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Diabetes Induced Oxidative Stress: A Comparative Study on Protective Role of *Momordica charantia* and Metformin

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ABSTRACT

Diabetes mellitus is associated with oxidative stress which plays an important role in development of chronic complications of diabetes. Due to various side effects associated with the drugs used for the management of diabetes, the development of new plant based drugs is the renewed interest of the present time. The new prospective anti-diabetic formulations should possess anti-oxidative potential to resist the oxidative insult of tissues during diabetes and their potential should also be comparable with standard drugs. *Momordica charantia* is extensively used in India, China and other parts of the world as vegetable. The present study was aimed to investigate the effect of *Momordica charantia* (MC) on antioxidant enzymes and lipid peroxidation in liver and kidney of alloxan induced diabetic rats. In a 30 days treatment, rats were divided into four groups (I-IV) of five animals in each, experiments were repeated thrice. Administration of MC (13.33 g pulp/kg body weight/day) extract in diabetic rats has remarkably improved the elevated levels of fasting blood glucose. A significant decrease in lipid peroxidation ($p<0.001$) and increase in the activities of key antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione-s-transferase (GST) and reduced glutathione (GSH) contents in liver and kidney tissues of diabetic rats were observed upon MC treatment. The anti-hyperglycemic and antioxidant properties of MC were comparable with Metformin, a standard hypoglycemic drug.

Keywords: Alloxan, diabetic rat, antioxidant status, lipid peroxidation, *Momordica charantia* and metformin.

INTRODUCTION

Diabetes mellitus (DM) is characterized by abnormal glucose metabolism which is usually associated with elevated blood glucose levels due to insulin deficiency or resistance, diminished glucose utilization in tissues that require insulin for glucose uptake, tissues in which glucose transport is not regulated by insulin (cardiac tissue, blood vessels, peripheral nerves, renal medulla and ocular lens) face severe and sustained hyperglycemia (1). The major complications of diabetes include atherosclerosis, retinopathy, nephropathy and neuropathy etc. Several studies had demonstrated that oxidative stress due to hyperglycemia-induced generation of free radicals, contributes to the development and progression of diabetes and related complications. Oxidative stress describes the condition where the amount of reactive oxygen species (ROS) overpowers the amount of neutralizing agents or antioxidants (2,3,4). Hyperglycemia can induce oxidative stress through advanced glycation end product (AGEs) formation, increased flux through the polyol pathway, increased activation of protein kinase C (PKC) and increased flux through hexosamine pathway (5).

Our body has variety of antioxidants to counteract the damaging effects of ROS. Enzymes such as superoxide dismutase (SOD) converts superoxide to hydrogen peroxide (H_2O_2) which is then transformed into water by catalase in lysosomes or by glutathione peroxidase (GPx) in mitochondria (6). The diabetes altered activities

of these enzymes and reduced level of GSH have been observed which affect the ability to defend against oxidative stress (7).

Degenerative changes occur in heart, kidney, brain and other neural tissues during diabetes. These include cardiomyopathy, nephropathy and neuropathy which are common complications of diabetes mellitus. This pathogenicity is believed to be due to oxidative damage of the tissues by oxygen free radicals. Several reports are available about the changes in antioxidant enzymes levels in various tissues (liver, kidney and heart) of diabetic rats (8). Several natural products such as *Momordica charantia*, *Azadirachta indica*, *Gymnema sylvestre*, *Pterocarpus marsupim*, *Coccinia indica*, *Trigonella foenum graecum, Allium sativum, Eugenia jambolana* and *Ocimum sanctum* etc are being used in India, China and other parts of the world for the management of diabetes and to overcome its complications. These plants are found to be effective and their low cost and minimal side effects have increased the interest of scientists to develop plant based drugs for managing diabetes. *Momordica charantia* (MC) is reported to have other beneficial effects such as antibacterial activity, inhibitory effects against insect larvae causing filaria, protection in gastric ulcer etc.(9,10,11). The present study has been planed on MC to evaluate its anti-hyperglycemic and anti-oxidative effects. These were compared with standard anti-hyperglycemic compound metformin to evaluate the potential of MC extract to be developed as anti-diabetic agent.

