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Antioxidants Status of STZ-Induced Diabetic Rats Treated With Extract of *Momordica Charantia*

Medical Science

KEYWORDS : Diabetes mellitus, Momordica charantia, Hyperglycaemia, Antioxidants, Glimepiride.

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ABSTRACT

The present study investigated the effects of Momordica charantia on hyperglycaemia and selected markers of antioxidants activities which include thiobarbituric acid reactants (TBARS), Catalase (CAT), Glutathione (GSH), Superoxide Dismutase (SOD), Glutathione Peroxidase (GPx) in streptozotocin-induced diabetic Wistar rats.

Forty healthy adult Wistar rats of both sexes were randomly assigned into five groups A, B, C, D and E of eight rats each. Group A were the control (normal rats); B were the experimentally-induced diabetic rats; C were diabetic rats treated with methanolic extracts of M. charantia for two weeks (withdrawal group); D were diabetic rats treated with methanolic extracts of M. charantia for four weeks. E was diabetic rats treated glimepiride for four weeks.

Results showed that extract of Momordica charantia has potent hypoglycaemic effects in diabetic rats. There was a significant reduction ($p< 0.05$) of TBARS in extract treated group when compared with untreated diabetic rats. There was a non significant increase in the activities of CAT, GSH, SOD and GPx in the extract treated group when compared with the untreated diabetic group.

In conclusion, this study revealed that M. charantia could restore to within normal levels, the observed changes in most antioxidants markers of diabetic rats

INTRODUCTION

Diabetes mellitus (DM) is frequently associated with cardiovascular diseases risk factors such as increased levels of total cholesterol triglycerides, and blood pressure, but much of the increased risk for cardiovascular diseases is not explained by these and other standard cardiovascular risk factors (Kannel et al.,1974; Uusitupa et al., 1990). This raises the possibility that other less recognized risk factors present in individuals with diabetes such as increased lipoprotein peroxidation and formation of advanced glycation endproducts (AGE) may enhance the atherogenic process. The mechanisms by which lipoprotein oxidation may accelerate atherosclerosis have been extensively reviewed (Berliner and Heinecke, 1995; Navab et al., 1996). Moreover, there is increasing evidence that lipoproteins from individuals with diabetes are more susceptible to oxidation (Tsai et al., 1994; Rabinivi et al., 1994) and that plasma from these individuals contains higher levels of lipid peroxides (Sato et al., 1979; Kaji et al., 1985). Oxidative stress is defined in general as excess formation and/or insufficient removal of highly reactive molecules such as reactive oxygen species (ROS) and reactive nitrogen species (RNS) (Turko et al., 2001, Maritim et al., 2003). ROS include free radicals such as superoxide ($\bullet O_2^-$), hydroxyl ($\bullet OH$), peroxyl ($\bullet RO_2$), hydroperoxyl ($\bullet HRO_2^-$) as well as nonradical species such as hydrogen peroxide (H_2O_2) and hydrochlorous acid (HOCl) (Turko et al., 2001, Evans et al., 2002). RNS include free radicals like nitric oxide ($\bullet NO$) and nitrogen dioxide ($\bullet NO_2^-$), as well as nonradicals such as peroxynitrite (ONOO-), nitrous oxide (HNO2) and alkyl peroxynitrates (RONOO) (Turko et al., 2001, Evans et al., 2002). Of these reactive molecules, $\bullet O_2^-$, $\bullet NO$ and ONOO- are the most widely studied species and play important roles in the diabetic cardiovascular complications. A multitude of *in vivo* studies have been performed utilizing antioxidants in experimental diabetic models. The effects of antioxidants on oxidative stress are measured through certain observable biomarkers. These markers include the enzymatic activities of Catalase (CAT), Superoxide Dismutase (SOD), Glutathione Peroxidase (GPx), and Glutathione (GSH), as well as thiobarbituric acid reactants (TBARS) levels (a lipid peroxidation marker), an indirect measurement of free-radical production that has been shown to be consistently elevated in diabetes. Normalization of the activity levels of any of these markers, and ultimately, the balance of free-radical production/removal, would be an effective method to reduce ROS-induced damage. Many animal studies have been completed with this aim in mind and indeed have shown that diabetes-induced alterations of oxidative stress indicators can be reversed when the animals are treated with various antioxidants.

