Indian Journal of Biochemistry & Biophysics
Vol. 47, August 2010, pp. 227-233

Anti-hyperglycemic and anti-oxidative effect of aqueous extract of *Momordica charantia* pulp and *Trigonella foenum graecum* seed in alloxan-induced diabetic rats

Uma Nath Tripathi and Deepak Chandra*
Department of Biochemistry, Lucknow University, Lucknow 226007, India

Received 13 October 2009; revised 12 April 2010

Diabetes is an oxidative stress disorder and oxidative damage to tissues such as heart, kidney, liver and other organs may be a contributory factor to several diabetic complications. *Momordica charantia* (family: Cucurbitaceae) and *Trigonella foenum graecum* (family: Fabaceae) are used traditionally in Indian folk medicine to manage diabetes mellitus. In the present study, the anti-hyperglycemic and anti-oxidative potential of aqueous extracts of *M. charantia* pulp and seed powder of *T. foenum graecum* were assessed in alloxan (150 mg/kg body weight) induced diabetic rats. Alloxan treatment to the rats could induce diabetes as the fasting blood glucose (FBG) levels were >280 mg/dl. Treatment of diabetic rats for 30 days with *M. charantia* and *T. foenum graecum* could significantly (p<0.001) improve the FBG levels to near normal glucose levels. Antioxidant activities (superoxide dismutase, catalase, reduced glutathione content and glutathione-s-transferase) and lipid peroxidation levels were measured in heart, kidney and liver tissues of normal, diabetic and experimental animals (diabetics + treatment). TBARS levels were significantly (p<0.001) higher and anti-oxidative activities were found low in diabetic group, as compared to the control group. Significant (p<0.001) improvement in both the TBARS levels and antioxidant activities were observed when *M. charantia* and *T. foenum graecum* were given to diabetic rats. Our results clearly demonstrate that *M. charantia* and *T. foenum graecum* are not only useful in controlling the blood glucose levels, but also have antioxidant potential to protect vital organs such as heart and kidney against damage caused due to diabetes induced oxidative stress.

Keywords: Alloxan, Diabetic rat, Anti-oxidative effect, *Momordica charantia*, *Trigonella foenum graecum*, Anti-hyperglycemic activity

Diabetes mellitus (DM) is the most common endocrine disease[1]. The worldwide prevalence of DM is expected to be more than 240 million by the year 2010[2]. DM leads to metabolic abnormalities and is characterized by hyperglycemia, resulting from defects in insulin secretion, insulin action or both[3-5]. It is associated with significant oxidative stress and the oxidative damage to tissues may be a contributory factor to several diabetic complications[6]. Some of the causes for the development of long-term complications in persistent hyperglycemia are the formation of glycated proteins, glucose auto-oxidation, and increased free fatty acids[7]. Reactive oxygen species (ROS) may also be involved in the initiation and development of vascular complications in diabetics[8]. Oxidative stress combined with mitochondrial dysfunction leads to the activation of inflammatory signaling pathways, which may damage insulin-producing cells and further aggrevate the complications of diabetes[9].

ROS generated due to oxidative stress in the cells can be scavenged by the enzymes involved in oxidative stress defense mechanism. Diabetes is known to induce changes in the activities of antioxidant enzymes, such as catalase (CAT), superoxide dismutase (SOD), glutathione reductase (GSSG-R), and glutathione peroxidase (GSHPx)[10,11]. There are many reports on perturbed plasma antioxidant levels in patients with diabetes and most of the studies indicate that supplementation of antioxidants provides greater protection against