MATERIAL AND METHODS

Plant material and preparation of extract

MC fruits were purchased from a local vegetable market in Lucknow. Fresh fruits (250 g) were taken and the seeds were removed. The fleshy parts were cut into small pieces and macerated with 250 ml TDW using electrical blander. This suspension was squeezed through a sterile muslin cloth and the filtrate was centrifuged at 5000 rpm for 30 minutes at 4°C. The supernatant was lyophilized at low temperature and reduced pressure by the method of Karunanayaka et al.(12) using Christ alpha 1-4 lyophilizer, Germany.

Animals and treatments

Male albino wistar rats weighing 150 to 200g were purchased from Central Drug Research Institute (CDRI), Lucknow, India, for study and housed at 25±5°C in the animal room of the department. They were provided a standard pellet diet (Hindustan Lever Ltd, Mumbai, India) and had free access to water. Prior permission for animal use and approval of the protocol were obtained from the Institutional Animal Ethical Committee. Rats were divided into four groups:

Group I	Normal control
Group II	Diabetic control
Group III	Diabetic + MC (13.33 g pulp/kg body weight/day)
Group IV	Diabetic + metformin (100 mg/kg body weight/day)

The body weight was measured on the first (after the diabetes was confirmed) and 30th day of the experiment. The MC extract and metformin were given orally by gastric intubation to the rats of groups III and IV, respectively, once daily for 30 days. Animals of groups I and II received the same amount of normal saline. After 30 days of treatment, rats were fasted overnight and sacrificed by cervical dislocation causing minimal pain. Liver and kidney tissues were collected separately and stored at -20°C.

Induction of diabetes

Diabetes in rats was induced with a single injection of alloxan monohydrate (150 mg/kg body weight), dissolved in sterile 0.15 M normal saline, by intraperitoneal route (13). Blood samples to measure FBG were obtained by tail vain puncture of all groups of rats, glucose levels were determined on different days using a glucometer (One touch ultra blood glucose monitoring system from Lifescan, Johonson and Johonson Company). After fifth day the development of diabetes was confirmed, rats with FBG range 250-300 mg/dl were considered as diabetic rats and included in the study.

Chemicals

Metformin, Alloxan monohydrate, 5,5'-Dithio-bis 2-nitrobenzeoic acid (DTNB), Epinephrine, glacial metaphosphoric acid and Glutathione were purchased from Sigma chemical company Inc., St Louis, Mo, USA. All other chemicals used were of analytical grade and obtained from SRL (India), Qualigens fine chemicals (India).

Preparation of homogenate

Liver and kidney tissues were washed thoroughly with ice cold saline, separately. 10% (w/v) homogenate of each was prepared in a Potter Elvehjem type homogenizer in ice-cold 50mM phosphate buffer pH 7.4 containing mammalian protease inhibitor cocktail. The homogenate was centrifuged at 10,000xg for 30 min at 4°C. The supernatant was used to assay the antioxidant activities/ levels and lipid peroxidation.

Estimation of lipid peroxidation

Lipid peroxidation was estimated in terms of MDA formed, according to the method of Ohkawa, et al. (14) using thiobarbituric acid (TBA) reagent. The reference used was 1, 1, 3, 3 tetraethoxypropane (TEP).

Estimation of antioxidant enzymes

The activity of catalase was determined by the method of Sinha (1971) (15). The activity was expressed as μmoles H_2O_2 decomposed/min/mg protein. Superoxide dismutase (SOD) activity was assayed by the method of Misra and Fridovich, 1972 (16). One unit of enzyme activity has been defined to cause 50% inhibition of auto-oxidation of epinephrine by 1.0 ml of homogenate.

GST was determined using 1-chloro-2, 4-dinitrobenzene (CDNB) as a substrate. The activity of GST is expressed as μmoles of GSH-CDNB conjugate formed/min/mg protein (Habig *et al*, 1974) (17).

GSH content

GSH content was estimated by the method of Chandra et al, 2002 (18) by using 5,5 dithiobis (2- nitrobenzoic acid); DTNB which gives yellow colour with compounds containing –SH groups.

