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Antihyperglycemic and antioxidant activities of active fraction from the aqueous extract of *Momordica cymbalaria* fruits in Streptozotocin induced diabetic rats

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ABSTRACT

The methanolic supernatant fraction (MSF) of the aqueous extract of *Momordica cymbalaria* fruits when given to streptozotocin (STZ) induced diabetic rats showed a significant reduction (65.9%, P<0.001) in fasting blood glucose levels at a dose of 0.5g/kg.b.w. These results were compared to that of glibenclamide, an oral hypoglycemic agent. The active MSF exhibited a dose dependent scavenging activity against 2, 2-diphenly-1-picrylhydrazyl (DPPH) radicals and nitric oxide radicals with an IC_{50} value of 42.5 and 157.2µg/ml, respectively. Further, the MSF had relatively lower reducing power, compared to that of ascorbic acid. The total phenolic content of the fraction was found to be 132mg/gm of dry fraction. In conclusion, MSF possess antihyperglycemic and antioxidant properties, which could be due to the presence of steroidal glycosides or phenolic compounds.

Keywords: Antihyperglycemic, Antioxidant, Diabetes mellitus, *Momordica cymbalaria,* Streptozotocin.

INTRODUCTION

Diabetes mellitus is a group of metabolic disorders characterized by alterations in carbohydrate, fat and protein metabolisms associated with absolute or relative deficiency of insulin secretion and/or insulin action. It is a growing public health problem throughout the world in which several pathogenic processes are involved. Due to the various side effects associated with the use of insulin and other oral hypoglycemic agents, there is an increasing demand for the natural products with antidiabetic activity and less side effects (1). Traditional crude remedies can serve as a powerful tool for drug development by leading to the discovery of pharmacologically active compounds (2). Studies indicate that hyperglycemia triggers the generation of free radicals and oxidative stress in capillary endothelial cells in the retina, mesangial cells in the renal glomerulus and neuron cells in the peripheral nerves (3). Treatment strategies that focus on decreasing oxidative stress as well as enhancing antioxidant defense systems might present important options for the treatment of diabetic complications. Hence compounds with both antihyperglycemic and antioxidative properties would be useful antidiabetic agents (4).

Momordica cymbalaria Hook. (MC) is one such plant that has been used as folklore medicine for the treatment of diabetes mellitus. It belongs to the family Cucurbitaceae. Nutritionally and morphologically MC is different from that of *Momordica charantia.* The fruits are fleshy, dark green and ribbed. Various phytochemical constituents were reported in the fruits, such as, carbohydrates, proteins, amino acids, phenols, alkaloids, tannins and vitamin C (5). The fruits are often used as pickles in southern parts of India (6).

Earlier it was reported that there was a significant reduction in blood glucose, cholesterol and triglycerides in alloxan induced diabetic rats after the treatment with the dried fruit powder of MC for 15 days without any hypoglycemic activity in the normal rats (7). Further it was observed that the aqueous extract of these fruits at a dose of 0.5 g/kg b.w. produced a significant fall in the blood glucose of the diabetic rats after 3 hours (hrs) of oral administration. It has also been shown that treatment with the aqueous extract (0.5 g/kg b.w) of MC fruit for six weeks produced significant antihyperglycemic and antihyperlipidemic activities (8). Therefore, an attempt was made to identify the active principle(s) in the aqueous extract of *Momordica cymbalaria* fruits.

MATERIALS AND METHODS

Collection of Plant Material

The fresh fruits of MC were collected from Western Ghats of Tirumala hills. They were identified and authenticated by a botanist at S.V.University, Tirupati. A voucher specimen of the plant (NN 886) was deposited in the herbarium of the Botany department, S.V.University, Tirupati, A.P, India. The fruits were dried under shade at room temperature.

