Pharmacognosy Magazine
ISSN: 0973-1296

PHCOG MAG.
An official Publication of Phcog.Net

Phcog Mag.: Research Article
Antidiabetic mechanisms of Saponins of *Momordica cymbalaria*

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ABSTRACT

Antidiabetic properties of *Momordica cymbalaria* is reported but not the mechanism of action. In the present study an attempt is made to study the possible mechanism of action. Saponins were separated from the Ethanolic extract, HPTLC fingerprinting was taken and used for the study. Diabetes was induced in 16 h fasted male Wistar rats by intraperitoneal injection of STZ (65 mg/kg).After 31 days treatment serum was analyzed for glucose, triglycerides, cholesterol, HDL-cholesterol, Insulin. The liver was analyzed for glycogen, mevalonate, HMG CoA, LPO, GSH, CAT and SOD. A portion of the pancreas was subjected to histopathology studies. In another batch of study Hyperinsulinemia was induced by feeding fructose rich diet for 15 days. After 15 days treatment serum was analyzed for glucose, triglycerides, cholesterol, and HDL-cholesterol; and Insulin. The liver was analyzed for HMG CoA and mevalonate. Alpha adrenergic antagonistic activity was studied on isolated rat's aortic strip and on anconccygeous muscle. The concentration of the drug required to bring rightward shift of NA concentration curve with suppression of maxima was observed. Diabetic rats treated with saponins fractions of MC showed significant decrease in serum glucose, cholesterol, triglyceride level where as significant increase in serum insulin and liver glycogen level. HMG CoA reductase activity also showed significant increase. They also exhibited significant reduction in lipid peroxidation or MDA content in the heart and significantly increased the reduced glutathione content, catalase activity and SOD activity in the liver as compared to Streptozotocin control group Histopathological sections of the pancreatic islets in MC treated rats showed increase in the number of pancreatic cell islets which were similar to that of healthy pancreatic islets. Treating FRD fed rats with saponins of MC; the serum glucose, Cholesterol, triglyceride level significantly decreased and significantly increased the ratio of HMG CoA vs Mevalonate. Saponins of MC displayed significant rightward shift of NA concentration curve with suppression of maxima on isolated rat's aortic strip and anconccygeous muscle preparation.

The drug may act by reversing the atrophy of the pancreatic islets of β-cells, as a result may increase insulin secretion, increases hepatic glycogen level and attenuates Hyperinsulinemia. α-adrenergic blocking effect might contribute to insulin secretion and sensitizing effect

KEY WORDS: Antidiabetic, *Momordica cymbalaria*, Saponin

INTRODUCTION

Ayurvedic physicians have treated diabetes for thousands of years using a combination of regulated lifestyle and herbal formulations. In recent times, the safety and efficacy of these herbs have been validated by laboratory. A Momordica genus of Cucurbitaceae family has the following species potential for antidiabetic activities *Momordica Charantia, Momordica balsamia, Momordica dioica, Momordica cochinchinesis, Momordica Schimperiana, Momordica subangulata blume, Momordica denudate, Momordica macrophylla gage, Momordica mixata, Momordica monodelpha, Momordica umbellate(*1-2) . *Momordica Charantia* is the most studied species.

Momordica cymbalaria Fenzl (MC) (Cucurbitaceae) is a species found in the states of Karnataka and Andra Pradesh, India. Its tuber is traditionally used as an abortifacient (1). Ethanolic extract is reported to have antiovulatory and abortifacient activity (3) and antiimplantation activity (4). The extracts and the dried form of fruit and leaves were shown to have antidiabetic and hypolipidemic properties (5-7). But the mechanism of antidiabetic and hypolipidemic properties is not elucidated. Hence in the present study an attempt is made to elucidate the possible mechanism of antidiabetic and hypolipidemic action of MC.

MATERIALS AND METHOD

Plant Material

The fresh roots of MC were collected from Gadag district, Karnataka, identified and authenticated by Dr. Sreenath, Department of Botany, Bangalore University; Bangalore. A specimen sample of the same was preserved in the herbarium of the Department of Botany, Bangalore University, Bangalore (voucher no. 18122003). The roots of MC were isolated, chopped into small pieces, dried under shade at room temperature for seven days and powdered. The powder was extracted with ethyl alcohol to get a yield of 14.1 % w/w. Ethanolic extract of MC was dissolved in hot distilled water and partitioned between water saturated n-butanol and water layer. Organic layer (n-butanolic layer) was separated and evaporated to get residue. This n-butanolic residue was dissolved in methanol and poured in diethyl ether (Et_2O) to obtain flocculent precipitate. This precipitate was separated by means of filter paper and washed with excess of Et_2O and dried to yield crude fraction of saponins (8). The phytochemical test indicates the presence of saponins-steroidal glycosides. The saponin mixture dissolved in distilled water was used for the study.

