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Hepatoprotective and antioxidant properties of *Momordica charantia* in cow urine on Streptozotocin-induced diabetic rat

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Abstract

The aim of the present study was to investigate the hepatoprotective and antioxidant activity of *Momordica charantia* in cow urine on streptozotocin-induced diabetic rats. The alcoholic extract of *Momordica charantia* was administered orally in cow urine at 200 mg/kg body weight to diabetic rats and the results were compared with standard antidiabetic drug, glinbenclamide. The results showed that there was a significant ($P<0.001$) increase in serum glucose, AST, ALT, cholesterol, triglyceride levels and significant ($P<0.001$) reduction in liver SOD, GPx and CAT activity indicating liver damage in diabetic control rats. The biochemical analysis was well supported by histopathological evaluation of the liver which showed necrosis and vacuolar degeneration of hepatocytes in diabetic rats. Oral administration of *Momordica charantia* in cow urine gradually and progressively improved the clinical condition of the diabetic rats. There was significant ($P\leq0.001$) reduction in the serum AST, ALT, cholesterol and triglycerides levels. The treatment also resulted in the significant ($P\leq0.001$) increase in reduced SOD, GPx and CAT activity in the liver of diabetic rats. Histopathology revealed a progressive improvement in the architecture and return to normalcy of hepatocytes. The results clearly suggested that fruit extract of *Momordica charantia* in cow urine at 200 mg/kg body weight may effectively normalize the hepatic damage and impaired antioxidant status in STZ-induced diabetic rats than glibenclamide treated groups.

Key words: *Momordica charantia*, cow urine, streptozotocin, hepatoprotective, antioxidant

Introduction

Diabetes mellitus is probably the fastest growing metabolic disorder in the world. Once regarded as a single disease entity, diabetes is now considered as a heterogeneous group of diseases characterized by a state of chronic hyperglycaemia, which causes a number of secondary complications like cardiovascular, renal, neurological and ocular (Thornally *et al*., 1996). There is increasing evidence that the complications related to diabetes are associated with the oxidative stress induced by the generation of free radicals (Garg *et al*., 2003). A free radical is any species capable of independent existence that contains one or more unpaired electrons.

In diabetes, oxidative stress has been found to be mainly due to an increased production of oxygen free radicals (OFRs) and a sharp reduction of antioxidant defences. OFRs exert their cytotoxic effect by peroxidation of membrane phospholipids leading to change in permeability and loss of membrane

integrity (Meerson *et al*., 1982). Pancreatic beta cell death underlies the pathogenesis of Type I diabetes mellitus and liver is an important organ which offers an adequate site for various metabolic functions. OFRs have been implicated in beta cell destruction as well as in liver injury in diabetes (Poli *et al.,* 1989; Robinovitch *et al.,* 1992; Roza *et al.,* 1995).

Many plant extracts and plant products have been shown to have significant antioxidant activity, which may be an important property of plant medicines associated with the treatment of several ill fated diseases including diabetes. *Momordica charantia* L. (Cucurbitaceae) known as bitter melon, balsam pear, Karela, etc. is a vegetable, indigenous to tropical areas including India, Asia, South America and Africa and cultivated throughout South America as food and medicine. Various preparations of *M. charantia* from injectable extracts to fruit juice to dried fruit bits have been traditionally used worldwide, particularly for blood-sugar lowering effects (Welihinda *et al.,* 1986; Raman and Lau, 1996). Ethanolic extracts of *M. charantia* (200 mg/ kg) showed an antihyperglycaemic and also hypoglycaemic effect when administered subcutaneously to langurs and humans (Shibib *et al*., 1993). In addition, it has been reported to exhibit diverse biological activities such as antioxidant, antimicrobial, antiviral, antihepatotoxic and antiulcerogenic activities. The main constituents of bitter melon which are responsible for these effects are triterpene, protein, steroid, alkaloid, inorganic, lipid, and phenolic compounds (Grover and Yadav, 2004). A steroid, charantin, contained mainly in the aerial parts, has been proven for its antidiabetic activity (Chanchai, 2003). The phenolic compounds from bitter melon extracted by solvent extraction were reported to exhibit antioxidant activity (Horax *et al*., 2005).