Momordica charantia (Linn Family: Cucurbaceae) is one of the popular herbs that grow in different regions of Nigeria. It is commonly called Bitter melon, Bittergourd, Balsam pear. Various parts of M. charantia such as the seed, fruit and even the whole plant has been reported to have beneficial effects in prevention and treatment of many diseases in folkloric medicine, especially in the treatment of DM in individuals with non-insulin dependent diabetes (Platel and Srinivasan 1997; Raman and Lau 1996). It has hypoglycaemic properties as it significantly suppressed the rise in blood glucose concentrations in albino rats (Platel and Srinivasan 1997; Nicholas et al., 2006).. Bitter melon contains an array of biologically active plant chemicals including triterpenes, proteins and steroids. In addition, a protein found in bitter melon, momordin, has clinically demonstrated anticancerous activity against Hodgkin's lymphoma in animals. Other proteins in the plant, alpha- and beta-momorcharin and cucurbitacinB, have been tested for possible anticancerous effects (Nagasawa et al.,2002). In numerous studies, at least three different groups of constituents found in all parts of bitter melon have clinically demonstrated hypoglycemic (blood sugar lowering) properties or other actions of potential benefit against diabetes mellitus

(Tan et al., 2007). These chemicals that lower blood sugar include a mixture of steroidal saponins known as charantins, insulin-like peptides, and alkaloids. The hypoglycemic effect is more pronounced in the fruit of bitter melon where these chemicals are found in greater abundance.

The present study investigated the effects of M. charantia on hyperglycaemia and selected markers of antioxidants activities (which include TBARS, SOD, GPx, Glutathione, Catalase) in streptozotocin-induced diabetic Wistar rats and compared the effects with those of glimepiride, an oral blood-glucose-lowering drug of the sulfonylurea class (Hamaguchi et al.,2004).

MATERIALS AND METHODS

Animal care:

Forty healthy adult Wistar rats of both sexes, with average weight of 134.4g were used for the experiment. The rats were bred in the animal holding of College of Health Sciences, Obafemi Awolowo University, Ile-Ife. They were maintained on standard rat pellet (Capsfeed, Ibadan, Nigeria) and water was provided ad libitum.

The animals were randomly assigned into five groups A, B, C, D and E of eight rats each.

- group A were the control (normal rats)
- group B were the experimentally-induced diabetic rats administered with 10% tween 80,
- group C were the experimentally-induced diabetic rats treated with methanolic extracts of Momordica charantia dissolved in10% tween 80 for two weeks (withdrawal group)
- group D were the experimentally-induced diabetic rats treated with methanolic extracts of Momordica charantia dissolved in 10% tween 80 for four weeks.
- group E were the diabetic rats treated with a standard diabetic drug (2mg/kg of glimepiride) dissolved in 10% tween 80 for four weeks

The animals received humane treatment as outlined in the "Care and Management of Laboratory Animals" published by the National Institute of Health . (NIH, 1985)

Plant material:

Matured leaves of Momordica charantia (Cucurbitaceae) were collected during the raining season from suburban villages of Ile-Ife metropolis in Osun State of Nigeria. The leaves were taken to the Herbarium in the Department of Botany, Obafemi Awolowo University, Ile-Ife to confirm identification and a voucher specimen number (UHI 16510) was placed in the Herbarium.

Preparation of methanolic extract of M. charantia:

Leaves of Momordica charantia (MC) were air dried and powdered in a warring blender. A 765 g of the powdered leaves were extracted in 1,950 mls of absolute methanol for 72 hours with intermittent shaking and filtered. The filtrate were concentrated in vacuo at 35^{0}C using a vacuum rotary evaporator (Bŭchi Rotavapor R110, Schweiz). The extract were partitioned between water and dichloromethane, the dichloromethane fraction (5.94%) was oven-dried at 37^{o}C and stored until it is ready to be used. The aqueous portion obtained was very little. Aliquot portions of the extract were weighed and dissolved in 10% tween 80 for use on each day of the experiment.

Induction of diabetes:

Diabetes mellitus was experimentally-induced in groups B, C, D and E by a single intraperitoneal injection of 65 mg/kg body weight of streptozotocin (Tocris Bioscience, UK) dissolved in 0.1M sodium citrate buffer (pH 6.3). Diabetes was confirmed in animals 48 hours after induction, by determining fasting blood glucose level using a digital glucometer (Accu-chek® Advantage, Roche Diagnostic, Germany) consisting of a digital meter and the test strips using blood samples obtained from the tail vein of the rats. The fasting blood glucose was subsequently monitored throughout the experimental period. Animals in group A were given equal volume of citrate buffer used in dissolving streptozotocin intraperitoneally.