Protein estimation

Protein was estimated by the method of Lowry et al.(19) using bovine serum albumin as standard, at 660 nm.

Statistical analysis

The results are expressed as mean ± SD (n=5). The data were subjected to one way Analysis-of-Variance (ANOVA) followed by Newman-Keuls Multiple Comparison Test for comparison between groups and values having $p<0.05$ were considered as significant.

Table 1: Effect of *Momordica charantia* and metformin on body weight of normal and alloxan induced diabetic rats.

Groups	Body weight (grams)	
	(0 day)	(30th day)
I	178.2±3.48	200.4±3.32
II	180.2±5.70	145.4±3.72*
III	177.6±3.72	197.0±3.74*
IV	185.2±4.06	195.4±3.66*

All values are expressed as mean ± SD, (n =5).
Group II is compared with Group 1 and group III and IV are compared with Group II,
** P<0.001,*
Group I = Normal control,
Group II = Diabetic control,
Group III = Diabetic + Momordica charantia, Group IV = Diabetic + metformin

RESULTS

All the rats in, all the four groups, chosen in the present study were having almost same body weight. Rats in group I (normal) showed normal growth as it was observed by increase in body weight during the period of study. Group II (diabetic) showed a significant decrease in body weight which was improved in groups treated with MC and metformin (Table 1).

An intraperitonial dose (150 mg/kg body weight) of alloxan resulted in increase in fasting blood glucose level from 91.20 ± 3.03 mg/dl to 287.6 ± 4.82 mg/dl after 96 hours of alloxan injection (group II), this high level of FBG was maintained in group II (diabetic) throughout the period of study, treatment of diabetic rats with MC extract (group III) and metformin (group IV) showed a time dependent decrease in FBG levels. (Table 2)

The TBARS level were significantly increased ($p<0.001$) in liver and kidney tissues of diabetic rats (group II) when compared with normal rats (group I). Treatment of diabetic rats with MC (group III) resulted in significant decrease ($p<0.001$) in TBARS levels. Similarly, metformin treatment to the diabetic rats (group IV) resulted in significant decrease ($p<0.001$) in TBARS levels. (Fig 1,2)

Table 2: Effect of *Momordica charantia* and metformin on fasting blood glucose levels in normal and alloxan induced diabetic rats.

Groups	Fasting blood glucose levels (mg/dl)				
	0 day	7th day	14th day	21st day	30th day
I	91.20±3.03	98.00±2.73	99.89±1.3	101.6±3.91	99.8±3.11
II	287.6±4.82*	289.6±3.5*	286.4±5.32*	282.2±5.8*	281.8±5.49*
III	287.8±4.08	238.6±4.09*	185.6±2.6*	143.0±5.32*	116.8±2.38*
IV	284.6±1.14	230.6±2.7*	168.8±6.41*	135.0±5.24*	107.0±4.74*

All values are expressed as mean ± SD, (n =5).
Group II is compared with Group I and group III and IV are compared with Group II,
** P<0.001,*
Group I = Normal control, Group II = Diabetic control,
Group III = Diabetic + Momordica charantia, Group IV = Diabetic + metformin