Cowpathy is an old system of medicine mentioned in ancient Indian literature (Ayurveda) as *Panchgavya Chikitsa*. The ayurvedic medicines of animal origin are mainly prepared from *Panchgavya* (five things from Indian cow viz., urine, dung, milk, butter oil and curd), which boosts up the body immune system and makes body refractory to various diseases (Chauhan *et al.*, 2004). Cow urine enhances the immunocompetence and improves general health of an individual; prevents the free radical formation and act as anti-aging factor; reduces apoptosis in lymphocytes and helps them to survive and efficiently repairs the damaged DNA (Dhama *et al.,* 2005). It has been found that cow urine and its distillate has antioxidant and antimicrobial activities and effectively alleviates CCL_4 induced hepatotoxicity in rats in a dose-dependent manner (Jarald *et al.,* 2008b; Gururaja *et al.,* 2009).

Since no previous attempts have been made to examine the hepatoprotective and antioxidant properties of *Momordica charantia* in cow urine on Streptozotocin-induced diabetic rats, the present study was undertaken.

Material and Methods

Experimental animals

Healthy female albino Wistar rats weighing 190±20 g were used for the present investigation. The animals were maintained under standard laboratory conditions, providing standard laboratory animal feed and clean drinking water *ad libitum*. The animals were acclimatized to the experimental conditions for two weeks after procurement. The study was carried out with a prior approval by the Institutional Animal Ethical Committee, Veterinary College Hebbal, Bangalore.

Preparation of STZ solution

The STZ of required quantity was dissolved in fresh 0.1M with pH 4.5 citrate buffer and injected intraperitoneally to rats immediately to avoid degradation.

Source of plant extracts

Alcoholic fruit extract of *Momordica charantia* (75% purity) used in the present study was obtained from Himalaya Herbals India, Bangalore.

Source of Cow urine

The distilled cow urine (Amruthasara) was commercially procured from Shri Kamadhenu Go Samrakshane Trust, Mandya, Karnataka, India.

Glibenclamide solution

Glibenclamide (Daonil®, 5 mg), an oral hypoglycaemic drug was dissolved in distilled water (82.33 ml) to give a concentration of 60 µg/ml and administered orally at a dose of 600 µg/ kg

Administration of plant extract and glibenclamide

For a period of 45 days of experimentation, the plant extract, cow urine and glibenclamide were administered orally for their respective groups during morning hours of the day.

Experimental design

Sixty female albino rats of Wistar strain were weighed and randomly distributed into six groups of ten rats each. Care was taken to maintain the intra-group weight variation to be less than 25 g and inter-group weight variation by 35 g.

The groups and treatments used were as follows,

Group I	Normal control: Used for studying the base line values of the parameters
Group II	Diabetic control: Streptozotocin induced diabetic rats
Group III	Diabetic rats supplemented with glibenclamide
Group IV	Diabetic rats supplemented with cow urine
Group V	Diabetic rats supplemented with extracts of *Momordica charantia* at the dose rate of 200 mg/kg body weight in distilled water
Group VI	Diabetic rats supplemented with extract of *Momordica charantia* at the dose rate of 200 mg/kg body weight in cow urine

Experimental induction of diabetes

The animals were fasted overnight and diabetes was induced in groups II to VI by a single intraperitoneal injection of a freshly prepared solution of streptozotocin (45 mg/kg body weight) in 0.1 M cold citrate buffer having a pH of 4.5 (Babu and Prince, 2004). The diabetic control animals received citrate buffer alone.

Confirmation of diabetes

The diabetic state was confirmed by estimating the blood glucose levels after 72 h of STZ injection using ready to using Span Diagnostic kit with semi-automatic biochemical analyzer. The animals that showed the blood glucose level above 200 mg/dl were considered as diabetic. After confirmation of diabetic state, the treatment was commenced.

Collection of serum samples

To evaluate the biochemical parameters, serum was collected for which blood was drawn from the retro-orbital plexus of the rats under ketamine anaesthesia at different time intervals on Day 3, 15, 30 and 45 post STZ injection of the study.

About 2 ml of blood from each animal of all groups was collected separately in clean test tubes. The separated serum was collected and subjected for glucose estimation immediately after collection and the remaining serum samples were stored at -20^0 C for further analysis.

