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Hypoglycemic, Antidiabetic and Toxicological Evaluation of *Momordica dioica* Fruit Extracts in Alloxan Induced Diabetic Rats

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ABSTRACT

The aim of the study was to evaluate the antidiabetic effect of *Momordica dioica* fruit extracts in alloxan induced diabetic wistar rats. Aqueous extract of *Momordica dioica* (AEMD) showed maximum fall (52.8%) in 0 to 1 h Fasting Blood Glucose (FBG) in Glucose Tolerance Test (GTT) compared to hexane (39%), chloroform (37.2%) and ethanol (37.7%) extract in normal healthy rats. Since AEMD exhibited maximum hypoglycaemic activity as compared to other extracts, it was further studied for antidiabetic effect in diabetic rats. The oral Effective Dose (ED) of AMED was 200 mg kg^{-1} body weight which produced a fall of 57.5% ($p<0.001$) in diabetic rats. The 200 mg kg^{-1} body weight AMED once daily for 21 days reduced the elevated Blood Glucose (BG) by 64.8% ($p<0.001$), Post Prandial Glucose (PPG) by 76.9% ($p<0.001$) and glycosylated hemoglobin (HbA1c) by 37.6% ($p<0.001$). Urea, creatinine and urinary sugar, total protein, AST, ALT, alkaline phosphatase and bilirubin activities were also reduced after AEMD treatment in diabetic rats. Above 3 g kg^{-1} b.wt was Lethal dose of AEMD i.e., 15 times of ED indicating high margin of safety. Our study suggests possible use of aqueous extract of fruits of *M. dioica* for the management of diabetes mellitus.

Key words: *Momordica dioica,* diabetes, alloxan, wistar rats, aqueous extract, glibenclamide, toxicity

INTRODUCTION

Diabetes mellitus is a chronic diseases affecting about 1% of the western countries and 5-10% people around the globe (Burke *et al*., 2003). The number of diabetic patients is expected to increase from 171 million in year 2000 to 366 million or more by the year 2030 (Wild *et al*., 2004). The disorder is already declared epidemic by WHO. Diabetes mellitus defined as acquired deficiency in production of insulin by the β-cells in pancreas (Type I) or by the ineffectiveness of the produced insulin (Type II). It is characterized by chronic hyperglycaemia with disturbances of carbohydrate, fat and protein metabolism and characteristic symptoms such as thirst, polyuria, blurring of vision and weight loss. In its most severe forms, ketoacidosis or a non-ketotic hyperosmolar state may develop and lead to stupor, coma and death in absence of effective treatment (WHO, 1999). The discovery of insulin has revolutionized the diabetes care and this hormone is currently the drug of choice for patients with type I and II diabetes as well. But insulin therapy suffers disadvantage of painful injections, weight gain, hypoglycemia and its excess dose may lead to coma or death

(UKPDS, 1998; Sinha *et al*., 1996). Hence there is always search for oral and safe antidiabetic drugs. Tolbutamide (Sulfonylurea), Metformin (Biguanides), Thiazolidinediones and few more antidiabetic drugs have been developed for treatment of diabetes in recent times. Side effects associated with prolonged use of insulin and other synthetic antidiabetic drugs (Al-Salman *et al*., 2000; Maeda, 2001; Watkins, 2003) necessitated search for safe and effective antidiabetic agents especially of herbal origin (Shukla *et al*., 2000; Grover *et al*., 2002; Qureshi *et al*., 2009). There are several reports of use of medicinal plants in treatment of diabetes across the globe particularly India (Iweala and Oludare, 2011; Ekor *et al*., 2010; Hossain *et al*., 2010). Several hundred medicinal plants have been investigated for their beneficial use in different types of diabetes in Ayurvedic System of Medicine (Joseph and Jini, 2011).

Momordica dioica Roxb. Ex. Wild. (Family: Cucurbitaceae) is commonly known spinegaurd (English), vahisi (Sanskrit), golkandra (Hindi) and kakora in folk language of Bundelkhand region of central India. The aqueous extract of the roots has spermicidal activity and anthelmintic activity (Satyavati *et al*., 1987). The roots are reported to possess moderate antimicrobial activity (Sadyojatha and Vaidya, 1996) and antifertility activity (Shreedhar *et al*., 2001). Antihyperglycemic effect of *M. dioica* fruits is reported on alloxan induced diabetic rats (Reddy *et al*., 2006) in short term 6 h study. Alkaloids, ascorbic acid, lectins, β-sitosterol, saponins, glycosides, triterpenes of ursolic acid, hederagenin, oleanolic acid, stearic acid, α-spiranosterol, gypsogenin and momodicaursenol have been isolated from fruits of *M. dioica* (Sadyojatha and Vaidya, 1996; Ali and Srivastava, 1998; Luo *et al*., 1998). In present study we explored antidiabetic potential of various fruit extract of this plant on diabetic rats and toxicity aspect of the active extract. To the best of our knowledge this is the first long-term antidiabetic and toxicity study of *Momordica dioica*.