*Corresponding author
Tel: 91-09415164388
E-mail: deepakvns@yahoo.com

Abbreviations: DM, diabetes mellitus; FBG, fasting blood glucose; GSH, reduced glutathione; GSHPx, glutathione peroxidase; GSSG-R, glutathione reductase; GST, glutathione-S-transferase; H_2O_2, hydrogen peroxide; MC, *Momordica charantia*; ROS, reactive oxygen species; SOD, superoxide dismutase; TBARS, thiobarbituric acid reactive substances; TFG, *Trigonella foenum graecum.*

oxidative stress-induced damages[12]. Several natural products, such as *Allium sativum, Azadirachta indica*, *Coccinia indica*, *Eugenia jambolana, Gymnema sylvestre*, *Momordica charantia*, *Pterocarpus marsupium*, *Trigonella foenum graecum* 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 have been 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) commonly referred to as bittergourd or Karela, (family: Cucurbitaceae) and *Trigonella foenum graecum* (TFG), commonly known as fenugreek or methi (family: Fabeaceae) are reported to have beneficial effects in management of diabetes[13-16], and also in other disease conditions. MC leaf extracts have been reported to possess antibacterial activity against *E. coli*, *Salmonella paratyphi, Shigella dysenterae* and *Streptomyces griseus* and fruit extracts have shown beneficial effects against *Helicobacter pylori* infection and gastric ulcers[17,18], The seed extracts of MC have shown inhibitory activity against the larvae of filarial vector *Culex quinquefasciatus*[19]. The TFG seed paste (applied externally) is used to treat abscess, boils, ulcers and burns and consumption of TFG seed powder possess beneficial effects against gastritis and gastric ulcers due to bacterial infections[20,21].

MC and TFG are commonly consumed in diet and have been reported to possess potent anti-hyperglycemic activity and anti-oxidative potential. Antioxidant and hypoglycemic potential in liver, kidney and pancreas have been reported in rats when treated with MC seed extract[22,23] and in plasma upon treatment with MC pulp[24]. Anti-diabetic effect of TFG seed powder has been reported in liver and kidney tissues[20]. Oxidative stress has been implicated in the pathogenesis of diabetes-related complications, such as cardiovascular, renal and nephropathy etc. and treatment with antioxidants seems to be a promising therapeutic option.

The reports on antioxidant potential of MC pulp and TFG seed extracts in heart tissue are not available. Most of the anti-diabetic drugs, including insulin are associated with side effects. Therefore, the administration of antioxidant from a natural origin may have a promising role in protection against cardiovascular and renal complication due to diabetes. In the present study, the anti-hyperglycemic and anti-oxidative effects of aqueous extract MC (fruit pulp) and TFG (seed) on different tissues (heart, liver and kidney) have been investigated in alloxan-induced diabetic rats.

Materials and Methods

Chemicals

Alloxan monohydrate, 5,5'-dithio-bis(2-nitrobenzeoic acid) or Ellman's reagent, epinephrine, mammalian protease inhibitor cocktail and glutathione were purchased from Sigma Chemical Co. Inc., St Louis, Mo, USA. All other chemicals used were of analytical grade and obtained from SRL (India) and Qualigens fine Chemicals (India).

Plant material and preparation of extract

MC fruits and TFG seeds were purchased from local market in Lucknow, India. Seeds were cleaned, dried and finely powdered. Fresh fruits of MC (250 g) were taken and the seeds were removed. The fleshy parts were cut into small pieces and macerated with 250 ml triple distilled water using electrical blender. This suspension was squeezed through a sterile muslin cloth, and the liquid was centrifuged at 8000 × *g* for 30 min in the cold. The supernatant was lyophilized at low temperature and reduced pressure by the method described previously[25] using CHRIST ALPHA 1-4 freeze dryer (from MARTIN CHRIST Gefriertrocknungsanlagen GmbH, An der unteren Söse 50, D-37520 Osterode, Germany). Lyophilized powder was resuspended in physiological saline and administered according to the dose mentioned above. 250 g of powdered TFG seeds were boiled in 2500 ml distilled water for 30 min and the decoction was cooled for 30 min at room temperature and filtered through a coarse sieve twice. Finally, the filtrate was concentrated by rotavapour (Buchi, R-210, Germany) at 50°C to a thick paste (weighing 100 g)[26].