Administration of extract and anti-diabetic drug:

Methanolic extracts of the leaves of M. charantia (100 mg/kg) was dissolved in10% tween 80 and administered daily (orally) by gastric intubation to the rats in groups C and D for 2 and 4 weeks respectively. The standard antidiabetic drug (glimepiride 2 mg/kg) was administered to group E rats for four weeks (Mir et al., 2008), while those in group B were left untreated.

Sacrifice of the animals:

At the end of the experimental period, all the animals were physically observed and anesthesized by chloroform inhalation. A midline incision was performed at the thoracic region. Blood samples were obtained by cardiac puncture using a 5 ml syringe with needle inserted into the left ventricle. The blood was centrifuged within 30 minutes of collection. The blood was centrifuged in a centurion scientific refrigerated centrifuge, R 8000 series (Oxford, UK) at 5000 R.P.M for 5 minutes to obtain the serum.

Biochemical Assays:

TBARS assay kit (BioAssay Systems USA) based on the reaction of TBARS with thiobarbituric acid (TBA) to form a pink coloured product, was use to determine serum TBARS level. The activity of catalase was assayed by measuring the catalase degradation of hydrogen peroxide using a redox dye according to the methods of Cowell et al., 1994. Serum Glutathione was measured by using sensitive colorimetric glutathione assay kit (BioAssay System, USA) which accurately measures reduced glutathione in biological samples. The Detect X® superoxide Dismutase (SOD) activity kit (Arbor Assays, USA), designed to quantitatively measure SOD activity in a variety of samples was used to assay for serum SOD levels. The activity of GPx (Bioassays System, USA) was measured in the serum by measuring NADPH consumption in the enzyme coupled reaction according to the methods of Pascual, et al. (1992).

Statistical analysis:

All values were presented as means ±SEM. Data were analyzed using one way analysis of variance (ANOVA) with Duncan multiple range test (DMRT) using Statistical Package for Social Science (SPSS 17).

RESULTS

Blood glucose level:

The initial (day 0) mean of the blood glucose level of all the animal groups were observed to be normoglycaemic and present a non significant ($p > 0.05$) difference. Forty- eight hours after the induction of STZ, the blood glucose levels of the rats in groups B, C, D and E were observed to have increased drastically which was significantly ($p < 0.05$) different when compared with the control animals in group A. The blood glucose levels of animals in groups B, C, D and E were not significantly different when compared ($p > 0.05$). Two weeks after STZ administration (14 days), Hyperglycaemia was observed in all animals. The blood glucose levels remains elevated till the 28 days after STZ administration. However, two weeks after the commencement of the extract administration to animals in groups C and D, the blood glucose levels was observed to have reduced significantly. Statistically, it was shown that the blood glucose levels of the rats in group C and D reduced by 50.34% and 47.39% respectively. After two months of the experimental period (56 days), the blood glucose levels of the diabetic rats in group B remains elevated (386.50 ± 36.97) while the blood glucose levels of the

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rats in group C (withdrawal group) showed a significant ($p<0.05$) 106.5% increase after two weeks of the extract withdrawal. This differs from groups D and E rats which maintained normoglycaemic levels of 233.20 ± 18.93 and 181.00 ± 60.67 respectively. (Figure 1).

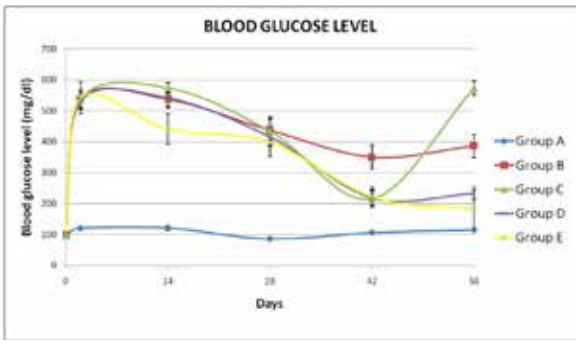

FIGURE 1: Showing the effect of M. Charantia on the blood glucose Level (mg/dL)

Lipid peroxidation marker:

Thiobarbituric Acid Reactive Substances (TBARS)

The result showed a significant ($p<0.05$) increase in the TBARS concentration of diabetic group (group B) as compared with the control. Administration of extract for four weeks (group D), significantly ($p<0.05$) reduced the TBARS concentration significantly ($p<0.05$) (17.01 ±8.56) when compared with diabetic group. Withdrawal of extract administration from animals in group C, increased the TBARS concentration (20.17 ±10.55) when compared with control (18.62 ±7.36). The antidiabetic drug reduced the TBARS concentration non-significantly as compared with the group treated with M.charantia for four weeks. (Table 1).