MATERIALS AND METHODS

This study was carried out between August 2009 to November 2010 at the Department of Biomedical Sciences, Bundelkhand University, Jhansi.

Chemicals: Alloxan was purchased from Sigma-Aldrich, USA. Kits for the estimation of various parameters were purchased from different sources: Total Cholesterol (TC), High-density Lipoprotein (HDL) and Low-Density Lipoprotein (LDL) and Bilirubin was assayed using standard kits from Span Diagnostics Ltd. India; triglycerides (TG) and glycosylated hemoglobin (Hb A1c) from Erba Diagnostics Mannheim GmbH, Germany; Serum insulin from Ranbaxy Diagnostics, India; Alkaline Phosphatase (ALKP) from Siemens Medical Solutions Diagnostics Ltd. India, serum Alanine Transaminase (ALT), serum Aspartate Transaminase (AST), total protein, creatinine and urea from Euro Diagnostics Ltd. India. One touch glucometer (Optium) of Roche Diagnostics, Germany and Uristx from Bayer Diagnostics India Ltd. were used for blood and urinary glucose respectively. All other chemicals used during the study were of high purity.

Animals: Wistar rats, weighing about 150-200 g obtained from Central Drug Research Institute (CDRI), Lucknow, were reared in animal house at Bundelkhand University. Animals were kept for acclimatization in the animal house at ambient temperature of 25°C and 45-55% relative humidity, with 12 h each of dark and light cycles and were fed pelleted diet and water *ad-libitum*. Animal experimental protocols were in accordance with the recommendations of the institutional animal ethical committee.

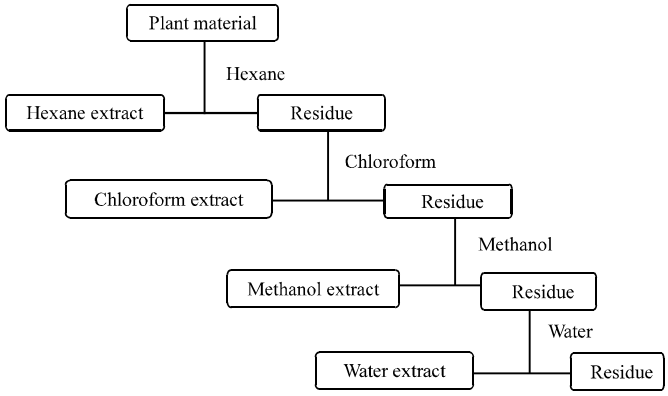

Fig. 1: Scheme for extraction of plant material

Plant material: The fresh fruits of *Momordica dioica* were collected in the rainy season, from the local hills of the Bundelkhand University, Jhansi. Experts in Department of Botany, Bundelkhand University, Jhansi identified the plant material and a voucher specimen is retained for future reference. The plant material was shade dried and grinded to obtained coarse power and subject to solvent extraction as per scheme in Fig. 1 (Singh *et al.*, 2002). The extracts were filtered and centrifuged (10,000 rpm, 10 min, at room temperature) to remove any residual or fibrous material. The organic solvent extracts were dried in rotary evaporator under reduced pressure. The aqueous extract was lyophilized.

Biochemical parameters: Blood glucose, glycosylated hemoglobin (Hb A1c), insulin, Total Cholesterol (TC), High-Density Lipoprotein (HDL), Low-Density Lipoprotein (LDL), Very-Low Density Lipoprotein (VLDL), Triglycerides (TG), Alkaline Phosphatase (ALKP), serum alanine transaminase (ALT), serum aspartate transaminase (AST), total protein, bilirubin, creatinine and urea were measured using diagnostic kits. Sugar was detected by reagent-based uristrix.

Induction of experimental diabetes: Diabetes was induced in rats using Alloxan. Alloxan solution was freshly prepared in neutral saline and kept in ice bath and used within half an hour of its preparation. Diabetes was induced in rats weighing 200 g, by single intraperitoneal injection of alloxan at the dose of 160 mg kg^{-1} body weight in overnight fasted rats. The diabetic state of animals was assessed after a period of one week. Animals with stabilized diabetes were used for the experiments. Fasting Blood Glucose (FBG) level was estimated at the time of induction of diabetes and postprandial glucose (PPG) was checked regularly till stable hyperglycemia is established. Animals with stabilized diabetes having FBG more than 250 mg dL^{-1} were used assessing antidiabetic effect of the plant and standard drug.