Experimental animals

Male albino Wistar rats weighing 100-120 g were purchased from NIMHANS (National Institute of Mental Health and Neurosciences) Bangalore. The animals were housed in polypropylene cages maintained in controlled temperature (27 ± 2°C) and light cycle (12h light and 12 h dark) and fed with standard rat pellet diet (Amrut rat and mice feed, India) and water *ad libitum*. The animals were given a week's time to get acclimatized with the laboratory conditions. All the experimental procedures were performed according to the committee for the purpose of control and supervision of experiments on animals (CPCSEA) and approved by the Institutional Animal Ethics Committee (IAEC). The oral acute toxicity study was performed using the up and down procedure (OPPTS guidelines) (6).

Experimental Procedure

Antidiabetic activity in streptozotocin induced Type 1 diabetes

Streptozotocin (STZ) was dissolved in 0.01 M cold sodium citrate buffer (pH 4.5) immediately before use. Diabetes was induced in 16 h fasted male Wistar rats (100-120g) by intraperitoneal injection of STZ (65 mg/kg). The rats were then given 5% w/v glucose solution in feeding bottles for the next 24 h to prevent hypoglycemia. After 72 h, rats with marked hyperglycemic fasting blood glucose > 200 mg/dL were selected and used for the study. All the animals were allowed free access to tap water and pellet diet and maintained at room temperature in polyethylene cages. The rats were divided into five groups consisting of six rats each.

Group 1: Vehicle (Sodium Citrate Buffer- Control).

Group 2: Streptozotocin (65mg/kg i.p) - STZ diabetic control

Group 3: Diabetic rats treated with reference standard, Insulin (6U/kg, s.c/day) for 30 days

Group 4: Diabetic rats treated with saponin fractions of MC (87.5mg/kg p.o/day/30days)

Group 5: Diabetic rats treated with saponin fractions of MC (175mg/kg p.o/day/30days)

Blood was collected on the 31st day by puncturing retro orbital plexus and serum was analyzed for glucose, triglycerides, cholesterol, HDL-cholesterol, Insulin. The liver was analyzed for glycogen, mevalonate, HMG CoA, LPO, GSH, CAT and SOD. A portion of the pancreas was fixed in formalin (10%) and subjected to histopathology studies.

Insulin sensitizing activity in fructose rich diet (FRD) induced Hyperinsulinemia

Male Wistar rats were randomly divided into four groups.

Group 1: Rats were fed with standard rat chow (Control).

Group 2: Rats received fructose rich diet (FRD control).

Group 3: FRD + Saponin fractions of MC (87.5mg/kg p.o/day / 15days)

Group 4: FRD + Saponin fractions of MC (175mg/kg p.o/day/15days)

Group 5: FRD + Metformin (200mg/kg p.o/day/15days)

The FRD was prepared by using 66% fructose, 12% fat, & 22% protein (casein) (9). After 15 days of the start of the FRD, saponin of MC was administered orally on a fixed time interval daily for remaining 15 days. Blood was collected on the 31st day by retro orbital bleeding and serum was analyzed for glucose, triglycerides, cholesterol, and HDL-cholesterol; and Insulin. The liver was analyzed for HMG CoA and mevalonate.

Effect on isolated rat's aortic strip and anconccygeous muscle

In vitro effect of MC on rat's aortic strip (10)

Rats were sacrificed by cervical dislocation; the thoracic aorta was isolated and cleaned from extraneous tissues, maintaining the tissue wet in the Krebs's solution. Two helical strips (20mm X 3mm) were cut from the aorta beginning from the end almost proximal to the heart. Vascular strips were then tied

with surgical threads and suspended in a jacketed tissue bath (25ml capacity) containing Krebs's medium gassed with carbogen (5%CO_2 – 95% O_2). The isotonic contractions were measured using an isotonic transducer (UGO Basile 7006), connected to a "Two channel recorder Gemini" (UGO Basile 7070). After at least an hour equilibration period under an optimal tension of 2 g, the experimental procedure was carried out. After equilibrium period cumulative NA concentration response curve was recorded, the first one was discarded and the second one was taken as control. The tissue was allowed to equilibrate with MC for 30 minutes before the generation of the third concentration response curve to NA. The concentration of the agonist was increased 2 or 3 fold at each step, with each addition made only after the response to the previous addition had attained maximal levels and remained steady. Finally the fourth CRR curve to NA was taken after 30 min repeated washings with the Krebs medium to assess for reversibility of the antagonist. NA solution contained 0.05% EDTA in 0.9% NaCl to prevent oxidation.