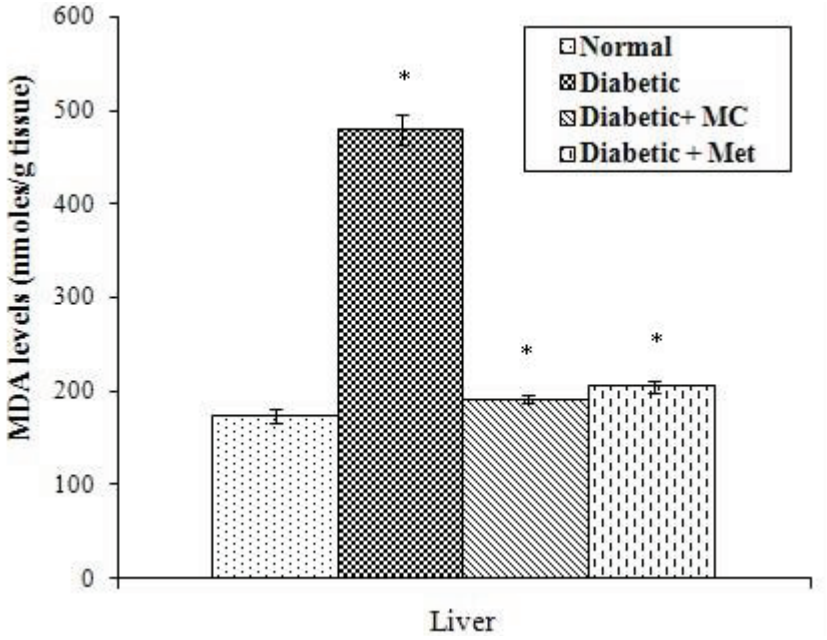

Figure 1: Effect of *Momordica charantia* and metformin on MDA levels in liver of diabetic rats

Each values is a mean ± SD (n=5 in each group). *Values are statistically significant at p<0.001. Diabetic control (group II) are compared with normal control (group I). Diabetic + MC treated (group III) and diabetic + metformin treated (groups IV) were compared with diabetic control (group II). The experiments were repeated thrice. MC = *Momordica charantia,* Met = Metformin, MDA = Malonaldehyde.

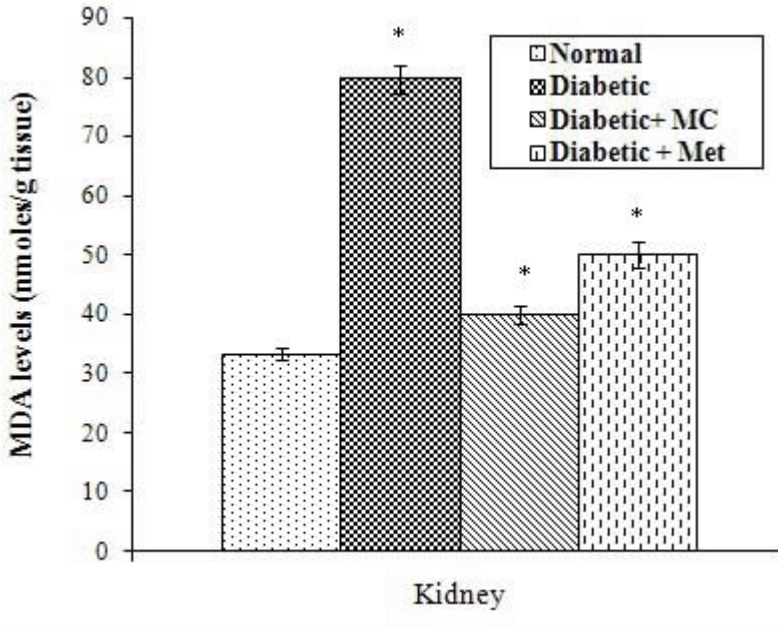

Figure 2: Effect of *Momordica charantia* and metformin on MDA levels in kidney of diabetic rats

Each values is a mean ± SD (n=5 in each group). *Values are statistically significant at p<0.001. Diabetic control (group II) are compared with normal control (group I). Diabetic + MC treated (group III) and diabetic + metformin treated (groups IV) were compared with diabetic control (group II). The experiments were repeated thrice. MC = *Momordica charantia,* Met = Metformin., MDA = Malonaldehyde