To study the progressive effects of the treatments given to different groups, two rats from each group were sacrificed using ketamine overdose on Day 15 and 30 and the remaining rats on Day 45 of the experiment.

Biochemical analysis

The serum samples collected at various intervals were subjected for biochemical estimation of serum levels of glucose, cholesterol, triglycerides, ALT and AST using Semi Automatic Biochemical Analyzer.

Estimation of antioxidant enzymes

The liver tissues were processed for the estimation of activity of antioxidant enzymes as per the method of Bruce and Baudry (1995). For estimation of antioxidant enzymes representative samples from liver were collected in ice cold normal saline and were blotted dry and stored at -20 °C for further analysis. Liver was crushed in tissue homogenizer with 0.05 M phosphate buffer (pH 7.4) to make it 10 % liver homogenate w/v (1 g of liver tissue homogenized in 10 ml of ice cold 0.05 M phosphate buffer (pH 7.4). The liver homogenate was centrifuged at 1500 g for 1 h at 4°C and the supernatant obtained was used for the estimation of total proteins, superoxide dismutase, catalase and glutathione peroxidase levels.

Estimation of superoxide dismutase (SOD)

Superoxide dismutase was estimated by the method described by Marklund and Marklund (1974). To 0.5 ml of tissue homogenate, 0.25 ml of ethanol and 0.15 ml of chloroform were added and mechanically shaken for 15 min. Then the contents were centrifuged at 13,000 g for 15 min at 4 °C. The supernatant was carefully separated and used for the test. Assay mixture consisted of 2 ml of 0.1M Tris HCl, 0.5 ml of homogenate, 1.5 ml of distilled water and 0.5 ml of pyrogallol. OD value was taken for 3 min at 420 nm wave length. The enzyme activity was expressed in terms of units per min per mg of protein. One unit of enzyme corresponds to the amount of enzyme that inhibits pyrogallol auto-oxidation reaction by 50 per cent.

Estimation of catalase (CAT)

Catalase was estimated by the method of Caliborne (1985). To 0.2 ml of homogenate, 1 ml of 30 mM H_2O_2 was added and the OD value was taken at 240 nm at an interval of 1 min for 3 min. Blank used contained 0.2 ml of distilled water plus 1 ml of 30 mM H_2O_2. Enzyme activity was expressed as µmol of H_2O_2 decomposed per min per mg of protein.

Estimation of glutathione peroxidase (GPx)

Glutathione peroxidase was determined by the method described by Rotruck *et al*. (1973). About 0.5 ml of 0.4 M phosphate buffer was taken and 0.1 ml of sodium azide, 0.2 ml of 4 mM GSH, 0.5 ml tissue homogenate, 0.1 ml of 30 mM H_2O_2 were added and the volume was made up to 2 ml with distilled water. It was incubated for 10 min in room temperature and the reaction was terminated by the addition of 0.5 ml 10 per cent TCA and centrifuged at 4 °C for 10 min at 1500 rpm. To determine the residual glutathione content, the supernatant was removed and to this 4 ml of 0.3 M disodium hydrogen phosphate and 1 ml dithio bis-nitrobenzoic acid (DTNB) (0.004%) reagent were added. The color developed was read at 412 nm against the reagent blank containing only phosphate solution and DTNB reagent. The enzyme activity was expressed as µmoles of glutathione utilized / min / mg protein.

Results

The present study was undertaken to verify and evaluate hepatoprotective and antioxidant properties of *Momordica charantia* in cow urine on streptozotocin-induced diabetic rats. The efficacy of the herbal extract in cow urine was also compared with that of glibenclamide, a reference hypoglycaemic drug.

In the present study diabetic control rats demonstrated polyphagia, polydipsia, polyuria and stable hyperglycemia. The diabetic control rats showed a significant increase in the serum glucose values on 3rd day of the study which progressively increased till the end of the study. Cow urine alone treated rats showed hypoglycaemic effect but was significantly lower compared to glinbenclamide treated rats, while in *M. charantia* treated rats, improvement was comparable to glibenclamide. In general *M. charantia* with cow urine showed better result than other treatment groups (Table 1).