Preparation of methanolic supernatant fraction

The aqueous extract of the dried fruits of MC was prepared according to the method of Kameswara Rao et al (9). The concentrated aqueous extract (50gms) was redissolved in methanol (100%) and left at room temperature for 3 days. The methanol soluble fraction was separated and centrifuged at 15,000 rpm for 20 minutes. The precipitate (MPF) and the supernatant (MSF) obtained after centrifugation was concentrated under reduced pressure using rotavapor R-200 and finally freeze dried. Then they were evaluated for their antihyperglycemic activity in experimental animals. The yield of methanolic supernatant fraction (MSF) was found to be 24.8% (w/w) of the starting material of the aqueous extract.

Induction of Diabetes mellitus

Diabetes was induced in Wistar albino rats aged 2-3months procured from Sri Venkateswara Agencies, Bangalore, by intra peritoneal administration of STZ at a dose of 50mg/kg.b.w. The animals were kept in polypropylene cages and maintained at room temperature under 12 hr dark/light cycles. The animals were fed with the standard pellet diet ad libitum. After 2 weeks of acclimatization, animals with a marked level of blood glucose > 250mg/dl were selected and used for the experimental study.

Evaluation of antihyperglycemic activity of MSF and MPF.

To evaluate the antihyperglycemic activity of MSF and MPF, the rats were divided in to 7 groups, each group consisting of 6 rats.

Group I	Normal untreated rats
Group II	Normal rats treated with the MSF (0.5gm/kg.b.w)
Group III	Normal rats treated with the MPF (0.5gm/kg.b.w)
Group IV	Diabetic untreated rats
Group V	Diabetic rats treated with the MSF (0.5gm/kg.b.w)
Group VI	Diabetic rats treated with the MPF (0.5gm/kg.b.w)
Group VII	Diabetic rats treated with glibenclamide (0.02gm/kg.b.w)

After an overnight fasting, the MSF and MPF were administered to the experimental animals in group II, III and V, VI by gastric intubation, using a force-feeding needle. Group I and IV were fed distilled water alone. Blood samples were collected from the tail vein for the measurement of blood glucose by GOD/POD method (10), at 0, 1, 2, 3, 4, 5, and 6 hrs after the administration of the partially purified active principles (MSF& MPF). The results were compared to that of glibenclamide, which is used as a standard oral hypoglycemic agent.

DPPH radical scavenging activity

The hydrogen atom or electron donation ability of the MSF was measured from the bleaching of the purple-coloured methanol solution of 2, 2-diphenly-1-picrylhydrazyl (DPPH). This spectrophotometric assay uses the stable radical DPPH as a reagent (11). 1ml of various concentrations of the MSF (25, 50, 75 and 100 µg/ml) was added to 4ml of 0.004 %(w/v) methanol solution of DPPH. After a 30 min incubation period at room temperature, the absorbance was read against blank at 517nm. The ability of MSF to scavenge DPPH radicals was calculated by the following equation

DPPH radical scavenging activity (%) = [(A control – A sample) / A blank] × 100

Where A control is the absorbance of the control reaction (containing all reagents except the test compound) and A sample is the absorbance of the test compound. Tests were carried out in triplicate. IC_{50} values for both MSF and BHT were calculated by plotting a graph concentration vs. percent of scavenging activity. IC_{50} value denotes the concentration of the MSF, which is required to scavenge 50% of DPPH free radicals.

Nitric oxide scavenging activity

Nitric oxide scavenging activity was measured by slightly modified methods of Green et al (12) and Marcocci et al (13). Nitric oxide radicals (N0˙) were generated from sodium nitroprusside. 1ml of sodium nitroprusside

(10mM) and 1.5 ml of phosphate buffer saline (0.2M, pH 7.4) were added to different concentrations (25, 50, 75 and 100µg/ml) of the MSF and incubated for 150 min at 25°C. After incubation 1ml of the reaction mixture was treated with 1ml of griess reagent (1% sulfanilamide, 2% H_3PO_4 and 0.1% napthalenediamine dihydrochloride). The absorbance of the chromatophore was measured at 546 nm. Butylated hydroxyl toluene was used as a standard. Nitric oxide scavenging activity was calculated by using the following equation

Nitric oxide scavenging activity (%) = [(A control – A sample) / A blank] × 100

Where A control is the absorbance of the control reaction (containing all reagents except the test compound) and A sample is the absorbance of the test compound. Tests were carried out in triplicate. The IC_{50} values for both MSF and BHT were calculated by plotting a graph concentration vs. percent of scavenging activity.