In vitro effect of MC on rat anconccygeous muscle

Anconccygeous muscle was isolated in a male rat. The ventral band was cut through and each muscle was mounted in a jacketed tissue bath (25ml capacity) containing Krebs's medium gassed with carbogen (5%CO_2 – 95% O_2).The isotonic contractions were measured using an isotonic transducer (UGO Basile 7006), connected to a "Two channel recorder Gemini" (UGO Basile 7070). After at least an hour equilibration period under an optimal tension of 1 g, the experimental procedure was carried out. After equilibrium period cumulative NA concentration response curve was recorded, the first one was discarded and the second one was taken as control. The tissue was allowed to equilibrate with the antagonist for 30 minutes before the generation of the third concentration response curve to NA. The concentration of the agonist was increased 3 fold at each step, with each addition made only after the response to the previous addition had attained maximal levels and remained steady. Finally the fourth CRR curve to NA was taken after a 30 min repeated washings with the Krebs medium to assess for reversibility of MC.

Biochemical procedures

Serum glucose, triglycerides, cholesterol, HDL-cholesterol was analyzed using *Auto span* diagnostic kit. Serum Insulin was measured using The ADVIA Centaur (IRI) and Ready Pack, of Bayer of corporation diagnostic kit. Liver Superoxide Dismutase was estimated using the method developed by Misra and Fridovick (1972) (11); Hepatic Catalase was estimated by the method of Hugo Aebi as given by Colowick et al. (1984) (12); Reduced Glutathione was estimated by the method of Moran et al. (1979) (13); Malanaldehyde formation was estimated by the method of Slater and Sawyer (1971) (14). Liver glycogen was estimated using anthrone reagent method as described by Carroll NV et al., (1952) (15). HMG-CoA reductase activity in liver tissue was estimated indirectly by measuring the ratio of HMG-CoA to mevalonate as described by Venugopal rao and Ramakrishnan (1975) (16).

Pancrea was perfused with chilled saline and heparin sulphate (50U/l) to remove blood and blood clots and fixed in 10%w/v formaldehyde. Sections of paraffin blocks were prepared and stained with eosin.

HPTLC fingerprinting

HPTLC fingerprinting of saponins of MC was performed. Silica gel 60F254 (Merck) was used as a stationary phase. Chloroform: Glacial acetic acid (9.5:0.5) was used as a mobile phase. The dried plate was scanned to visualize the migrated components under UV radiation at 254 nm, 336 and 540 nm using Reprostar 3 with a digital camera (CAMAG).

Statistical Analysis

The results of the above estimations have been indicated in terms of ± SEM. Difference between the groups was statistically determined by analysis of variance (ANOVA) with Tukey-Kramer post comparison test using Graph Pad InStat version 3.00, Graph Pad Software, California USA. The levels of significance was set at $p<0.05$

RESULTS

Acute toxicity test

Mortality in the acute toxicity test of saponins of MC was seen in the limit test at the dose of 5000 mg/kg. Mortality was not seen in the main test up to dose of 1750 mg/kg and hence 1/10th and 1/20th of 1750mg/kg (87.5 & 175 mg/kg) were selected for the study.

Effect on streptozotocin induced Type 1 diabetes

Diabetic rats treated with saponins fractions of MC (87.5&175mg/kg, p.o/day/30days) showed significant ($p<0.01$, $p<0.001$), decrease in serum glucose, cholesterol, triglyceride level where as significant ($p<0.001$) increase in serum insulin and liver glycogen level. HMG CoA reductase activity also showed significant increase ($p<0.01$) when compared to diabetic control rats (Table 1). Treatment with saponins of MC (87.5 and 175mg/kg p.o/day/30days) in

STZ rats significantly ($p<0.05$) reduced lipid peroxidation or MDA content in the liver and significantly($p<0.05$) increased the reduced glutathione content, catalase activity and SOD activity in the liver as compared to Streptozotocin control group (Table 3)

Histopathological sections of the pancreatic islets of diabetic rats showed irregularly shaped, small and scanty islets. Severe vacuolation and degranulation were present in the β-cells of a number of islets (Fig 1). Histopathological sections of the pancreatic islets in MC saponins (175mg/kg p.o/day/30days) treated rats showed increase in the number of pancreatic cell islets (Fig 1) which were similar to that of healthy pancreatic islets.

