups containing 5 animals in each group.

The treated animals were monitored for 14 days for mortality and various responses like behavioral, neurological and autonomic responses. No death was observed up to the end of the study. The test samples were safe up to the dose of 2,000 mg/kg and from the results, 400 mg/kg was chosen as the maximum dose for further experimentation.

Antidiabetic Activity in Alloxan-Induced Diabetic Rats: Alloxan-induced diabetic model was selected to confirm the utility of active antihyperglycaemic extract and fraction in diabetic conditions. Diabetes was induced by injecting 120 mg/kg of alloxan monohydrate intraperitoneally in 0.9% w/v NaCl to overnight-fasted rats. 10% glucose solution bottles were kept in their cages for the next 24 h to prevent hypoglycemia. After 72 h of injection, fasting blood glucose level was measured. Animals which did not develop more than 300 mg/dl glucose levels were rejected [12]. Diabetic animals were divided into 6 groups (n = 5) and one more group of normal non-alloxanized animals was also added in the study.

Group 1: was kept as normal control (non-alloxanized rats)

Group 2: was kept as the negative control, alloxan-induced.

Group 3: diabetic was treated with glibenclamide (5 mg/kg) as a reference drug.

Group 4: diabetic-induced were treated with aqueous extract of *Momordica charantia* Linn.

Group 5: diabetic-induced were treated with polysaccharide fraction of *Momordica charantia* Linn.

Group 6: diabetic-induced were treated with the non-polysaccharide fraction of *Momordica charantia* Linn.

Collection of Blood and Estimation of Blood Glucose Parameters: The blood glucose level was measured using the Accu-chek Active ™ Test meter on blood from the rat tail vein.

Statistical Analysis: The values are expressed as mean ± SEM. The results were analyzed for statistical significance using one-way ANOVA followed by Dunnett's test. $p<0.05$ was considered significant.

TABLE 1: PERCENTAGE YIELD OF VARIOUS EXTRACTS AND FRACTIONS OF *M. CHARANTIA*

S. no.	Test parameter	Aqueous extract	Polysaccharide fraction	Non-polysaccharide fraction
1	Colour	Yellowish brown	Blackish brown	Yellowish brown
2	Consistency	Semi solid	Semi solid	Semi solid
3	Odor	Characteristic	Characteristic	Characteristic
4	Yield (% w/w)	4.91% w/w	43.75% w/w	31.54% w/w

TABLE 2: PRELIMINARY PHYTOCHEMICAL SCREENING OF EXTRACT & FRACTIONS OF *M.CHARANTIA*

S. no.	Chemical Constituents	Aqueous extract	Polysaccharide fraction	Non-polysaccharide fraction
1	Alkaloid	+	+	+
2	Carbohydrates	+	+	+

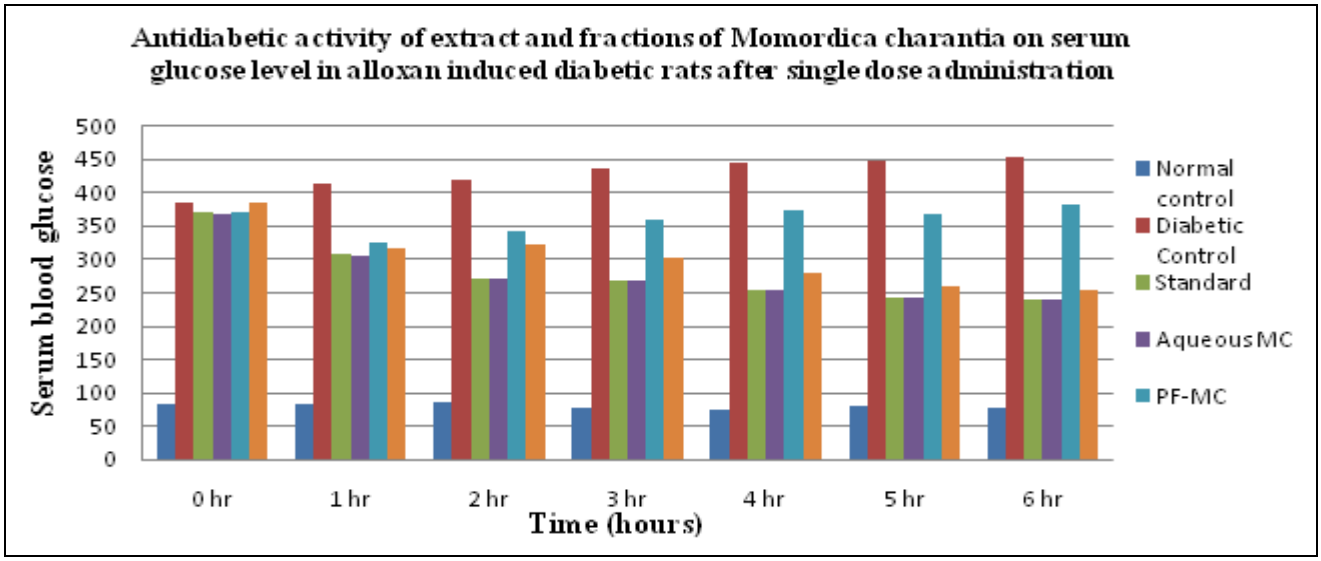
3	Glycosides	+	-	-
4	Flavanoids	+	-	+
5	Tannins & Phenolic compounds	-	-	+
6	Amino acids	+	-	-
7	Saponins	+	-	+
8	Steroids	+	-	+
9	Resins	-	-	-