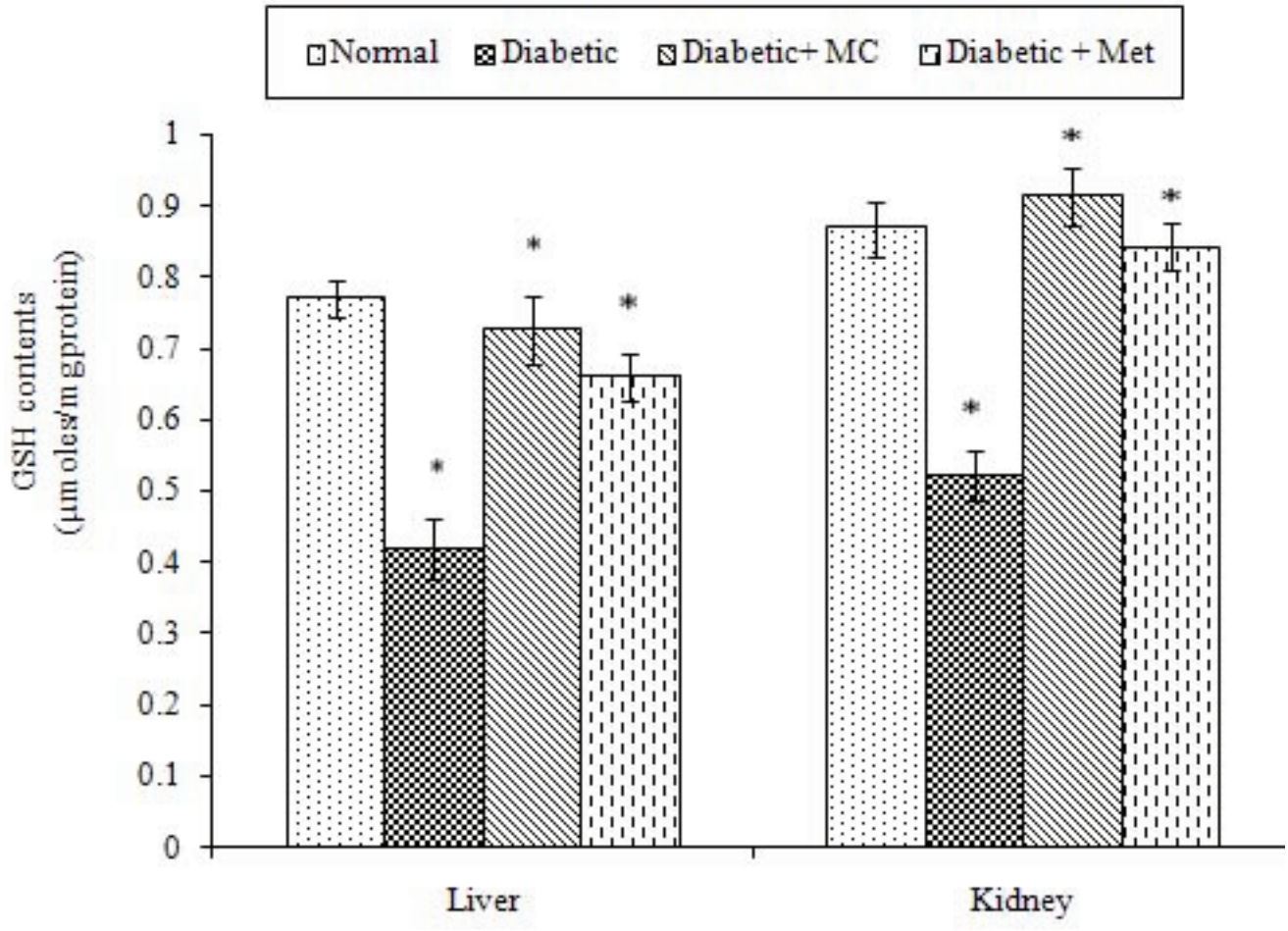

Figure 3: Effect of *Momordica charantia* and metformin on GSH contents in liver and kidney of diabetic rats
Each values is a mean ± SD (n=5 in each group). *Values are statistically significant at p<0.001. Diabetic control (group II) are compared with normal control (group I). Diabetic + MC treated (group III) and diabetic + metformin treated (groups IV) were compared with diabetic control (group II). The experiments were repeated thrice. MC = *Momordica charantia,* Met = Metformin., GSH = Reduced glutathione

The GSH contents were significantly decreased (p<0.001) in liver and kidney tissues of diabetic rats (group II) when compared with normal rats (group I). Treatment of diabetic rats with MC (group III) resulted in significant increase (p<0.001) in GSH content when compared with normal rats. Similarly, metformin treatment to the diabetic rats (group IV) resulted in significant increase (p<0.001) in GSH contents (Fig. 3)

The activities of antioxidants (SOD, CAT and GST) were significantly decreased (p<0.001) in liver and kidney tissues of diabetic rats (group II) when compared with normal rats (group I). Treatment of diabetic rats with MC (group III) resulted in significant increase (p<0.001) in antioxidants activities. Similarly, metformin treatment to the diabetic rats (group IV) resulted in significant increase (p<0.001) in antioxidants activities when compared with diabetic rats (group II).