Dosing of plant extract and standard antidiabetic agent: Plant extracts were given orally by gavaging. Hexane, chloroform and ethanol extract were dissolved in DMSO and aqueous extract was dissolved in distilled water. Glibenclamide at a dose of 5 mg kg^{-1} b.wt (Djomeni *et al.*, 2006) was prepared in distilled water and given orally with the help of feeding cannula.

Experimental design

Hypoglycemic effect of different solvent extracts on glucose tolerance in normal healthy rats during Glucose Tolerance Test (GTT): Solvent extracts of *M. dioica* prepared as per scheme-I were assayed for hypoglycemic activity by GTT in normal healthy rats. Four groups (Group I-IV) of six rats each with each group having individual control (C) of six rats were used in the experiment. Animals of group I-IV were Treated (T) with hexane, chloroform, ethanol and aqueous extracts at a dose of 300 mg kg^{-1} b.wt, respectively where as control of each group received the vehicle in which the extract was given to treated rats (DMSO for group I-III and water for group IV). GTT was performed in overnight fasted rats, initial Fasting Blood Samples (FBG) were taken from tail end and then different extracts were given orally to different groups of animals. After 90 min, blood was again drawn that gives the 0 h value. The animals were then given a glucose solution (2 g kg^{-1} b.wt) orally and blood samples were drawn at 1, 2, 3 h after glucose administration which gives the 1, 2, 3 h values (Gupta *et al*., 2005).

Determination of Effective Dose (ED) of AEMD by GTT in diabetic rats: Animals with stabilized diabetes having FBG more than 250 mg dL^{-1} were used in the study. Animals were divided in seven groups of six rats each. Group I served as normal control, group II served diabetic control, group III-VI received different doses of AEMD viz., 150, 200, 250 and 300 mg kg^{-1} body weight and group VII was given glibenclamide, 5 mg kg^{-1} b.wt. GTT was conducted with different doses of aqueous extract, in overnight fasted rats. The dose which produced maximum effect, was taken as ED and was used in subsequent experiments.

Treatment of diabetic rats with AEMD: Four groups of six rats each were used in this experiment. Group I served as normal healthy control, group II served as diabetic control, group III was treated with effective dose (200 mg kg^{-1} b.wt) of AEMD and group IV was treated with 5 mg kg^{-1} b.wt of glibenclamide once daily for 21 days. FBG, PPG, body weight and urinary sugar were estimated at 0, 7, 14 and 21 days of the experiment. Hb1Ac, serum insulin, TC, TG, HDL, LDL, VLDL, serum total protein, ALT, AST, alkaline phosphatase, bilirubin, urea and creatinine levels were estimated at the beginning and end of the experiment (i.e., 0 day and 21 day).

Toxicity test: The toxicity test for determining median lethal doses 50 (LD 50) in healthy rats was carried out in three steps. Animals were divided into three groups (I-III) of six rats (3 females and 3 males) and were administered orally a single dose of 5, 10, 15 times of ED of AEMD, respectively. The rats were observed for food consumption, behavioral changes such as excitement, nervousness, dullness, alertness, ataxia or death (if any) continuously for 1 h after treatment and then 4 h and thereafter over a period of 24 h. Careful observation of the animals was continued up to one week.

Statistical analysis: Results were expressed as Mean±SD. The statistical analysis involving two groups was performed by means of paired t-test and analysis of variance (one way ANOVA) followed by Tukeys test. All the data were processed with GraphPad InSat software. $p<0.01$ was considered significant and $p<0.001$ was considered highly significant.

RESULTS

Hypoglycemic activity of different solvent extracts of *M. dioica*: Aqueous extract significantly ($p<0.01$) reduced blood glucose rise from 0 to 1 h during GTT by 52.8% as compared

to control. Hexane, chloroform and ethanol extracts produced a fall of 39.0, 37.2 and 37.7% respectively as compared to control values (Table 1). Aqueous extract (AEMD) was found most active and was used in subsequent experiments.

Effective dose of AEMD: AEMD at 200 mg kg^{-1} b.wt produced highest fall of 57.5% ($p<0.01$) in the peak blood glucose at 1h during GTT as compared to untreated control group while 150, 250, 300 and glibenclamide produced fall of 37.3, 50.5, 29.2 and 44.4%, respectively (Table 2). Thus 200 mg kg^{-1} b.wt was taken as ED in subsequent experiments.

Effect of AEMD on FBG, PPG, urinary sugar and body weight in alloxan induced experimental diabetic rats: Administration of ED of AEMD for 21 days to diabetic rats significantly ($p<0.001$) brought down the FBG from the initial value by 64.8% where as glibenclamide produced a fall of 58.8% ($p<0.001$). PPG was also bought down from initial value by 76.9% by AEMD which is higher than the fall 72.6% ($p<0.001$) produced by glibenclamide (Fig. 2). No urine sugar was observed after the treatment with AEMD while untreated control showed 3.0 g L^{-1} and while in glibenclamide it was 1.0 g L^{-1}. A reduction in body weight was observed in diabetic rats but when the rats were treated with extract, the decrease in body weight was minimized to almost nil and the improvement in body weight was observed (Table 3).