Insulin sensitizing activity of MC on fructose rich diet (FRD) induced Hyperinsulinemia

Feeding FRD for 15 days significantly ($p<0.001$) increased the serum insulin level when compared to normal diet fed control rats. FRD rats treated with saponins of MC (175mg/kg p.o./day/15days) significantly ($p<0.001$) decreased the insulin levels when compared to FRD control rats.

Treating the FRD fed rats with saponins of MC (87.5 and 175 mg/kg p.o/day/15days), the serum glucose, Cholesterol, triglyceride level significantly ($p<0.05$ & $p<0.001$ respectively) decreased when compared to FRD control rats and significantly ($p<0.01$) increased the ratio of HMG CoA vs Mevalonate when compared to FRD control rats (Table 2).

Effect on isolated rat's aortic strip and anconccygeous muscle

Saponins of MC (1µg /ml, 3µg /ml and 10µg /ml) displayed significant ($p<0.05$, $p<0.01$, $p<0.01$ respectively) rightward shift of NA concentration curve with suppression of maxima. The effect was not reversed after 30 minute of washing period (Fig No 3a). Saponins fractions of MC (10µg/ml, 20µg/ml and 40µg/ml) displayed significant ($p<0.05$, $p<0.01$, $p<0.01$ respectively) rightward shift of NA concentration curve with suppression of maxima. The effect was not reversed after 30 minute of washing period (Fig No 3b).

DISCUSSION

The mechanisms of both allopathic medicines and the traditional herbal medicines to lower blood glucose are (17), 1. To stimulate beta -cell of pancreatic islet to release insulin; 2, to resist the hormones which rise blood glucose; 3, to increase the sensitivity of insulin receptor site to insulin; 4, to decrease the leading-out of glycogen; 5, to enhance the use of glucose in the tissue and organ; 6, To clear away free radicals, resist lipid peroxidation and 7, correct the metabolic disorder of lipid and protein;

Streptozotocin is well known for its selective pancreatic islet of ß-cell cytotoxicity and has been extensively used to induce diabetes mellitus in animals. It interferes with cellular metabolic oxidative mechanisms (18).

MC increased serum insulin level significantly in overnight fasted diabetic rats. This observation indicates that MC enhances insulin release from destroyed pancreatic ß-cells, either by regenerating the partially destroyed pancreatic beta cells or by the release of insulin stored in the granules. In order to know whether the increase in serum insulin level after administration of saponins of MC was due to the release of insulin stored in the granules or due to direct effect on the pancreatic islets of ß-cells, histopathology of the pancreas was studied. Histopathological sections of the pancreatic islets of diabetic rats showed irregularly shaped, small and scanty islets with severe vacuolation and degranulation in the ß-cells of a number of islets. Histopathological sections of the pancreatic islets in saponins of MC treated rats showed increase in the number of pancreatic cell islets which were similar to that of healthy pancreatic islets and reversed the atrophy of the pancreatic islets of ß-cells. The regeneration of the ß-cells of the STZ-destructed islets is probably due to the fact that pancreas contains stable (Quiescent) cells which have the capacity of regeneration. Therefore, the surviving cells can proliferate to replace the lost cells (19). *Gynmnema Sylvestre* also increases insulin secretion probably by regeneration of pancreatic beta cells (20). Many other plants like *Teucrium polium(*21,22), *Aegle marmelos(*23) *Ephedra sinica Stapf., and Ephedra distachya L(*24), *Prunella vulgaris L.*(25) are reported to regenerate atrophied pancreatic islets, restore the secretion of insulin, and thus correct hyperglycemia. *Momordica Charantia* fruit is reported to have insulin secretagogue and insulinomimetic activity (26). *Trigonella foenum-graecum* and *Allium sativum* L (27) are reported to act by stimulating insulin secretion. One of the main constituent of Momordica charantia is a steroidal saponins charantin, and is responsible for the fruit's anti-diabetic effects; it also contains Momordicine and insulin-like steroidal saponins(28) .

Insulin resistance is a common phenomenon of the patient with type 2-diabetes and it usually antedates

Table 1:Effect of saponin fractions of MC (87.5 &175 mg/kg, p.o,/daily) in Streptozotocin treated rats on serum Glucose, Insulin, Cholesterol, Triglyceride, and hepatic glycogen, HMG CoA activity after 30 days treatment.