Reducing Power

The reducing power was determined according to the method of Oyaizu (14). Different concentrations of the MSF (25, 50, 250 and 500µg/ml) were mixed with phosphate buffer (2.5 ml, 0.2M, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (2.5ml, 1%). The mixture was incubated at 50°C for 20 min and 2.5ml of trichloroaceticacid (10%) was added to the mixture, which was then centrifuged at 3000 rpm for 10 min. The upper layer of the solution (2.5 ml) was mixed with distilled water (2.5 ml) and $FeCl_3$ (0.5 ml. 0.1%) and the absorbance was measured at 700nm. Increased absorbance of the reaction mixture indicated increased reducing power. Ascorbic acid was used as a standard.

Determination of total phenolic compounds

Total phenolic content of the MSF was determined by Folin-Ciocalteau reagent according to Singleton and Rossi (15), using gallic acid as a standard. 0.1ml (100µg) of sample solution was made up to 3 ml with distilled water. About 0.5 ml of Folin-Ciocalteau reagent was added and mixed thoroughly and incubated for 3 min at room temperature. After incubation 3ml of 20% Na_2CO_3 was added and mixed thoroughly and incubated in boiling water bath for 1min. The absorbance was measured at 650 nm.The concentration of total phenols was expressed in terms of micrograms of gallic acid equivalents.

Phytochemical analysis

Phytochemical analysis was carried out according to the methods of Harborne (16).

Statistical analysis

The statistical analysis was carried out using student t-test.

RESULTS

There was a significant decrease (65.9%, $p<0.001$) (Table 1) in fasting blood glucose levels in the diabetic treated rats at 5th hr after the administration of the MSF at a dose of 0.5g/kg.b.w., where as only 10% (statically not significant at $P<0.01$) reduction was observed in fasting blood glucose levels of diabetic treated rats at 2nd hr after the administration of MPF at a dose of 0.5g/kg.b.w. The MSF has potent action than that of the glibenclamide (30%, $p<0.01$). Both of the methanolic fractions did not show any hypoglycemic activity either in normal or in diabetic treated rats. Phytochemical analysis revealed the presence of phenols, carbohydrates, sterols and glycosides in the MSF.

In the present study, it was observed (Figure1) that purple colour of DPPH was bleached completely and very rapidly by the MSF at all concentrations (25-100 µg/ml) in a dose dependent manner. The maximum free radical (DPPH) scavenging activity was exerted by MSF (74.9%) at a concentration of 100 µg/ml, while the standard BHT showed 57.53% at the same concentration. The IC_{50} values of standard BHT and MSF were 72.5 and 42.5 µg/ml respectively.

The MSF had a better nitric oxide scavenging ability compared to the standard BHT (Figure 2). The IC_{50} values of BHT and MSF were 357.14 and 157.23 µg/ml respectively. The reducing power of the MSF was dose-dependent (Figure 3). The maximum activity was exerted by the fraction at a concentration of 500 µg/ml; this activity was lower than that of ascorbic acid at the same concentration. The amount of total phenolics present in MSF was 132 mg/g of dry fraction.

DISCUSSION

The present study was conducted to identify the possible active principle(s) present in the aqueous extract of *Momordica cymbalaria* fruits, which has showed a potent antihyperglycemic and antihyperlipedimic activities (8). Hence as an initial step of partial purification, the aqueous extract was redissolved in 100% methanol for 3 days, the methanolic soluble fraction was separated and subjected to centrifugation at a maximum speed of 15,000 rpm for 20 minutes at 4°C. The precipitate and supernatant obtained were then checked for their antidiabetic activity in STZ induced diabetic rats, by comparing with that of the standard oral hypoglycemic agent, glibenclamide.