Effect of AEMD on glycosylated hemoglobin and serum insulin: AEMD and glibenclamide treated groups showed increase in serum insulin levels by 31.3 and 31.6%, respectively (Fig. 3). In

Table 1: Hypoglycemic activity of different solvent extracts of M. dioica fruits during GTT in normal healthy rats

Extract		Blood glucose (mg dL^{-1})					(%) fall between
treated	Group (n = 6)	FBG	0 h	1 h	2 h	3 h	0 and 1 h
Hexane	C	85.6±2.08	88.6±1.5	105±3.0	99.6±1.5	90±3.6	39
	T	89.3±2.5	94±2.0	104±2.5	100.6±1.1	99±2.6	
Chloroform	C	89.3±2.5	89±3.6	107±5.1	100±2.0	97.3±2.08	37.2
	T	91.3±3.05	93±2.0	104.3±2.5	98.3±8.1	99±7.9	
Ethanol	C	88±2.6	90.3±1.5	108.6±2.5	101.3±2.5	91.6±2.5	37.7
	T	98.3±2.5	89.6±1.5	101±1.0**	97.6±1.5	91.3±2.5	
Aqueous	C	88.3±3.5	86.6±2.5	109±4.1	105.3±3.05	95±3.0	52.8
	T	87.6±2.08	87.3±3.7	98±2.0**	95.3±1.5*	84.6±2.5	

n = No. of animals per group. The values represent the Mean±SD. *$p<0.01$, **$p<0.01$ as compared with control values at the same time

Table 2: Effective Dose (ED) determination of AEMD during GTT in diabetic rats

Group	Treatment	Blood glucose (mg dL^{-1})					(%) fall between
(n = 6)	(mg kg^{-1})	FBG	0 h	1 h	2 h	3 h	0 and 1 h
I	Normal control	88±7	89.3±4.5	109.3±3.0	95.6±5.8	94±5.5	-
II	Diabetic control	249±8.5	251±3.6	350±4.04	336±7.6	352.3±6.8	-
III	150	255.6±6.6	264.6±4.5	326±5.2	301±3.6	297±2.5	37.3
IV	200	257.3±9.2	243.3±7.6	285±5**	270±5.5	258.6±3.2	57.5
V	250	247.3±7.5	266±10.5	315.6±2*	296.3±5.6	275.6±4	50.5
VI	300	249±9	227.3±6.0	297.6±2.5**	277.3±2.5	269±3.6	29.2
VII	Glibenclamide (5 mg kg^{-1} b.wt)	258±11	245±5	300.3±5.5**	243.3±5.7	235±5	44.4

n = No. of animals per group. The values represent the Mean±SD. *$p<0.05$, **$p<0.01$ as compared with group II values at the same time

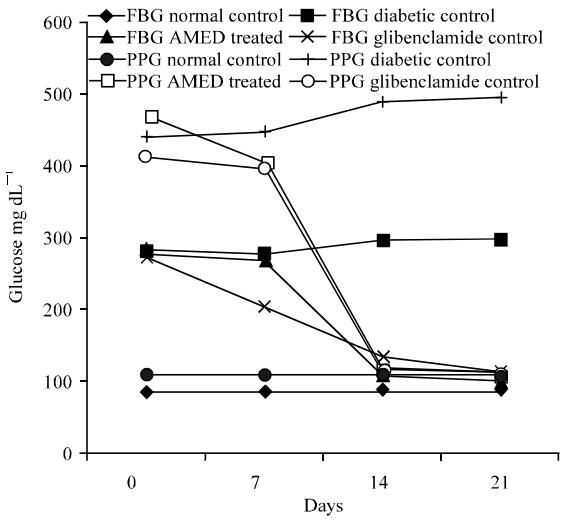

Fig. 2: Effect of 21 days treatment of AEMD and glibenclamide on FBG and PPG

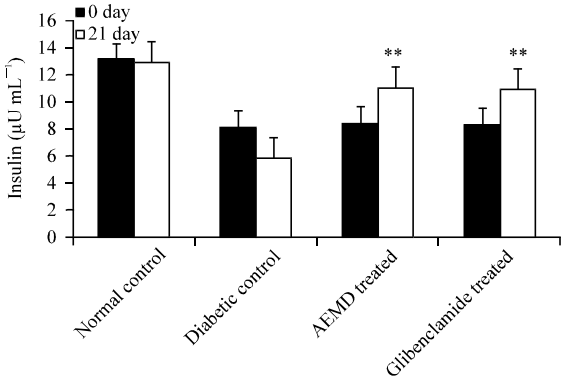

Fig. 3: Effect of 21 days treatment of AEMD and glibenclamide on serum insulin. **Indicates highly significant difference ($p<0.001$) as compared with 0 day at the same time. The results were expressed as Mean±SEM