Groups	Treatment	glucose (mg/dl)	TG (mg/dl)	Chole sterol (mg/dl)	HDL (mg/dl)	Liver glycogen (mg/ 100gm tissue)	HMG CoA activity*	Serum Insulin (µu/ml)
G1	Sodium citrate buffer (Control)	83.8 ±1.984	64.33 ±1.22	55.68 ±1.80	42.1 ±1.49	1330.33 ±5.17	6.91 ±0.09	20.41 ± 0.19
G2	STZ-Control (65mg/kg i.p.single dose)	274.08± 7.27†††	88.11± 2.47†††	128.58± 2.85†††	30.36± 1.46††	779.0± 8.11†††	3.9± 0.09†††	6.91 ± 0.23†††
G3	STZ+ Saponin fraction of MC(87.5mg/kg)	172.1 ±2.33**	76.53 ±2.37	126.4 ±1.71	43.34 ±2.24	990.83 ±4.84***	3.95 ±0.15	8.98 ± 0.29*
G4	STZ+ Saponin fraction of MC (175mg/kg)	131.63 ±1.09***	66.8 ±1.35***	73.18 ±1.84***	55.98 ±3.15***	1115.0 ±17.87***	5.71 ±0.10**	13.45 ±0.12***
G5	STZ+ insulin (6 I.U)	122.13 ±1.23***	69.5 ±0.67***	67.0 ±1.17***	55.96 ±1.69***	1074.66 ±5.60***	5.85 ±0.14***	14.91 ± 0.26***

Values expressed as mean ±SEM for six animals
$^{\dagger\dagger\dagger}p<0.001$, $^{\dagger\dagger}p<0.01$, $^{\dagger}p<0.05$ when compared to normal control group.
****p<0.001, **p<0.01, *p<0.05 when compared to STZ control group*
**expressed as HMG CoA vs Mevalonate ratio*

Table 2 : Effect of saponins of MC in fasting Plasma Triglyceride, Cholesterol, Glucose, Insulin and HMG CoA reductase on rats fed with Fructose Rich Diet (FRD) after 15 days treatment

Groups	Treatment	glucose (mg/dl)	TGS (mg/dl)	Cholesterol (mg/dl)	Insulin (mU/l)	HMG CoA reductase*
G1	Normal Diet (Control)	80.06 ±0.90	64.5 ±1.67	62.56 ±1.06	28.5 ±1.19	3.11 ±0.03
G2	FRD-Control	133.45 ±1.06†††	319.23 ±3.62†††	132.8 ±1.52†††	69.25 ±1.10†††	2.03 ±0.02†††
G3	FRD + Saponins MC(87.5 mg/kg p.o)	125.55 ±1.77*	145.45 ±1.84***	113.75 ±1.48**	63.0 ±0.70	1.98 ±0.02
G4	FRD + Saponins MC(175 mg/kg p.o)	98.23 ±2.99***	84.28 ±1.70***	82.01 ±1.50***	37.75 ±1.93***	2.44 ±0.07**
G5	FRD + Metformin (200 mg/kg p.o)	88.7 ±2.57***	78.78 ±1.78***	72.4 ±2.61***	33.25 ±0.75***	2.99 ±0.02***

Values expressed as mean ±SEM for six animals,
$^{\dagger\dagger\dagger}p<0.001$, $^{\dagger\dagger}p<0.01$, $^{\dagger}p<0.05$ when compared to normal control group.
****p<0.001, **p<0.01, *p<0.05 when compared to FRD control group*
**HMG CoA vs mevalonate*

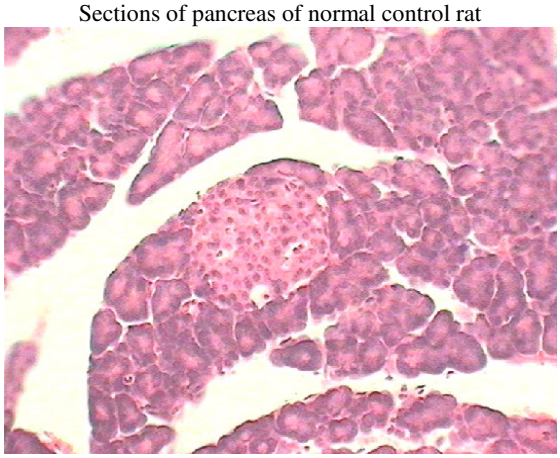
Table 3 : Effect of saponins of MC on hepatic LPO, GSH, CAT and SOD on streptozotocin (STZ) induced diabetic rats

Group	Treatment	LPO ηm of MDA / mg of protein	GSH µg/mg of protein	CAT µm H_2O_2/mg of protein	SOD U/mg of protein
I	Normal control	0.616 ±0.008	5.36 ±0.11	10.43 ±0.16	12.7 ±0.16
II	STZ(65 mg/kg, i.p) - control	0.89 ±0.026*	2.66 ±0.08***	4.17 ±0.102***	8.33 ±0.08***
III	STZ+ Insulin (6U/kg, i.p 30days)	0.74 ±0.005†	4.44 ±0.17†††	8.55 ±0.09†††	10.58 ±0.11†††
IV	STZ +MC (87.5 mg/kg p.o 30days)	0.86† ±0.01	2.59 ±0.09†	6.59††† ±0.12	8.09† ±0.29
V	STZ +MC (175 mg/kg p.o. 30days.)	0.615 ±0.01†††	4.22 ±0.06†††	7.24 ±0.17†††	10.29 ±0.17†††