Antioxidants:

Catalase (CAT)

The result showed a significant ($p<0.05$) reduction in the catalase activity of diabetic group (group B) as compared with the control. Administration of extract for four weeks (group D), increased the catalase activity non-significantly ($p>0.05$) (13.56 ±4.73) when compared with diabetic group. Withdrawal of extract administration from animals in group C, further increased the catalase activity (39.03 ±19.62). The antidiabetic drug increased the catalase activity non-significantly ($p>0.05$) as compared with the diabetic group. (Table 1).

Glutathione (GSH)

The result showed a non significant ($p>0.05$) reduction in the glutathione concentration of diabetic group (group B) as compared with the control. Administration of extract for four weeks (group D), increased the glutathione concentration non-significantly ($p>0.05$) (25.28 ±12.98) when compared with diabetic group. Withdrawal of extract administration from animals in group C, reduced the glutathione concentration (13.24 ±13.24). The antidiabetic drug increased the glutathione concentration significantly ($p<0.05$) as compared with the diabetic group. (Table 1).

Superoxide dismutase (SOD)

The result showed a non significant ($p>0.05$) reduction in the SOD activity of diabetic group (group B) as compared with the control. Administration of extract for four weeks (group D) increased the SOD activity non-significantly ($p>0.05$) (0.64 ±0.25) when compared with diabetic group. Withdrawal of extract administration from animals in group C reduced the SOD activity (0.30 ±0.17). The antidiabetic drug increased the SOD activity non-significantly ($p>0.05$) as compared with the diabetic group. (Table 1).

Gluthatone Peroxidase (GPx)

The result showed a non significant ($p>0.05$) reduction in the GPx activity of untreated diabetic group (group B) as compared with the control. Administration of extract for four weeks (group D), increased the GPx activity non-significantly ($p>0.05$) (0.49 ±0.22) when compared with diabetic group. Withdrawal of extract administration from animals in group C, reduced the GPx activity (0.41 ±0.22). The antidiabetic drug increased the GPx activity non-significantly ($p>0.05$) as compared with the diabetic group. (Table 1).

TABLE 1: The effect of M. charantia on Lipid peroxidation and antioxidants

	TBARS (μM)	CATALASE (U/L)	GLUTATHIONE (μM)	SOD (U/mL)	GPX (U/L)
Group A	18.62 ±7.36^{ab}	72.30 ±12.62^{b}	18.99 ±9.05^{ab}	0.38 ±0.19^{a}	0.81 ±0.36^{a}
Group B	36.03 ±6.78^{b}	9.38 ±5.75^{a}	13.57 ±8.42^{ab}	0.37 ±0.18^{a}	0.44 ±0.27^{a}
Group C	20.17 ±10.55^{ab}	39.03 ±19.62^{a}	13.24 ±13.24^{ab}	0.30 ±0.17^{a}	0.41 ±0.22^{a}
Group D	17.01 ±8.56^{ab}	13.56 ±4.73^{a}	25.28 ±12.98^{ab}	0.64 ±0.25^{a}	0.49 ±0.22^{a}
Group E	17.94 ± 11.32^{ab}	15.52 ± 11.88^{a}	41.20 ± 25.40^{b}	0.47 ±0.30^{a}	0.64 ±0.16^{a}

Values are given as mean ± SEM for 5 biochemical parameters named as TBARS, Catalase, Glutathione, SOD and GPX in each group.a, b, ab within column signifies that means with different letters differs significantly at $p < 0.05$ while means with the same letters does not differ significantly at $p < 0.05$ (using one way ANOVA with Duncan multiple range test).