DISCUSSION

Diabetes is known to cause oxidative stress that has been implicated in the progression of pathogenesis of the cardiovascular complications, retinopathy, neuropathy and other complications accordingly antioxidants were considered as promising therapeutic strategy (20). A large number of anti-diabetic pharmaceuticals are available in the market, there is a latest trend in researchers to look for remedies from plant sources because of their lesser side effects and low cost. In the present study, anti-diabetic and antioxidant potentials of aqueous extract of *Momordica charantia* has been evaluated in liver and kidney tissues in alloxan induced diabetic rats and compared with the effects of metformin treatment.

A dose of alloxan 150 mg/kg body weight was chosen in this study is in accordance with other reports and non

lethal(21). At this alloxan dose pancreatic β cells are so damaged that insulin is secreted in insufficient amounts to regulate blood glucose levels, resulting in significant increase in fasting blood glucose levels. The suggested mechanism of action of alloxan is generation of oxidative stress in pancreatic tissues (21). Treatment of diabetic rats with aqueous extract of MC (13.33 g pulp/ kg body weight/ day) for 30 days after establishment of hyperglycemia resulted in significant reduction of FBG levels. The decrease in FBG levels were comparable to metformin's effect. Although the conclusive experiments are required but we propose that anti-hyperglycemic effect of MC might be due to enhanced peripheral glucose utilization or these plant extracts potentiate the insulin effect by rejuvenation of damaged pancreatic β cell. Metformin (a biguanide derivative) is considered as an anti-hyperglycemic rather than a hypoglycemic agent which makes the metformin a drug of choice. Various mechanisms have been proposed to account for anti-hyperglycemic action of metformin like suppression of basal hepatic glucose production, increase peripheral glucose up take, and increase in non-oxidative glucose metabolism (22,23,24). However, the major concern with the biguanide therapy has been the risk of lactic acidosis and gastrointestinal disturbances like abdominal discomfort and diarrhea (25). Biguanides are contradicted in the patients with renal impairment, hepatic disfunction and cardiac failure.

Hypoinsulinemia due to alloxan induced diabetes leads to several biochemical alterations including lipid peroxidation (26). Increased lipid peroxidation impairs membrane functions, its product are harmful to most of the cells in the body and associated with a variety of diseases (27). Our present study showed a significant ($p<0.001$) increase in TBARS content in liver and kidney tissues of diabetic rats suggesting that peroxidative injury may be involved in tissues due to diabetes. The aqueous extract of MC could significantly lower the elevated tissue lipid peroxidation products levels (fig. 1,2) suggesting that that aqueous extract of MC may contain potent inhibitory molecule (s) to protect tissues from oxidative damage. The use of metformin has also resulted in similar effect which was in accordance with previous report (28).

Reduced glutathione (GSH) is known to protect the cellular system against the toxic effects of lipid peroxidation (29). GSH functions as direct free radical scavenger, as a co-substrate for glutathione peroxidase (GPx) activity and as a cofactor for many enzymes and forms conjugates in endo and xenobiotic reactions (30). Decreased GSH contents were estimated in liver and kidney tissues because of hyperglycemia induced oxidative stress as demonstrated by increased lipid peroxidation. Decreased GSH content was significantly recovered in diabetic rats who were fed with MC extract. These findings are in accordance with previous report where treatment of diabetic rats with MC extract was found to restore the GSH content in RBC (31).