Table 3: Effect of AEMD and glibenclamide on urinary sugar and body weight in diabetic rats

Urinary sugars				Body weight (g)			
0 day	7 day	14 day	21 day	0 day	7 day	14 day	21 day
0	0	0	0	200±0	206.3±1.5	253±5.7	295±5
3+	3+	3+	3+	180.6±2	175.6±4	175±13.2	183.3±5.7
3+	2+*	1+**	0**	189.7±4.6	187.7±6.3	238.7±4. **	246.2±4.7**
3+	3+	2+*	1+**	188±2.6	211.6±7.6	230±17.3*	243.3±5.7**

The values represent the Mean±SD. *$p<0.01$, **$p< 0.001$ as compared with control values at the same time. +Indicates the level of sugar

healthy controls, there was no significant change. No change in level of glycosylated hemoglobin (measured as (%) of HbA1c) was observed in untreated diabetic controls where as significant ($p<0.001$) reduction of 37.6 and 39.3% was observed in AEMD and glibenclamide treated groups (Fig. 4).

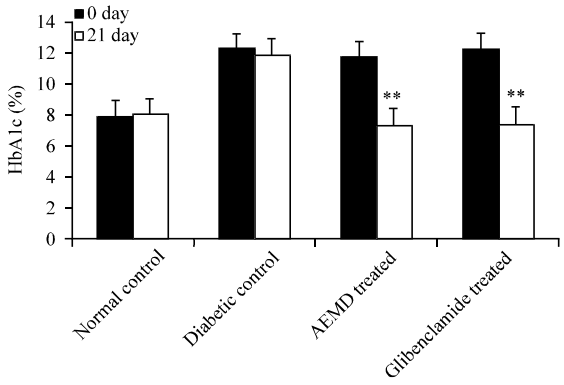

Fig. 4: Effect of 21 days treatment of AEMD and glibenclamide on glycosylated hemoglobin. **Indicates highly significant difference ($p<0.001$) as compared with 0 day at the same time. The results were expressed as Mean±SEM

Table 4: Effect of AEMD and glibenclamide on total protein and bilirubin

	Normal control		Diabetic control		Diabetic treated		Glibenclamide treated	
Parameter	Initial	Final	Initial	Final	Initial	Final	Initial	Final
Total proteins ($g\ dL^{-1}$)	6.6±0.40	6.9±0.43	10.4±0.49	11.6±0.52	10.8±0.36	6.9±0.45** (36.1%↓)#	11.56±0.4	7.5±0.36** (35.1%↓)#
Bilurubin ($mg\ dL^{-1}$)	0.2±0.02	0.18±0.05	0.53±0.04	0.57±0.02	0.49±0.01	0.19±0.01 (61.2%↓)#	**0.47±0.02	0.19±0.01** (59.5↓)#

The values represent the Mean±SD. *$p<0.01$, **$p<0.001$ as compared with control values at the same time. # % fall when compared with untreated diabetic control. ↓shows (%) fall when compared with untreated diabetic control

Effect of AEMD on lipid profile, liver and kidney function tests: TC and TG values were brought down significantly ($p<0.001$) 52.2 and 32.5% after treatment with AEMD. Glibenclamide treated animals showed 37.5 and 22.8% decrease in TC and TG levels, respectively. An interesting observation is that, there was an increase of 40.6 and 65.2% in HDL cholesterol in AEMD and glibenclamide treated diabetic rats respectively in contrast to the untreated diabetic rats. Also there was fall of 56.7 and 32.5% in LDL cholesterol and 57.6 and 33.9% in VLDL cholesterol levels in AEMD and glibenclamide treated rats (Fig. 5).

Reduction of 36.1 and 35.1% in total protein levels of AEMD and glibenclamide treated rats respectively were observed (Table 4). The 39.2 and 56.6% in AEMD treated decreased AST and ALT levels and 43.07 and 56.6% in glibenclamide treated rats. Alkaline phosphatase also showed same results with fall of 10.5 and 23.9% in AEMD treated and glibenclamide treated rats, respectively (Fig. 6). Bilirubin levels show a fall of 61.2 and 59.5% in AEMD and glibenclamide treated rats, respectively (Table 4).

Urea and creatinine values fall by 41.4 and 32.04% in AEMD and 45.4 and 25.4% fall in glibenclamide treated rats (Fig. 7).