DISCUSSION

The chronic hyperglycaemia (high blood glucose level) of diabetes mellitus is associated with long-term damage, dysfunction and failure of various organs especially the eyes, kidneys, nerves, heart and blood vessels (Gavin et al., 2003). In the present study, the blood glucose level was observed to increase significantly in groups B, C, D and E 48 hr after the induction of STZ. This hyperglycemic state was maintained until two weeks before the commencement of M. charantia and glimepiride treatment to animals in group C, D and E. Following treatment with M. charantia to animals in group D for four weeks, the blood glucose level dropped significantly by 43.81% while the group administered with glimepiride dropped by 54.58%. The reduction in BGL by M. charantia in this investigation corroborates other studies (**Szkudelski 2001;** El Batran et al., 2006; Virdi, et al., 2003). For instance, Chaturvedi (2005) reported a statistically significant

reduction in the BGL to normal by day 15 in a dose dependent manner. Baby et al., (2013) reported that a dose of 0.5g/kg of M. charantia, the BGL produced a significant decrease. Following the withdrawal of the extract from animals in group C, the blood glucose level went up by 166.51% which is significantly different from the group in which the extract administration continued for four weeks. The reversal of the BGL from a normoglycemia to hyperglycemic status following the withdrawal of the M. charantia treatment to animals in group C, underscores the insulin-like properties of M. charantia. Baby et al., (2013) in his investigation of the effect of M. charantia on blood glucose level of normal and alloxan-diabetic rabbits concluded that the mechanism by which M. charantia reduced the BGL is similar to that of insulin. Active components through which M. charantia reduces BGL are thought to be charantin, vicine, and polypeptide-p (an unidentified insulin-like protein similar to bovine insulin) (Dans et al., 2007).

Numerous studies have demonstrated that oxidative stress is mediated mainly by hyperglycemia-induced generation of free radicals which contributes to the development and progression of diabetes and its related complications (Giugliano et al., 1996; Ceriello; 2003; Ceriello, 2004). In view of this, it become imperative to search for an effective means through herbal therapy for reducing oxidative stress generated in diabetes.

Increase in TBARS level has been reported in diabetic condition. This increase occurs as a result of oxidative stress. Oxidative stress is the formation and/or insufficient removal of highly reactive molecules such as reactive oxygen species (ROS) and reactive nitrogen species (RNS) (Turko et al; 2001; Maritimi et al 2003). In this study, TBARS concentration was observed to be significantly increased in the diabetic rats (group B). A significant increase in TBARS level in Streptozotocin induced diabetic rats was also reported (Sathishsekar and Subramanian 2005a; Sathishsekar and Subramanian 2005b; Prince and Menon 2001; Jain et al., 2010). Treatment of diabetic rats with M. charantia significantly reduced the concentration of TBARS while its withdrawal increased its concentration. This established that M. charantia inhibits oxidative stress as shown in this study. Mekinova et al.,1995 demonstrated that supplementation of streptozotocin (STZ) diabetic rats with vitamins C, E, and beta-carotene for 8 weeks produced a significant reduction of TBARS levels. A study in type-2 diabetic rats revealed that troglitazone and pioglitazone reduced TBARS levels and increased the aortic vasorelaxation response (Lida et al., 2003). The efficacy of M. charantia in the management of diabetic complications is further affirmed by a non significant reduction in TBARS concentration in group D when compared with the group treated with glimepiride.

Antioxidant defence mechanisms involve both enzymatic and nonenzymatic strategies. Common antioxidants include the vitamins A, C, and E, glutathione, and the enzymes superoxide dismutase, catalase, glutathione peroxidase, and glutathione reductase. Other antioxidants include α-lipoic acid, mixed carotenoids, coenzyme Q10, several bioflavonoids, antioxidant minerals (copper, zinc, manganese, and selenium), and the cofactors (folic acid, vitamins B1, B2, B6, B12). They work in synergy with each other and against different types of free radicals (Maritimi et al., 2003).