Table 1: Antihyperglycemic activity of methanolic fractions in STZ induced diabetic rats.

Groups	Blood glucose (mg/dl) at different hours after the treatment with methanolic fraction						
	0h	1h	2h	3h	4h	5h	6h
I	75.16±1.4	74.33±4.1	71.33± 4.6	68.16±4.6	70.66±6.05	69 ±6.54	68.5 ± 8.0
II	70± 6.3	70.66±6.4	71.33±6.4	68.33±5.4	73.16±9.15	68.83±3.6	69.0 ± 3.7
III	75±8	73±6.8	76±7.3	72±9.8	70±8.1	74±4.9	71±10.2
IV	282.8±28*	263.16±31	266.0±32.9	279.8±35	276.6±48.3	273.6±29.0	305.16±42
V	348±35.4*	302±24.1†(13%)	279± 22††(19%)	225.6±45.8††(35.1%)	158.3±50††(54.5%)	118.5±9.5††(65.9%)	138.14±10.85
VI	258±31*	246±36	232±16(10.07%)	245±38	253±53	256±31	260±22
VII	283.8±21*	255.3±17†(10.21%)	236.6±11.5††(16.61%)	223.5±8.1††(21.1%)	214.5±9.7††(24.4%)	199.3±8.8††(29.78%)	285.6 ± 42.9

*P<0.001 compared with the initial level of blood glucose (0hr) of normal rats

†P<0.05 compared with the initial level of blood glucose (0hr) in the respective group

††P<0.001 compared with the initial level of blood glucose (0 hr) in the respective group.

Numbers in parenthesis indicate the percentage of fall in 0hr blood glucose.

The results indicated that at a dose of 0.5g/kg.b.w. the efficacy of MSF in reducing blood glucose level in diabetic rats (table 1) was observed from 1st hr after the treatment and it was continued till the end of the 5th hr (65.9%, p<0.001), where as at the same dose the MPF showed only 10% of reduction in fasting blood glucose levels of diabetic rats and it was observed at 2nd hr after the treatment. This indicates the fact that the active principle(s) was more concentrated in the supernatant fraction and sufficient to elicit the antihyperglycemic action. The antihyperglycemic activity could be due to its stimulatory effect on the remnant β-cells or insulin mimetic activity. The precipitate did not show a significant antihyperglycemic action since the active principle(s) might not be present in the precipitated fraction.

The phytochemical analysis has revealed the presence of glycosides, sterols, phenols and carbohydrates in the MSF. It was reported that Lotliker and Rajarama Rao (17), isolated stigmaoidal sitosterol glycoside called 'Charantin' from *Momordica charantia*. Charantin exhibited a significant fall in blood glucose levels when administered orally or intravenously. Charantin was the earliest reported active constituent of *Momordica* family. *Momordica foetida* a potent hypoglycemic plant contains a compound identical to charantin called '*Foetidin'* from the whole plant (18). The blood glucose lowering effect of '*Foetidin'* was comparable to that of insulin (19). Recent reports (20), revealed that saponin-steroidal glycosidal fraction from the ethanolic extract at a dose of 87.5 mg/kg p.o/day/30 days showed significant decrease in serum glucose, cholesterol, triglyceride level, with an increase in insulin and glycogen levels. There was reversal of the atrophy of the pancreatic β-cells, which were destroyed earlier due to streptozotocin. It was also found that the antihyperglycemic action of MSF was higher than that of the glibenclamide. No hypoglycemia was noticed in either of the methanolic fractions (supernatant and precipitate) of the aqueous extract during the treatment period. Drugs that normalize function, without causing hypoglycemia, would make attractive targets for diabetes (21). The selected dose of the methanolic fractions is the same as that of the aqueous extract of the fruits of *Momordica cymbalaria*. Hence MSF of the aqueous extract was identified as the active fraction. Further purification and characterization of the active principle(s) is going on in our laboratory by performing the bioassay guided fractionation.