Table 1: The mean (±SE) serum glucose (mg/dL) values of normal control, diabetic and diabetic treatment groups at different intervals of time.

Groups	Days post-treatment			
	3	15	30	45
Group I	110.83±4.15	109.33±3.52	105.33±3.45	108.00±5.58
Group II	403.66±8.99 [a]	437.66±9.30 [a]	462.33±7.86 [a]	485.16±6.89 [a]
Group III	400.50±6.23 [a]	331.83±4.20 [ab]	248.00±3.17 [ab]	193.50±2.74 [ab]
Group-IV	399.33±9.78 [a]	336.66±1.30 [ab]	277.33±5.04 [abc]	223.83±1.85 [abc]
Group-V	397.83±7.95 [a]	326.00±2.64 [ab]	259.50±5.01 [ab]	206.83±2.95 [ab]
Group-VI	401.16±8.55 [a]	321.83±2.60 [ab]	253.83±4.22 [ab]	196.33±3.29 [ab]

All values are mean (± SE), Mean values with superscript differ significantly, [a](Comparison with Group I), [b](Comparison with Group II), [c](Comparison with Group III), Values are statistically significant at P≤ 0.001

There was a significant increase in the mean serum cholesterol, triglyceride, AST and ALT levels in diabetic control rats compared to normal rats. Various treatments used in the present study were effective in improving serum cholesterol, triglyceride, AST and ALT levels, but *Momordica charantia* in cow urine treated group showed significant improvement in reducing the serum cholesterol and triglyceride levels compared to glibenclamide, cow urine and *Momordica charantia* in distilled water treated rats. Cow urine alone treated rats also showed significant improvement in AST and ALT compared to glibenclamide treated rats (Table 2-5).

The diabetic control rats showed drastic reduction in the liver antioxidant enzyme (SOD, CAT and GPx) activities post streptozotocin treatment. In the present study all the treatment groups showed significant improvement in the liver antioxidant enzyme activities compared to diabetic control

Table 2: The mean (±SE) serum cholesterol (mg/dL) values of normal control, diabetic and diabetic treatment groups at different intervals of time

Groups	Days post-treatment			
	3	15	30	45
Group I	80.68±0.61	75.84±0.88	77.82±0.61	78.16±0.58
Group II	146.17±1.05 [a]	166.20±0.79 [a]	177.21±0.88 [a]	186.46±0.74 [a]
Group III	147.25±0.80 [a]	128.87±1.71 [ab]	116.63±1.34 [ab]	106.80±1.13 [ab]
Group-IV	146.82±1.54 [a]	138.14±1.13 [abc]	127.04±1.44 [abc]	111.10±0.81 [ab]
Group-V	146.72±0.95 [a]	124.14±1.14 [abc]	113.45±0.88 [ab]	103.73±0.92 [ab]
Group-VI	145.87±0.87 [a]	117.73±1.87 [abc]	110.36±0.63 [abc]	96.63±1.75 [abc]

All values are mean (± SE), Mean values with superscript differ significantly, [a](Comparison with Group I), [b](Comparison with Group II), [c](Comparison with Group III), Values are statistically significant at P≤ 0.001

Table 3: The mean (±SE) serum triglyceride (mg/dL) values of normal control, diabetic and diabetic treatment groups at different intervals of time

Groups	Days post-treatment			
	3	15	30	45
Group I	94.41±1.19	95.33±0.79	94.71±0.96	93.82±0.93
Group II	186.49±1.02 [a]	197.70±0.89 [a]	216.72±1.38 [a]	238.23±0.81 [a]
Group III	185.98±0.69 [a]	165.92±0.74 [ab]	157.05±0.86 [ab]	146.46±0.88 [ab]
Group-IV	185.52±0.79 [a]	165.50±0.93 [ab]	156.62±0.95 [ab]	144.57±0.96 [ab]
Group-V	186.09±0.88 [a]	164.52±1.03 [ab]	149.67±0.60 [abc]	135.60±0.76 [abc]
Group-VI	186.17±0.61 [a]	156.99±0.64 [abc]	141.35±0.56 [abc]	130.12±0.41 [abc]

All values are mean (± SE), Mean values with superscript differ significantly, [a](Comparison with Group I), [b](Comparison with Group II), [c](Comparison with Group III), Values are statistically significant at P≤ 0.001