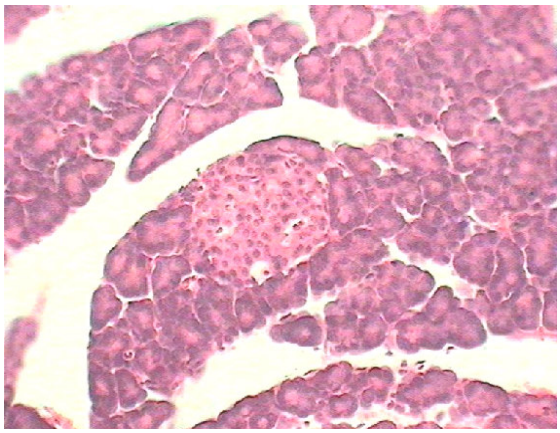
Values expressed as mean ±SEM, n=6,
* p<0.05, *** p<0.001 When compared to normal control group.
† p<0.05, ††† p<0.001 When compared to STZ control group

Figure 1 Pictograms of rat Pancreatic sections showing the effect of MC (500 mg/kg p.o/day/30days) in Streptozotocin (65mg/kg i.p/single dose) treated rats after 30 days of treatment (H & E =100), n=6

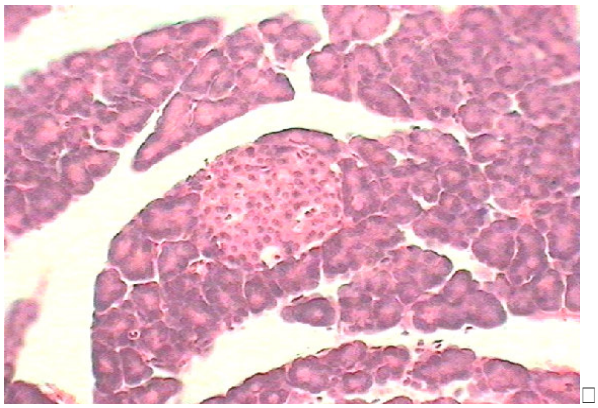
Sections of pancreas of normal control rat



Sections of diabetic control rat



Sections of pancreas of MC (87.5mg/kg) treated diabetic rats



Sections of pancreas of MC (175mg/kg) treated diabetic rats

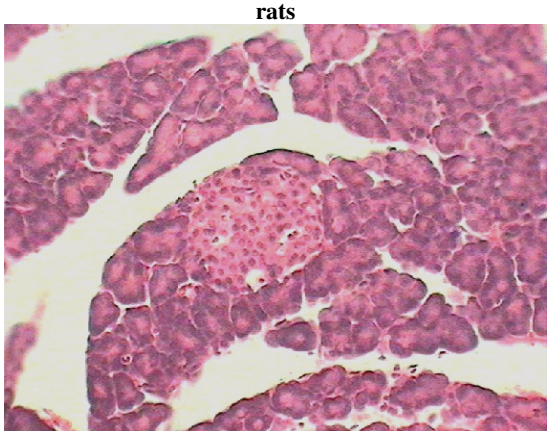
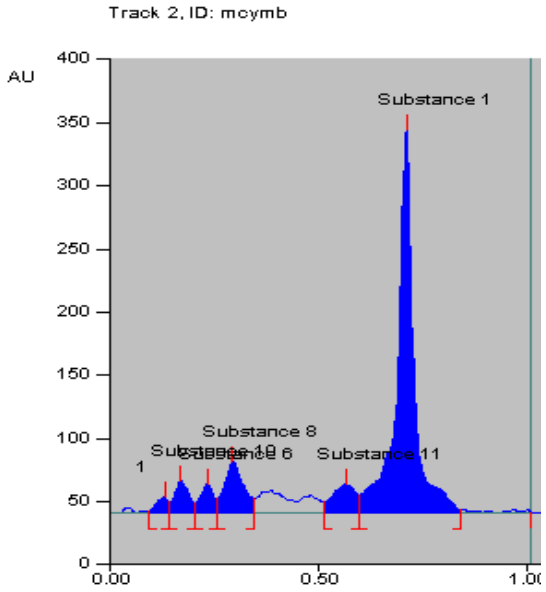