Animals and treatment

Male albino wister rats weighing 120-150 g were obtained from Central Drug Research Institute (CDRI), Lucknow, India, and housed at about 25 ± 2°C in the animal room in the Department. They were provided a standard pelleted diet (Hindustan Lever Ltd., Mumbai, India) and had free access to water. Fourth day after alloxan injection (intra-peritoneal)[27], the development of diabetes was confirmed by measuring blood glucose levels. Rats

with fasting blood glucose (FBG) ranged above 280 mg/dl were considered as diabetic rats, which was in accordance with previous report[28].

Rats were divided into four groups containing 5 rats each viz: Group I or control were given sterile physiological saline; Group II or alloxan-induced diabetic control were given 150 mg alloxan/kg body weight, dissolved in sterile physiological saline[27]; Group III or diabetic-treated with TFG were given 9 g seed powder/kg body weight/once daily by oral gavage; and Group IV or diabetic-treated with MC were 13.33 g pulp/kg body weight/once daily by oral gavage were given.

The plant extracts were administrated orally by oral gavage to rats of respective groups (III and IV) once daily for 30 days. Normal and diabetic control animals (groups I and II) received the same amount of normal saline (0.9%). Prior permission for animal use and approval of the protocol were obtained from the CPCSEA, Animal Ethics Committee of University of Lucknow, Lucknow.

Preparation of tissue homogenate

For preparation of homogenate (10% w/v), tissues were washed thoroughly with ice cold saline and homogenized in a homogenizer (Potter-Elvehjem type) using ice-cold 50 mM phosphate buffer (pH 7.4) containing mammalian protease inhibitor cocktail from Sigma Chemical Co. [containing 4-(2-Aminorthyl) benzenesulfonyl fluoride or AEBSF, pepstatin A, E-64, bestatin, leupeptin and aprotinin]. The homogenates were centrifuged at 12,000 × *g* for 30 min at 4°C and the supernatant was used for assay of antioxidant enzymes and TBARS.

Biochemical assay

Animal were fasted overnight, blood samples were obtained by tail vain puncture of all groups of rats and fasting blood glucose (FBG) levels were determined using a one touch ultra glucometer (Johonson and Johonson Co., USA) 4th day after alloxan injection and 30 days after treatment. Urine glucose was assessed in fresh urine using glucose indicator sticks (Boehringer Mannheim, Germany).

Effect on antioxidant parameters

Catalase (CAT) and superoxide dismutase (SOD) activity

The activity of CAT was determined by the method of Sinha[29]. CAT was assayed colorimetrically at 570 nm. The reaction mixture in a total volume 1.6 ml contained 1.0 ml of 0.01 M, phosphate buffer (pH 7.0), 0.1 ml of tissue homogenate-supernatant and 0.5 ml of 2.0 M H_2O_2. The reaction was stopped by the addition of 2.0 ml dichromate acetic acid reagent (5% potassium dichromate and glacial acetic acid mixed in a 1:3 ratio). The activity was expressed as μmoles H_2O_2 decomposed/min/mg protein.

SOD activity was assayed by the method of Misra and Fridovich[30]. 3.0 ml reaction mixture contained 1.5 ml 0.1 M carbonate-bicarbonate buffer (pH 10.3), 0.1 ml 30 mM EDTA, suitable aliquot of enzyme preparation and water to make up the volume to 2.94 ml. The reaction was started by addition of 0.06 ml of 15 mM epinephrine. Change in absorbance was recorded at 480 nm for 1 min at 15 s interval. Control consisting of all the ingredients, except enzyme preparation was run simultaneously. One unit of enzyme activity was defined which caused 50% inhibition of auto-oxidation of epinephrine by 1.0 ml of homogenate.