Table 4 : The mean (±SE) serum alanine aminotransferase (ALT) (IU/L) values of normal control, diabetic and diabetic treatment groups at different intervals of time

Groups	Days post-treatment			
	3	15	30	45
Group I	62.81±0.73	63.95±0.75	63.80±0.94	63.72±0.90
Group II	132.13±0.71[a]	147.16±0.83[ab]	167.44±0.85[ab]	182.32±0.64[ab]
Group III	131.14±0.64[a]	119.65±0.60[ab]	114.14±0.83[ab]	107.32±0.83[ab]
Group-IV	132.22±0.75[a]	115.16±0.71[abc]	108.36±0.86[abc]	100.28±0.45[abc]
Group-V	131.89±0.44[a]	119.35±0.48[ab]	109.30±0.54[abc]	103.92±0.68[abc]
Group-VI	131.73±0.52[a]	112.91±0.69[abc]	105.83±0.45[abc]	98.53±0.72[abc]

All values are mean (± SE), Mean values with superscript differ significantly, [a](Comparison with Group I), [b](Comparison with Group II), [c](Comparison with Group III), Values are statistically significant at P≤ 0.001

Table 5 : The mean (±SE) serum aspartate aminotransferase (AST) (IU/L) values of normal control, diabetic and diabetic treatment groups at different intervals of time

Groups	Days post-treatment			
	3	15	30	45
Group I	83.67±0.68	83.09±0.85	84.62±0.97	84.63±0.53
Group II	161.44±0.50 [a]	186.12±0.80 [a]	205.57±0.96 [a]	225.75±1.26 [a]
Group III	161.65±0.65 [a]	150.07±0.54 [ab]	139.99±0.83 [ab]	131.44±0.65 [ab]
Group-IV	161.61±0.59 [a]	146.75±1.00 [abc]	136.73±0.69 [abc]	128.10±0.44 [abc]
Group-V	161.61±0.44 [a]	142.44±0.44 [abc]	128.12±0.69 [abc]	120.67±0.26 [abc]
Group-VI	161.50±0.59 [a]	138.60±0.73 [abc]	125.99±0.71 [abc]	116.69±1.40 [abc]

All values are mean (± SE), Mean values with superscript differ significantly, [a](Comparison with Group I), [b](Comparison with Group II), [c](Comparison with Group III), Values are statistically significant at P≤ 0.001

rats. Cow urine alone and *M. charantia* in cow urine as well as distilled water treated group's revealed better improvement compared to glibenclamide treated group (Fig.1).

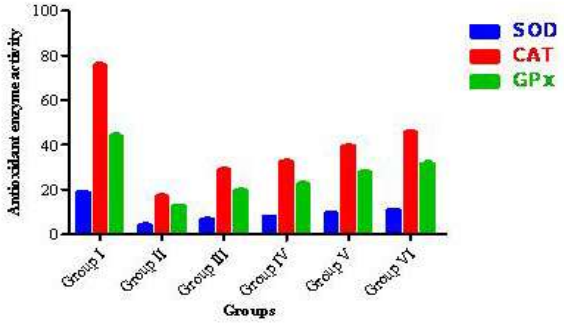


Fig.1. The mean (+SE activities of Syperoxide dismutase (SOD) (U/min/mg/of protein), Catalase(CAT) (µmoles of H_20_2/min/mg of protein) and Glutathione peroxidise (GPx) (µmoles of glutathione utilized/min/mg protin) in the liver of normal control, diabetic and diabetic treatment groups on 45th day of experiment

Microscopically, in diabetic rats pancreas revealed lesions both in endocrine as well as exocrine portion. In the endocrine portion there was a reduction in the number of islets which were irregular with loss of demarcation between islets and adjacent exocrine portion. The islets revealed reduced number of β-cells that were either highly swollen with vacuolated cytoplasm or elongated and fusiform with condensed nucleus. Streptozotocin induced effects on islets gradually subsided in all the treatment groups with *M. charantia* in cow urine showing better response. There was an improvement in the number, size and architecture of the islets (Fig 2-5). There was a loss of architecture of the liver with increase in cytoplasmic granularity, vacuolations and obliteration of sinusoidal space which became more severe as the time advanced in diabetic control rats. The various treatment groups especially *M. charantia* in cow urine, protected the liver from STZ induced effects throughout the study (Fig 6-9).