Changes in catalase activity in chemically induced diabetic animals as been investigated by several authors (Rauscher et al., 2000; Sanders et al., 2001; Mekinova et al., 1995). For example, catalase activity is consistently found to be elevated in heart (Kaul et al., 1996; Rauscher et al., 2000; Sanders et al., 2001) and aorta (Kocak et al., 2000), as well as brain (Kedziora-Kornatowska et al., 2000) of diabetic rats. Catalase (CAT) is a hemeprotein which catalyses the reduction of hydrogen peroxides and protects the tissues from highly reactive hydroxyl radicals (Sathishsekar and Subramanian 2005b). In this study, a significant decrease was observed in the activity of catalase in the diabetic rats compared with the control group. Decrease in catalase is an indication that there might be generation of an active radical—a factor which is eminent in the existence of this enzyme. Treatment with extract for four weeks slightly increased catalase activity. Previous studies have also reported an increase in catalse activity following treatment with M. charantia in diabetic rats (Sathishsekar and Subramanian 2005b, Chaturvedi and George, 2010). The reason for the increase suggests the presence of potent antioxidant activity in M. charantia (Sathishsekar and Subramanian 2005b).. Withdrawal of the extract further elevated the catalase activity. The reason for this is unknown and calls for further investigation. The efficacy of M. charantia is further affirmed by a non significant increase in catalase activity in group D as compared with the group treated with glimepiride.

Glutathione (GSH) functions as a direct free-radical scavenger, as a co-substrate for glutathione peroxidase activity, and as a cofactor for many enzymes, and forms conjugate in endo- and xenobiotic reactions (Josephy 1997, Gregus et al., 2006). GSH concentration is found to be decreased in the liver (Aragno et al., 1999; Sanders et al., 2001), kidney (Aragno et al., 1999), pancreas (Abdel-Wahab et al., 2000), plasma and red blood cells (Montilla et al., 1998), nerve and precataractous lens (Obrosova and Stevens 1999) of chemically induced diabetic animals. However, there is also some contradictory evidence of increased GSH concentration in diabetic rat kidney (Mekinova et al., 1995) and lens (Borenshtein et al., 2001). This present study showed a significant decrease in the concentration of GSH in the diabetic rats compared with control. Treatment with glimepiride and M. charantia for four weeks increased GSH concentration. Withdrawal of the extract decreased the GSH concentration. This indicates that the M. charantia extracts and glimepiride can either increase the biosynthesis of GSH or reduce the oxidative stress leading to less degradation of GSH, or has both effects (Sathishsekar and Subramanian 2005b).

SOD is an important enzyme in the enzymatic antioxidant defense system which catalyses the dismutation of superoxide radicals to produce H_2O_2 and molecular oxygen (Sathishsekar and Subramanian 2005b). The observed decrease in SOD activity in the diabetic rats in this study could be as a result of inactivation by H_2O_2 or by glycation of enzymes (Sozmen et al., 2001; Sathishsekar and Subramanian 2005b). Several authors have reported either an increase or decrease in SOD activity in diabetic rats (Kaul et al., 1996; Kaul et al., 1995; Kedziora-Kornatowska et al., 1998; Aragno et al., 1999; Obrosova et al., 2000; Sailaja et al., 2000). Treatment with glimepiride and M. charantia for four weeks increased SOD activity. Withdrawal of the extract decreased the SOD activity. Alterations of SOD activity in diabetic animals were also reported to be normalized by probucol (Kaul et al., 1995) captopril, (Kedziora-Kornatowska et al., 1998), DHEA (Sailaja et al., 2000), -lipoic acid (Stefek et al., 2000), melatonin (Jang et al., 2000), boldine (Obrosova et al., 2000), stobadine (Aragno et al., 1999). This indicates that the Momordica charantia extracts can reduce oxidative stress by boosting the activity of this enzyme.

Glutathione peroxidase activity is seen to be elevated in liver (Rauscher et al., 2000 ; Aragno et al., 1999; Sanders et al., 2001), kidney (Rauscher et al., 2000; Rauscher et al., 2001; Aragno et al., 1999; Sanders et al., 2001; Mekinova et al., 1995), aorta (Kocak et al., 2000), pancreas (Jang et al., 2000), blood (Mohan et al., 1998; Kedziora-Kornatowska et al., 1998), and red blood cells (Jang et al., 2000), whereas decreased activity was seen in heart(Kaul et al., 1996, Kaul et al., 1995) and retina (Stefek et al., 2000).. Reduced activities of GPx in diabetic rats have been observed in this study. The decrease may be due to increased production of

reactive oxygen radicals that can themselves reduce the activity of GPx enzyme. Treatment with glimepiride and M. charantia for four weeks increased GPx activity. Withdrawal of the extract decreased the GPx activity.

This present study has thus reaffirmed the possible potent hypoglycaemic effect of M. charantia in diabetic state and the strong potential to restore the antioxidant level in serum to within normal thus reversing the effects of oxidative stress in diabetic state. Thus suggesting that Momordica charantia could be used for the management of diabetes mellitus.

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