The capacity of MSF to scavenge DPPH was measured and the results were shown in Figure 1. The antioxidants react with DPPH, a purple coloured stable free radical, and convert it into a colourless α- α- diphenyl- β- picryl hydrazine. The amount of reduced DPPH could be quantified by measuring the decrease in absorbance at 517 nm. In the present study, it was observed that purple colour of DPPH was bleached completely and very rapidly by MSF at all concentrations (25–100 µg/ml) in a dose dependent manner. The maximum free radical (DPPH) scavenging activity was exerted by the supernatant fraction (74.9%) at a concentration of 100 µg/ml, it was greater than that of standard BHT (57.53%) at the same concentration. IC50 value of the standard BHT and MSF were 72.5µg/ ml and 42.5µg/ml, respectively. The DPPH scavenging ability of the fraction may be attributed to its hydrogen donating ability.

The nitric oxide scavenging activity is based on the principle that sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide, which interacts with oxygen to produce nitrite ions that can be estimated using griess reagent. The MSF as a scavenger of nitric oxide competed with oxygen, leading to reduced production of nitrite ions. The MSF had a better nitric oxide scavenging ability compared to that of standard BHT (Figure 2). At a concentration of 100 µg/ml, MSF scavenged almost 47.5 % of nitric oxide where as BHT scavenged only 14% at the same concentration. The IC_{50} value of MSF and standard BHT were 157.23 and 357.14

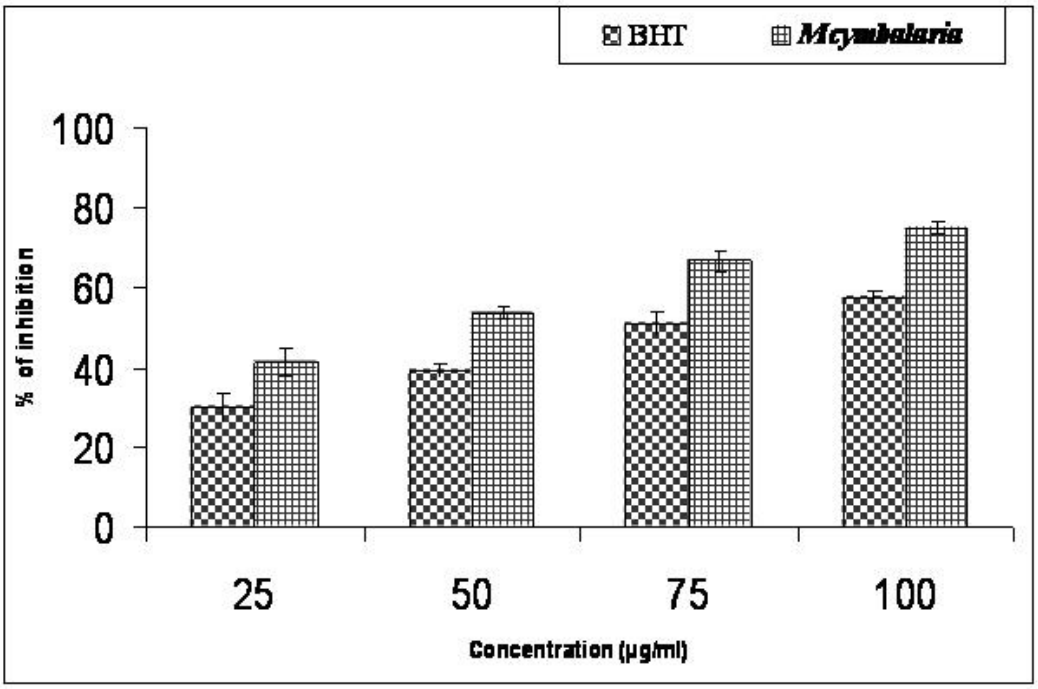

Figure 1: Scavenging effect of MSF of *Momordica cymbalaria* fruit aqueous extract and standard BHT on 2, 2′-Diphenyl-1-picryl hydrazyl (DPPH) radical. Results are mean ± S.E of three parallel measurements