Fig 2: Pancreas from normal control animal showing normal architecture with compact arrangement of β-cells and α-cells on 45th day of the study. (H&E 200X)

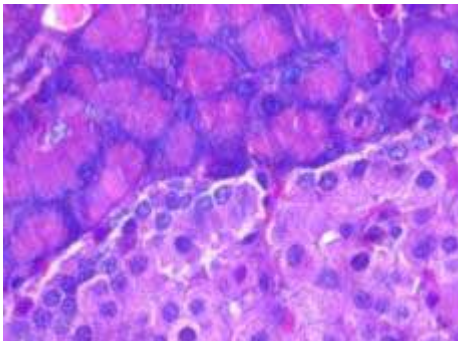


Fig 3: Islet of Langerhans from diabetic control rat showing loss of normal architecture with vacuolated and necrotic β-cells on 45th day of the study. (H&E 200X)

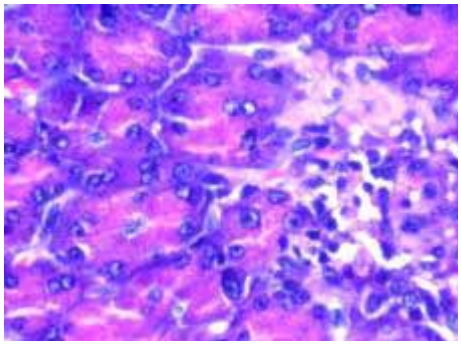


Fig 4: Pancreas from glibenclamide treated rat showing improvement in the architecture of the islet with hypercellularity mainly comprising α-cells on 45th day of the study. (H&E 200X)

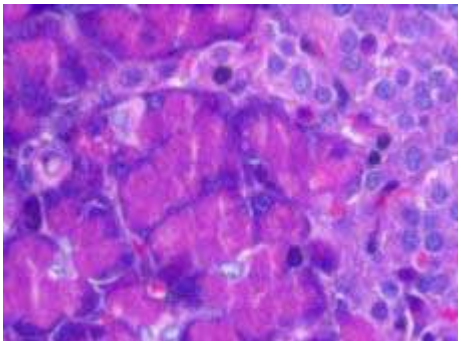


Fig 5: Section of pancreas from *M. charantia* 200 mg/kg in cow urine showing improvement in the islet size and compact arrangement of the cells with increase in the number of β-cells on 45th day of the study. (H&E 200X)

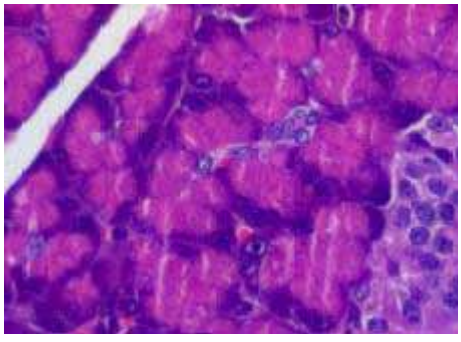


Fig 6: Liver of normal control animal showing normal architecture on 45th day of the study. (H&E 100X)

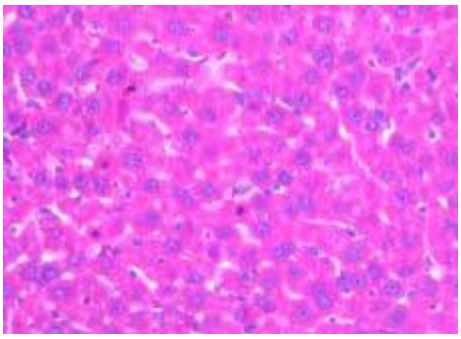


Fig 7: Section of liver in diabetic control animal showing swollen hepatocytes with cytoplasmic vacuolations and obliteration of sinusoidal space on 45th day of the study. (H&E 400X)