Reduced Activities of SOD, CAT and GST in the tissues of diabetic rats have been observed in our study. The decrease in activities of SOD and CAT in diabetic rats may be due to increased production of reactive oxygen radicals that can themselves reduce the activities of these enzymes (32). SOD is an important defense enzyme which converts superoxide to H_2O_2 (33). CAT is hemeprotein, which decomposes H_2O_2 and protects the tissues from highly reactive OH^- (34) The reduction of these enzymes in these tissues may lead to number of deleterious effects. Administration of MC and metformin restores the activities of these enzymes and may help to avoid the deleterious effects of free radicals generated during diabetes.

CONCLUSION

In conclusion, the present study demonstrates that *Momordica charantia* and metformin exhibit antiperoxidative and antioxidant activities in liver and kidney tissues of alloxan induced diabetic rats by decreasing the levels of lipid peroxidation products and increasing the levels/activities of antioxidants. Other plants such as *Trigonella foenum graecum*, *Eugenia jambolana* are reported to exert their anti-hyperglycemic effect due to presence of relatively high alkaloid content (35,36). The anti-oxidative potential of *Eugenia jambolana* , *Albizzia lebbeck* and other plants is reported and which is mainly attributed by high amount of flavanoids present in them. Alkaloids, flavonoids and tannins are reported to be absent or present in negligible amounts in MC (37). This suggests that anti-hyperglycemic and anti-oxidative activities present in MC is due to constituent(s) other than alkaloids, flavonoids and tannins. The anti-hyperglycemic and anti- oxidative activities are comparable to the protective effect offered by the established anti- hyperglycemic compound metformin. As metformin is associated with some deleterious side effects, (26) development of anti-diabetic compounds from MC extract may be proposed. Thus, the role of MC extract in management of diabetes is of paramount importance as this plant extracts may serve various purposes in diabetics- lower the blood glucose levels, delay complications (atherosclerosis, nephropathy, neuropathy and gastroparesis etc). Its anti-infective properties may be an added benefit as diabetic are known to be more susceptible to infections. Further

Table 3: Effect of *Momordica charantia* and metformin on antioxidant activities in liver of normal and alloxan induced diabetic rats

Groups	SOD (U/mg protein)	CAT (U/mg protein)	GST (U/mg protein)
I	61.15±2.44	46.38±1.47	12.51±0.84
II	22.43±1.78*	30.63±1.86*	5.96±0.23*
III	48.47±1.57*	44.83±1.91*	11.34±1.57*
IV	42.14±1.37*	40.89±1.38*	8.08±0.48**

All values are expressed as mean ± SD, (n =5).

Group II is compared with Group 1 and group III and IV are compared with Group II. U (SOD)= 50% inhibition of auto-oxidation of epinephrine/min,U (CAT)= µmoles H_2O_2 decomposed/min, U (GST)= µmoles of GSH-CDNB conjugate formed/min.

** P<0.001,*

*** p<0.01*

Group I = Normal control, Group II = Diabetic control,

Group III = Diabetic + Momordica charantia, Group IV = Diabetic + metformin

Table 4: Effect of *Momordica charantia* and metformin on antioxidant activities in kidney of alloxan induced diabetic rats

Groups	SOD (U/mg protein)	CAT (U/mg protein)	GST (U/mg protein)
I	29.75±1.12	40.06±0.87	16.12±1.80
II	17.75±1.51*	24.29±1.79*	9.27±0.56*
III	25.02±1.94*	37.39±1.01*	15.53±1.37*
IV	22.29±1.44*	33.78±2.45*	13.54±0.72*

All values are expressed as mean ± SD, (n =5)

Group II is compared with Group 1 and group III and IV are compared with Group II. U (SOD)= 50% inhibition of auto-oxidation of epinephrine/min,U (CAT)= µmoles H_2O_2 decomposed/min, U (GST)= µmoles of GSH-CDNB conjugate formed/min.

** P<0.001*

Group I = Normal control, Group II = Diabetic control,

Group III = Diabetic + Momordica charantia, Group IV = Diabetic + metformin

investigations are necessary to find out the active components (s) present in the plant extract of MC and their mechanism of action and to establish their therapeutic potential in the treatment of diabetes and related complications.

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