Toxicity test: After administration of 5, 10 and 15 times of ED of AEMD no behavioral signs such as excitement, nervousness, dullness, alertness, ataxia or death were observed with all the three doses. Thus AEMD seems to have no toxic effects even with 15 times of ED. Food consumption was also normal in all animals treated with all the three doses.

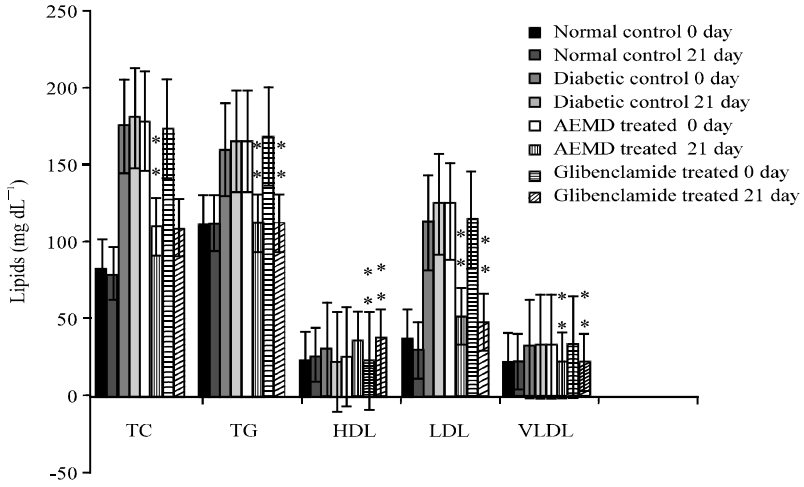

Fig. 5: Effect of 21 days treatment of AEMD and glibenclamide on lipid profile. **Indicates highly significant difference ($p<0.001$) as compared with 0 day at the same time. The results were expressed as Mean±SEM

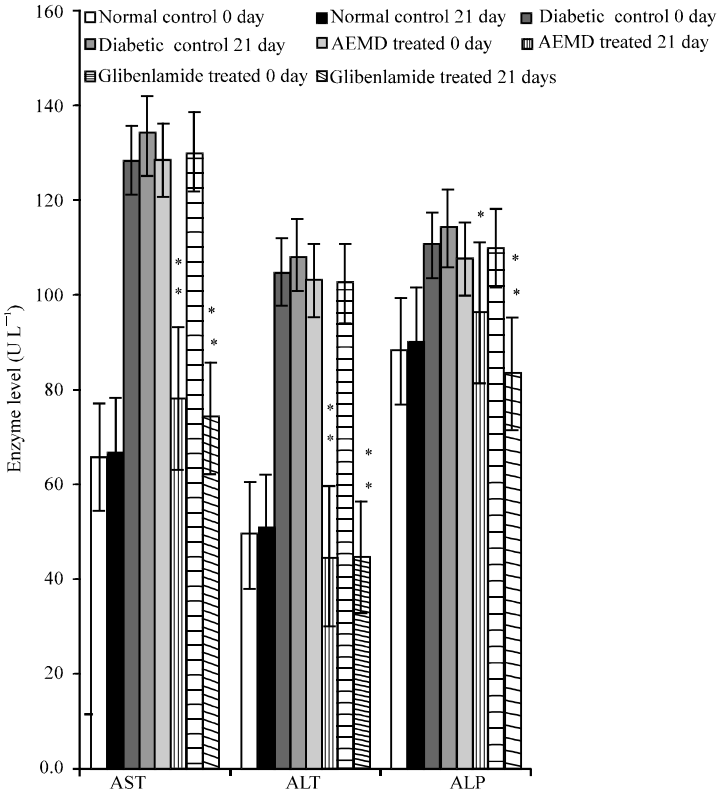

Fig. 6: Effect of 21 days treatment of AEMD and glibenclamide on liver function markers. *Indicates significant difference ($p<0.01$), **Indicates highly significant difference ($p<0.001$) as compared with 0 day at the same time. The results were expressed as Mean±SEM