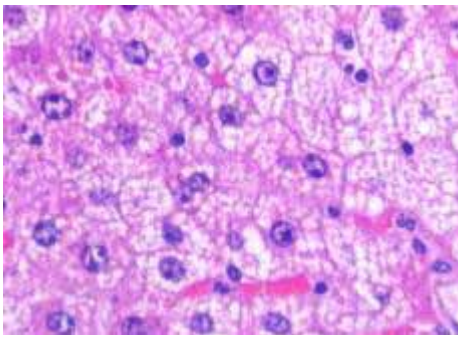


Fig 8: Liver of diabetic rat treated with glibenclamide showing mild vacuolations on 45th day of the study. (H&E 200X)

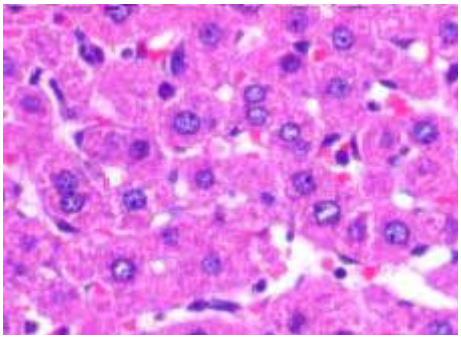
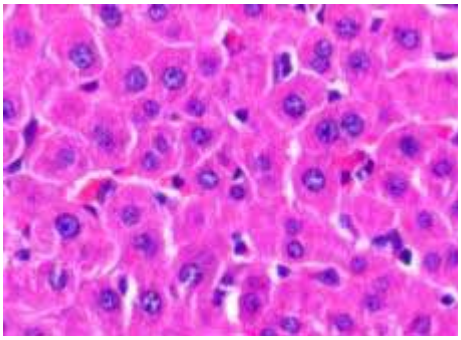


Fig 9: Liver sectionfrom *M. charantia* 200 mg/kg in cow urine treated rat showing attainment of normal architecture on 45th day of the study. (H&E 200X)



Discussion

Streptozotocin (STZ) is a naturally occurring compound produced by the bacterium *Streptomyces achromogenes* that exhibits broad spectrum antibacterial properties. It is assumed that the toxicity of streptozotocin is dependent upon the DNA alkylating activity of its methylnitrosourea moiety, especially at the O_6 position of guanine and the transfer of the methyl group from streptozotocin to the DNA molecule that causes damage, which along a defined chain of events, results in the fragmentation of the DNA. The DNA damage induces activation of poly ADP-ribosylation which leads to depletion of cellular NAD+ and ATP. The depletion of the cellular energy stores ultimately results in beta cell necrosis (Lenzen, 2008).

In the present investigation, all animals in the normal control group remained healthy at different intervals of the study. All the values of various parameters analysed were within the normal range and indicated their healthy status. In the diabetic control animals (Group-II), hyperglycaemia, which characterises diabetes, is directly related to insulin deficiency caused by relative destruction of beta cells of pancreas by STZ which causes loss of glucose homeostasis (Gu *et al.,* 1997). In addition various metabolic derangements that occur in response to hypoinsulinemia in diabetes are contributable for hypercholesterolemia and hypertriglyceridemia (Fernandes *et al.,* 2007). ALT and AST are the leakage enzymes of hepatocytes and are reported to elevate in their concentration in serum in STZ induced diabetes as STZ causes damage to plasma membrane and organellar membrane especially that of RER and mitochondria (Abdollahi *et al.,* 2010).

Diabetes is usually accompanied by an increased production of free radicals due to hyperglycaemia induced glucose auto-oxidation and protein glycosylation (Wolff *et al.,* 1991) which quench off the antioxidants, a feature also observed in present study that showed a significant reduction in SOD, CAT and GPx activities.

The pathological examination of diabetic rats in the present study showed progressive atrophic changes in the pancreas grossly and microscopically, necrosis of beta cells specifically due to toxic effects of STZ (Atangwho *et al.,* 2010). In addition, vacuolar change indicative of fatty change was observed in liver which could be due to increased influx of fatty acids induced by hypoinsulinemia (Ohno *et al*., 2000).