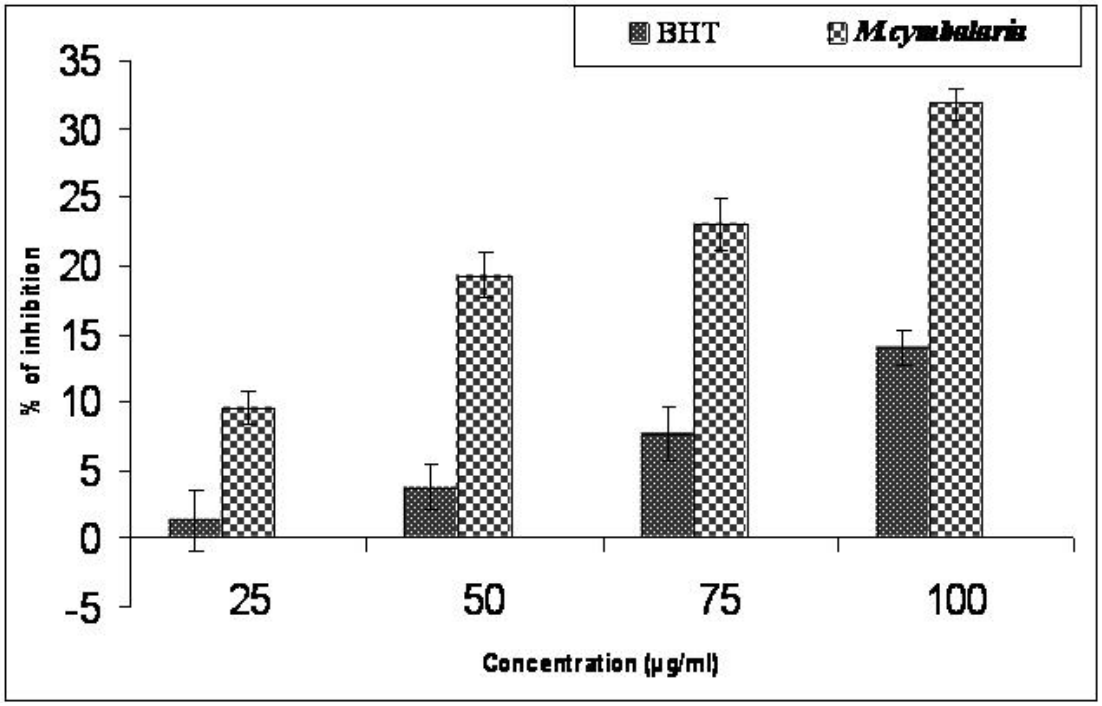

Figure 2: Scavenging effect of MSF of *Momordica cymbalaria* fruit aqueous extract and standard BHT on nitric oxide radical. Results are mean ± S.E of three parallel measurements

µg/ml respectively. MSF showed a greater potency than BHT in this study. In addition to reactive oxygen species, nitric oxide is also a factor involved in inflammation, cancer, and other pathological conditions (22). Natural extracts may have the property to counteract the effect of NO formation and, in turn, may be of considerable interest in preventing the ill effects of excessive NO generation in the human body.