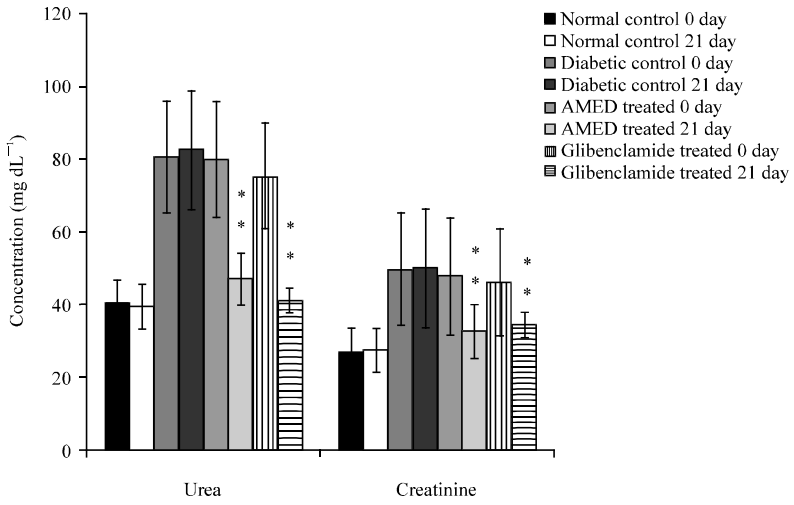

Fig. 7: Effect of 21 days treatment of AEMD and glibenclamide on kidney function markers. **Indicates highly significant difference ($p<0.001$) as compared with 0 day at the same time. The results were expressed as Mean±SEM

Administration of 5, 10 and 15 times of ED of AEMD in healthy rats showed no behavioral signs of toxicity such as excitement, nervousness, dullness, alertness, ataxia or death. Food consumption was also normal in all animals treated with all the three doses. Thus AEMD seems to have no toxic effects even with 15 times of ED.

DISCUSSION

Various species of the genus *Momordica* are widely explored for antidiabetic activity in animal models (Rao *et al.*, 1995; Krawinkel and Keding, 2006; Basch *et al.*, 2003) however, *Momordica dioica* is relatively less studied. To the best of our knowledge, there is only one report of antihyperglycemic activity of organic solvent extracts of this plant in a short 6 h study (Reddy *et al.*, 2006). First time we are reporting a detailed study of antidiabetic effect of organic and aqueous extract of this plant. Reddy *et al.* (2006) reported antidiabetic activity from ethyl acetate and ethanol extract of *M. dioica* however, we observed better activity in aqueous extract. Aqueous extract was not explored for antidiabetic activity by Reddy *et al.* (2006). Literature survey reveals that antidiabetic activity is reported from aqueous extracts of a number of medicinal plants (Grover *et al.*, 2002).

Aqueous extract showed maximum hypoglycemic effect in normal healthy rats as compared to other extracts (Table 1). Aqueous extracts produced 52.8% fall in blood glucose with in 1 h during GTT and hypoglycemic effect is maintained for at least 3 h. This indicate that it takes around 2.5 h for the active principal of extract for absorption and its effect remains around 4-5 h.

Studies with different doses (150-300 mg kg^{-1} b.wt) of the aqueous extract (Table 2) on FBG of alloxan diabetic rats during GTT indicated that the most effective dose was found to be 200 mg kg^{-1} b.wt produced 57.5% fall while 250 mg kg^{-1} b.wt has also showed good activity (50.5% fall). Whereas 300 mg kg^{-1} b.wt showed lesser fall. Such a phenomenon of less hypoglycemic effect at higher dose is not uncommon with medicinal plants and has been observed in *Brassica nigra* (Anand *et al.*, 2007), *Murraya koenigii* (Kesari *et al.*, 2005), *Cinnamomum tamala*

(Sharma *et al.*, 1996) and *Aegle marmelose* (Rao *et al.*, 1995). The effect of 200 mg kg^{-1} b.wt of aqueous extract was better than other doses during GTT, hence further studies were carried out with aqueous extract given at a dose of 200 mg kg^{-1} b.wt.

Treatment of diabetic rats with AEMD for 21 days brought down the elevated blood glucose levels from more than 250 mg dL^{-1} to nearly normal range. Alloxan, a β-cytotoxin induces diabetes in a wide variety of animal species through damage of insulin secreting cell (Rerup, 1970). Alloxan not only destroys the pancreatic β-cells but also damages the kidney which is however reversible (Goldner and Gomori, 1943). The elevated blood glucose levels in severely diabetic animals (FBG≥250 mg dL^{-1}) with considerable pancreas damage resemble type 1 diabetes. The study shows that AEMD is able to bring down FBG level considerably, hence it is very useful in management type I diabetes. The extract might have additional advantage over the existing sulfonylurea drugs which are able to lower blood glucose only when there is a functional pancreas as proposed by Sharma and coauthors while studying antidiabetic effect of *Cesalpinia bounducella* (Sharma *et al.*, 1997). The AEMD effectively decreased the blood glucose in alloxan-induced diabetic rats (FBG 64.8% and PPG 76.9%) which is even better than the effects produced by standard drug glibenclamide (Fig. 2). This glucose lowering effect may be by activation of β-cells and granulation returns to normal giving insulinogenic (Kedar and Chakrabarti, 1982). Perhaps AEMD bring about its hypoglycemic action through stimulation of surviving pancreatic β-cells to release more insulin. This is clearly evidenced by the increased level of serum insulin in diabetic rats treated with AEMD. There are reports that hyperglycaemia increases the generation of free radicals by glucose auto-oxidation and the increment of free radicals may lead to liver damage. The increase in oxygen free radicals in diabetes could be primarily due to the increase in blood glucose levels and secondarily due to the effect of diabetogenic agent alloxan. The study showed that AEMD is not only able to reduce blood glucose levels but also exhibits hepatoprotective effect. Some plants extracts have shown hypoglycemic activity by protecting β-cell from alloxan induced damage through scavenging free radicals (Kim *et al.*, 2006), hence this protective action of AEMD is also not ruled out in our study. Further studies are required to study the effect of AEMD on liver lipid peroxidation and antioxidant enzyme level to confirm this hypothesis. A reduction in body weight observed in alloxan induced rats may be due to excessive breakdown of tissue proteins (Chatterjea and Shinde, 2002). Treatment with AEMD, the decrease in body weight was minimized to almost nil and the improvement in body weight was observed indicating prevention of muscle wasting due to hyperglycemic condition, similar results were reported by Prakasam *et al.* (2003) in case of *Casearia esculenta* root extract. Also after treatment with AEMD, urine sugar was nil and in trace (1+) amounts, respectively in aqueous extract and glibenclamide treated groups while in the untreated animals it was 3+.