In the glibenclamide treated group, the progressive improvement in several parameters could be attributed to the effect of glibenclamide in enhancing insulin secretion by β-cells of pancreas and also increasing sensitization of the peripheral tissues to insulin (Hribal *et al.,* 2001; Abdollahi *et al.,* 2010). Glibenclamide is a second generation sulfonylurea used in the treatment of noninsulin dependent diabetes. Its hypoglycemic effect is mainly due to stimulation of insulin release from pancreatic beta cells and sensitization of the peripheral tissues to insulin (Sweetman, 2002).

Glibenclamide has been shown to bind to the surface receptors of β-cell membrane inhibiting ATP-sensitive K^+ channels and cause depolarization of cell membrane. Depolarization leads to opening of K^+ channels which enables extracellular Ca^{2+} to enter the cell. Increased intracellular Ca^{2+} concentration enhances the binding of Ca^{2+} to the transport protein calmodulin which leads to microfilament contraction and release of insulin containing granules. Increased insulin causes subsequent reduction in serum glucose levels which improves β-cells sensitivity to glucose and potentiates insulin secretion (Ling *et al*., 2006).

To determine the synergistic hepatoprotective and antioxidant effect of *Momordica charantia* fruit extract and cow urine, group VI was used. When compared with *M. charantia* in distilled water, *M. charantia* in cow urine treated rats showed better improvement which indicated synergistic effect of *M. charantia* and cow urine. The improvement in various parameters in *M. charantia* in cow urine treated groups could be attributed to the combined hypoglycaemic and antioxidant activity of *M. charantia* and cow urine leading to better utilization of glucose, amino acids and nutrients (Singh *et al*., 1989; Jarald *et al*., 2008a; Kumar *et al.,* 2010; Gururaja *et al.,* 2011).

The hypoglycaemic effect of *M.charantia* fruit extract could be because of its property of enhancing insulin production from pancreatic β-cells (Welhinda *et al.,* 1982) and improving peripheral glucose utilization resulting in increased glycogen storage by liver (Welhinda *et al.,* 1986). Ahmad *et al.* (1998) reported an increase in the number of pancreatic β-cells in the islets of Langerhans in *M. charantia* treated animals. Some previous studies have also revealed that *M. charantia* increases the glucose uptake in liver via promoting glucose-6-phosphate dehydrogenase and declining glucose-6-phosphatase activities (McCarty, 2004). In addition, *M. charantia* is also supposed to increase the mRNA expression of glucose transporter 4 (GLUT4) proteins in skeletal muscles (Shih *et al*., 2009). Mahomoodally *et al*. (2007) suggested that *M. charantia* fruit extract can reduce glucose transport via the brush border of small intestine in albino rats.

Several hypoglycemic components of *M. charantia* have been identified, which consist of a steroidal glycosides (charantin), insulinomimetic proteins (p-insulin or v-insulin) and alkaloids that are concentrated in the fruit of the plant (Kumar *et al.,* 2010), which all could be contributing for antidiabetic effect of *M. charantia*.

The hepatoprotective effect of *Momordica charantia* has been reported by many workers who have indicated that the hepatoprotection is through elaboration of antioxidants by *Momordica charantia* which prevent lipid peroxidation (Abdollahi *et al.,* 2010). The antioxidant compounds of *M. charantia* include phenolic phytochemicals and vitamins such as C and A (Grover and Yadav, 2004). Recently, cucurbitane-type triterpenoids were isolated from the stems of *M. charantia* and their antioxidant activity was documented (Liu *et al*., 2010).

The chemo-profiling of cow urine by Jarald *et al.,* (2008b) has shown presence of protein, urea, uric acid, creatinine, phenol, aromatic acids, enzymes and vitamins. Randhawa (2010) reported that antioxidant property of urine was due to presence of uric acid. Cow urine has been reported to have bioenhancing ability thus possibly increasing the availability of the active principles of *M. charantia* to the animal (Jarald *et al*., 2008b). It was also observed in the present study that, the *M. charantia* extract had better solubility in cow urine compared to that in distilled water.

Conclusion

The present study has shown that:

- Glibenclamide has a substantial hypoglycaemic activity but lacks adequate antioxidant activity.
- *M. charantia* in cow urine was more effective in protecting the liver and restoring its antioxidant status in STZ induced diabetic rats compared to *M. charantia* and cow urine alone, so a synergism was observed between *M. charantia* and cow urine in alleviating the STZ induced effects.

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