In the reducing power assay, the presence of antioxidants in the MSF would result in the reduction

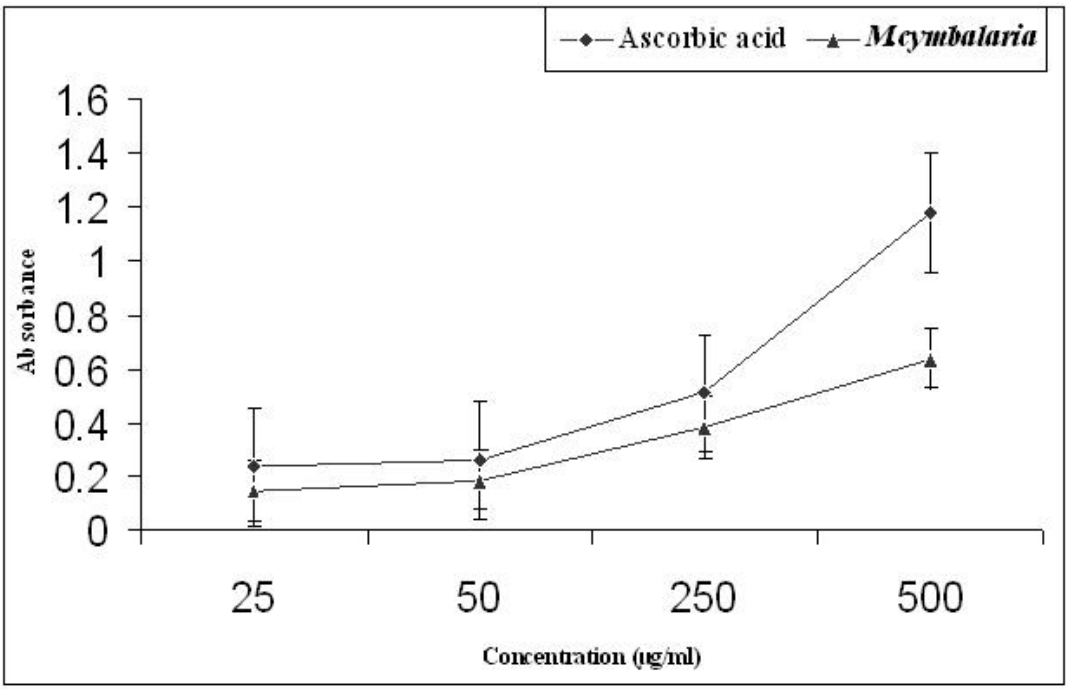

Figure 3: The reductive ability of MSF of *Momordica cymbalaria* fruit aqueous extract and ascorbic acid. Results are mean ± S.E of three parallel measurements

of Fe^{3+} to Fe^{2+} by donating an electron. Amount of Fe^{2+} complex can then be monitored by measuring the formation of Perl's Prussian blue at 700 nm. Increasing absorbance at 700 nm indicates an increase in the reductive ability. Figure 3 shows the dose response curves for the reducing power of the MSF and standard ascorbic acid. The MSF exhibited a good reducing power at 0.5 mg/ml. The reducing power of MSF was lower than that of standard ascorbic acid.

The amount of total phenolics present in methanolic fraction was 132 mg/g of dry fraction. Recent studies have shown that many flavonoids and related polyphenols contribute significantly to the total antioxidant activity of many fruits and vegetables (23). We have found correlation between antioxidant activity and total phenolic content.

CONCLUSION

In conclusion, we have investigated the antihyperglycemic and antioxidant properties of the partially purified active principle(s) from aqueous extract of *Momordica cymbalaria* fruits. The MSF at a dose of 0.5g./kg.b.w is effective for reducing blood glucose levels to near normal in the diabetic rats Unlike insulin, insulin secretogogues or small proteins or peptides isolated from *M.charantia*, the treatment with the MSF did not produce hypoglycemia in either diabetic or normal rats. This study also suggested that the MSF possesses antioxidant activity, which might be helpful in preventing or slowing the progress of various oxidative stress related diseases including diabetes. Further investigation on the isolation and identification of both antihyperglycemic and antioxidant component(s) in the MSF may lead to chemical entities with potential for clinical use.

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