(-) Absent (+) Present

TABLE 3: EFFECT OF VARIOUS EXTRACTS OF *MOMORDICA CHARANTIA* IN GLUCOSE LOADED HYPERGLYCEMIC RATS

Group	Drug	Dose mg/kg	0 h	1 h	2 h	3 h	4 h	5 h	6 h
I	Normal Control	-	84.67 ±22.70 ***	83.67 ±20.95 ***	87.00 ±20.40 ***	77.33 ±20.37 ***	75.00 ±21.50 ***	79.67 ±20.23 ***	77.67 ±20.22 ***
II	Diabetic Control	1 % w/v Tween 80	387.40 ±21.29	414.00 ±20.66	421.40 ±20.59	438.00 ±20.15	447.20 ±20.36	449.80 ±20.55	454.60 ±20.55
III	Standard Drug (Glibenclamide)	5	372.80 ±20.86	310.40 ±20.12*	271.60 ±21.62***	270.80± 20.57***	254.60 ±20.55***	242.60 ±22.53***	240.00 ±20.98***
IV	Aq. - MC	400	369.40 ±21.44	307.80 ±25.75**	291.20 ±21.37***	270.60± 20.54***	295.60 ±22.79***	360.20 ±20.27	333.60 ±20.55**
V	PF - MC	400	372.40 ±20.35	325.20 ±23.27*	343.80 ±24.56	359.00± 29.05	375.20 ±29.89	368.80 ±29.76	384.40 ±23.37
VI	NPF - MC	400	385.40 ±20.84	318.20 ±21.20*	322.20 ±23.18*	304.20± 27.36***	279.20 ±20.75***	261.40 ±21.88***	253.20 ±22.54***

Each value represents the mean ± SEM of five observations. *P<0.05; **P<0.01; ***P<0.001 Versus Control (ANOVA followed by Dunnett's test). Aq. – Aqueous, MC – *Momordica charantia*, PF – Polysaccharide Fraction, NPF – Non - Polysaccharide Fraction.



GRAPH 1: ANTIDIABETIC ACTIVITY OF EXTRACT AND FRACTIONS OF *MOMORDICA CHARANTIA* ON SERUM GLUCOSE LEVEL IN ALLOXAN INDUCED DIABETIC RATS AFTER SINGLE DOSE ADMINISTRATION

RESULT AND DISCUSSION:

Percentage Yield & Preliminary Phytochemical Screening: Percentage yield & phytochemicals of various extracts and fractions of *M. charantia* are given in **Table 1** and **2**.

Effect of Extracts and Fractions in Alloxan-Induced Diabetic Rats: Table 3 shows the antihyperglycaemic impact on alloxan-induced diabetic rats, after administration of plant extracts and fractions at a dose of 400 mg/kg. The basal blood glucose levels of all the groups were statistically not different from each other. Three days after alloxan administration, blood glucose values were 5-folds higher in all the groups and were not statistically different from each other. After 7 days, values of blood glucose decreased in all the treated groups, and the diabetic rats showed a slight increase in blood glucose level.

The administration of plant extracts, fraction and glibenclamide to diabetic rats restored the level of blood glucose significantly ($p<0.01$). Both the extract and the fraction were effective in alleviating diabetes. The activity of the non-polysaccharide fraction was more active, and the activity was comparable with that of the standard drug glibenclamide **Table 3**.

CONCLUSION: **Table 3** show the antihyperglycaemic effect of *Momordica charantia* Linn. extracts and fractions at a dose of 400 mg/kg in alloxan-induced diabetic rats. After the alloxan administration, there was a significant rise in the blood glucose level of control animals. The blood glucose level was checked after the administration of *Momordica charantia* Linn. extracts and fractions declined at the 4 h. The aqueous extract of *Momordica charantia* Linn. was more active after these we went for fractionation. On comparing the fractions, we found that the non- polysaccharide fraction of *Momordica charantia* Linn. was exhibited the significant activity as compared to the other fractions.

ACKNOWLEDGEMENT: Authors are thankful to the AICTE, New Delhi for providing necessary facilities and fundings to carry out this work. The authors are also thankful to Department of Herbal Drug Research, B. R. Nahata College of Pharmacy and Research Center, Madhya Pradesh, India for giving facilities, help, and support.

CONFLICT OF INTEREST: Nil

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How to cite this article:
Kaur M, Gupta R and Jarald E: Phytochemical screening and fractionation of Momordica charantia Linn. fruit to show antihyperglycaemic activity. Int J Pharmacognosy 2016; 3(2): 92-96. doi: 10.13040/IJPSR.0975-8232.3(2).92-96.