HbA1c is found to increase in patients with diabetes mellitus and the amount of increase is directly proportional to the fasting blood glucose level (Koenig *et al.*, 1976). During diabetes, the excess glucose present in the blood reacts with hemoglobin to form HbA1c (Al-Yassin and Ibrahim, 1981). HbA1c is used as a marker for estimating the degree of protein glycation in diabetes. HbA1c alters the structure and function of antioxidant enzymes such that they are unable to detoxify free radicals, exacerbating oxidative stress in diabetes (Maritim *et al.*, 2003). A fall in HbA1c is considered as indicator of antidiabetic activity of test drug and it is more reliable diagnostic tool. Administration of AEMD considerably reduced HbA1c by 37.6% (Fig. 4) by virtue of its normoglycaemic activity. This normalization of glycosylated hemoglobin indicates decreased glycation of proteins. These finding again suggests ameliorating role of AEMD in

reducing hyperglycaemia induced oxidative stress. AEMD also increased serum insulin levels by 31.3%, indicating that it enhances insulin release from stressed pancreatic β-cells, either by regenerating the partially destroyed cells or by the stimulating release of insulin stored in the granules which in turn improves glucose tolerance.

There is increase in TC, LDL and VLDL cholesterol and TG while decrease in HDL cholesterol in diabetes, all of which contribute to the coronary artery disease (Arvind *et al*., 2002; Palumbo, 1998). Several studies propose that most of the drugs that decrease total cholesterol also decrease HDL cholesterol. From this point of view, it is encouraging that the AEMD brought down the elevated TC levels after treatment of 21 days (Fig. 5) and also increased the HDL levels which is commonly considered good cholesterol. An increase in triglyceride may be due to the lack of insulin under diabetic condition, while insulin activates the enzyme lipoprotein lipase and hydrolysis TG under normal condition. In the present study, AEMD effectively reduced TG possibly by decreasing the Non-Esterified Fatty Acids (NEFA) in alloxan-induced diabetic rats. Similarly, reduction in NEFA and TG was observed in the arterial wall of untreated diabetes rats (Reinila, 1981).

An increase in total protein, AST, ALT, Alkaline phosphatase and Bilirubin in alloxan-induced diabetic rats is an indication of liver damage (Dame, 1981) reflecting hepatocellular necrosis as they are released into the blood after cell membrane damage (Kim *et al*., 2006) in diabetic animals. Administration of AEMD effectively reduced total protein, AST, ALT, alkaline phosphatase and bilirubin activities in diabetic rats, indicating that the aqueous extract may have hepatoprotective effect.

Administration of AEMD significantly reduced the creatinine and urea levels in diabetic animals, indicating that it more effectively inhibit the incidences of diabetic nephropathy. Diabetic nephropathy is mainly associated with excess urinary albumin excretion, abnormal renal function as represented by an abnormality in serum creatinine and urea (Rao and Nammi, 2006) in diabetic animals.

CONCLUSION

The Aqueous extract of *Momordica dioica* possesses good antidiabetic activity. It increases serum insulin, HDL and decreases glycosylated hemoglobin as well as FBG, PPG, TC, VLDL and LDL level. It effectively reduced total protein, AST, ALT, alkaline phosphatase and bilirubin activities in diabetic rats as compared to control, indicating that the aqueous extract have hepatoprotective effect. Urea, creatinine and urinary sugar levels were reduced exhibiting its nephroprotective effect. The extract is non toxic up to 15 times of ED 50 as per this study indicating high margin of safety. Hence, our study suggests possible use of AEMD in management of diabetes.

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