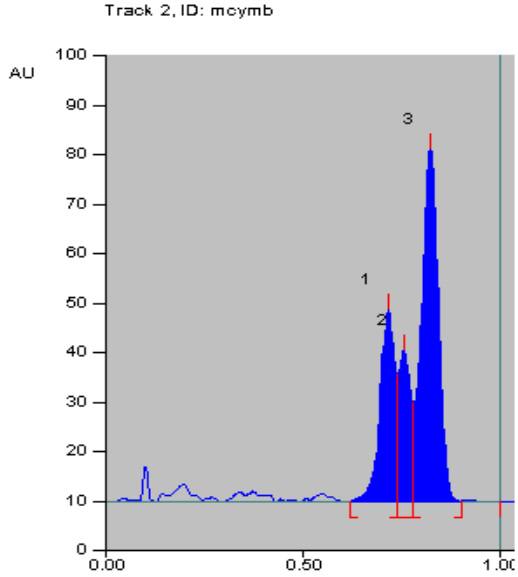
Fig 2. HPTLC Spectra of MC

HPTLC Spectra of Saponins of MC at 254 ηm



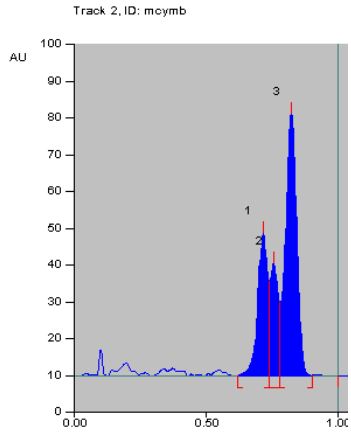
Peak	Maximum Rf	% area
1	0.17	4.34
2	0.23	4.76
3	0.29	10.28
4	0.38	5.91
5	0.45	3.42
6	0.55	6.01
7	0.75	65.21

HPTLC Spectra of Saponins of MC at 336 ηm



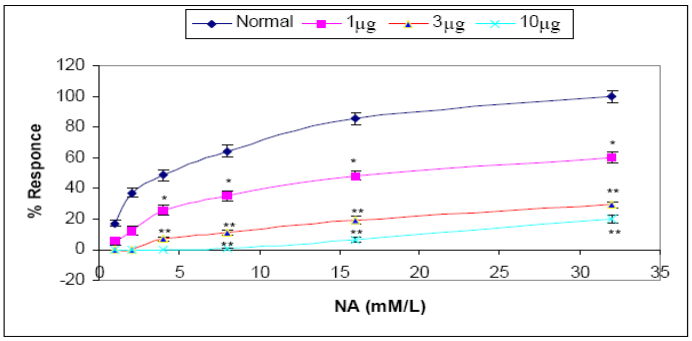
Peak	Maximum Rf	% area
1	0.71	37.53
2	0.75	21.31
3	0.82	40.67

HPTLC Spectral Details of saponins of MC



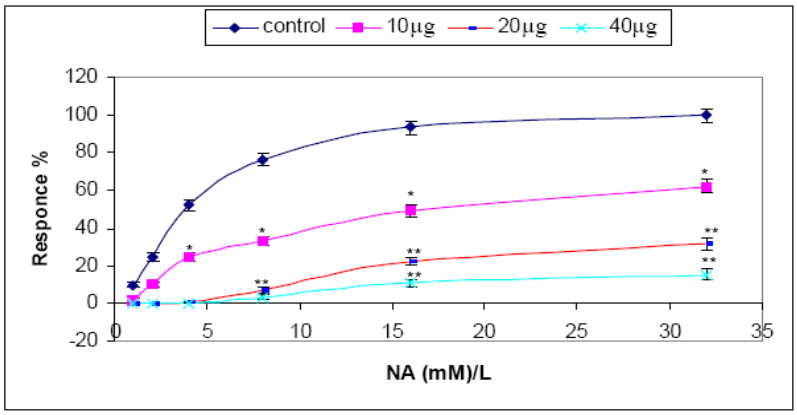
Peak	Maximum Rf	% area
1	0.17	4.34
2	0.23	4.76
3	0.29	10.28
4	0.38	5.91
5	0.45	3.42
6	0.55	6.01
7	0.75	65.21

Figure3a. The concentration response curve of NA on the isolated rat aortic strip in the presence and absence of MC



The concentration *response curve of NA on the isolated rat aortic strip in the presence and absence of Saponins 1µg/ml, 31µg/ml and 101µg/ml; * p< .05,**p<.01 when compared to normal control response.(before administration of saponins)vertical lines represent SEM N=6*

Fig3b. The concentration response curve of NA on the isolated rat anoconccygeous muscle in the presence and absence of MC



*The concentration response curve of NA on the isolated rat anoconccygeous muscle in the presence and absence of Saponin 10µg/ml, 20µg/ml and 40µg/ml; *p< .05,**p<.01 when compared to normal control response.(before administration of Saponin)vertical lines represent SEM N=6*

the onset of the disease. Insulin resistance prevents the target tissues (especially the muscle and liver) from responding to normal circulating concentrations of insulin. High concentration of fructose can result in a relatively unregulated concentration of dihydorxy acetone phosphate (DHAP) and d-glyceraldehyde(29) . The induced production of DHAP and D-glyceraldehyde individually results in uncontrolled synthesis of glucose, acetyl CoA, triglyceride and fatty acids (30) .This stimulated triglyceride synthesis is likely to lead to hepatic accumulation of glycerol, fatty acids and triglycerides that have been shown to reduce insulin sensitivity [31]. Increased fructose may also alter insulin sensitivity partially by altering insulin binding. Thus fructose rich diet results in a state of hyperglycemia, hypertrigylceridemia and increased free fatty acid levels. Hyperglycemia has been implicated in the activation of additional biochemical pathways, which are linked to the development of IR and ß-cell dysfunction (32). MC in the present study significantly attenuated hyperinsulinemia in FRD rats; hence it may act as insulin sensitizers. *Brassica juncea* (Rai), *A. senticosus* significantly prevented the development of insulin resistance in FRD rats(33). Hepatic HMG-CoA reductase is responsible for the majority of the body's regulatable cholesterol synthesis. The expression of this enzyme is affected by cholesterol, insulin, thyroid hormone, bile acids, fasting and re feeding, and also varies diurnally (34). HMG-CoA reductase (HMGR) and mRNA levels are both decreased in diabetic animals, and can be rapidly restored with insulin treatment (35). Saponins fractions of MC showed a significant inhibition of HMG CoA reductase activity. Hence the antihypercholesteremic

effect seen may be due to increased secretion of insulin in diabetic animals and HMG CoA reductase inhibitory activity of MC.

Hepatic glycogen is decreased in diabetes mellitus and this is the most important cause of diabetes ketosis and coma because of impoverishment of liver with respect to glycogen. Increase in glycogen in liver can be brought about by an increase in glycogenesis and/or a decrease in glycogenolysis. Insulin acts on liver cells by stimulating them to take up glucose from the blood and convert it into glycogen and inhibiting glycogenolysis and gluconeogenesis. In the present study a significant decrease in hepatic glycogen level was seen in the streptozotocin induced diabetic rats. Treatment with MC significantly increased the hepatic glycogen level. The increased glycogen level may be due increased level of insulin in the MC treated diabetic rats which has increased glycogenesis and decreased glycogenolysis and gluconeogenesis.

Oxidative stress may play a role in the pathophysiology of diabetes and cardiovascular disease. Mekinova et al. (1995) (36) demonstrated that supplementation of STZ diabetic rats with vitamins C, E, and beta-carotene for 8 weeks produced a significant reduction of TBARS levels, GHS, and GHS-Px, an increase in Cu-SOD, and no change in catalase activity in kidneys. In the present study MC has brought in regenerative changes in the diabetic pancreatic cell islets. The antioxidant effect of MC may have protected the organs from degeneration.

Cherksey et al. (1982) (37) have reported that the adrenergic receptors on rat pancreatic islet cells are of the alpha 2-subtype.A. Kashiwagi et al (1986) (38) report that New alpha 2-adrenergic blocker (DG-5128) improves insulin secretion. Ortiz-Alonso(1991) (39) have reported that, MK-912, a potent new selective [alpha]2-adrenergic receptor antagonist was shown to decrease of fasting plasma glucose, increase of fasting plasma insulin, improve of ß-cell function due to an increase in maximal ß-cell secretory capacity; and increase in basal and stimulated glucagon. Hence insulin secretion seen in the present study may also be due to the alpha adrenergic inhibitory effect of MC.

Hence the probable mechanism of antihyperglycemic action saponins of MC may include, an increase the serum insulin level, a reverse the atrophy of the pancreatic islets of ß-cells, as a result may increase insulin secretion, an increase hepatic glycogen level in the STZ diabetic rats. This may be by increasing glycogenesis and decreasing glycogenolysis and gluconeogenesis. Insulin is reported to increase glycogenesis, and decrease glycogenolysis & gluconeogenesis, attenuates hyperinsulinemia in FRD induced insulin resistant rats and α-adrenergic blocking effect might contribute to insulin secretion and sensitizing effect.

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