GSH content and GST activity

The 0.2 ml homogenate was mixed in 0.3 ml precipitating regent (0.2 M glacial meta-phosphoric acid, 5.1 M NaCl and 5.9 m*M* EDTA). After centrifugation at 10,000 × *g* for 15 min, 0.2 ml supernatant was added to 0.8 ml 0.3 M Na_2HPO_4, followed by the addition of 0.1 ml of 0.04% DTNB prepared in 1% sodium citrate). The change in optical density (OD) at 412 nm was recorded using UV-vis spectrophotometer[31].

GST activity was determined using 1-chloro-2, 4-dinitrobenzene (CDNB) as a substrate[32]. The assay mixture contained 1 m*M* GSH, 1 m*M* CDNB and 100 m*M* phosphate buffer (*p*H 6.5). The reaction was started by the addition of enzyme in linearity range and the rate of increase in absorbance due to formation of CDNB conjugate of GSH was monitored at 340 nm for 3 min. The activity of GST was expressed as μmoles of GSH-CDNB conjugate formed/min/mg protein.

TBARS content

Lipid peroxidation was estimated in terms of MDA formed using thiobarbituric acid (TBA) reagent[33]. Briefly, to 0.2 ml of the homogenate, added 0.2 ml sodium dodecyl sulphate [8.1% (w/v)], 1.5 ml glacial acetic acid and thiobarbituric acid [0.8% (w/v)] and the final volume was made to 3.0 ml. The contents of the tubes were vortexed vigorously, heated in a water bath at 90°C for 1 h and then cooled immediately under running tap water. To each tube, 1.0 ml of

water and 5.0 ml of a mixture of *n*-butanol and pyridine (15:1, v/v) were added and the tubes were vortexed and centrifuged at 800 × *g* for 20 min. The upper layer was aspirated out to measure the color intensity at 532 nm and 1, 1, 3, 3 tetraethoxypropane (TEP) was used as reference.

Protein estimation

Protein was estimated by the method of Lowry *et al*[34] using bovine serum albumin as standard at 660 nm.

Statistical analysis

Results were expressed as mean ± S.E.M. Statistical analysis was carried out by using One-way-analysis-of-variance (ANOVA), followed by Newman-Keuls multiple comparison test. A values of $p<0.05$ were considered as significant.

Results

Effect of TFG and MC on blood and urine glucose and body weight

The blood glucose levels and urine glucose were measured in normal and alloxan-induced diabetic rats. The results are shown in Table 1. There was a significant ($p<0.001$) increase in blood glucose levels in diabetic rats (Group II). Administration of TFG and MC significantly ($p<0.001$ and $p<0.001$) decreased blood glucose levels in diabetic rat (Groups III and IV). Body weight was also decreased ($p<0.001$) in diabetic rats, while administration of TFG and MC increased ($p<0.001$ and $p<0.01$) body weight in alloxan-induced diabetic rats.

Effect of TFG and MC on antioxidant parameters

CAT and SOD activities

The activity of CAT in experimental rats tissues is shown in Table 2. There was a significant ($p<0.001$) reduction in the CAT activity in heart, liver and kidney ($p<0.001$) during diabetes. Administration of TFG increased the activity of CAT in heart, liver and kidney ($p<0.001$). MC treatment also increased the CAT activity in heart, liver and kidney $p<0.001$, $p<0.05$ and $p<0.01$) in groups III and IV. A significant reduction in the activity of SOD was observed in the heart, liver and kidney ($p<0.001$) in diabetic animals. Administration of TFG significantly increased the activity of SOD in heart, liver and kidney ($p<0.001$, $p<0.001$ and $p<0.01$ respectively). Similarly, in MC-treated diabetic group, SOD activity was increased in heart, liver and kidney ($p<0.001$, $p<0.01$ and $p<0.01$ respectively) (Table 2).

GSH contents and GST levels

There was a significant ($p<0.001$) decrease in the concentration of GSH in diabetic control group when compared with the normal. Administration of TFG significantly increased the levels of GSH in liver, heart and kidney ($p<0.001$) during diabetes and MC also increased GSH content in heart, liver and kidney ($p<0.001$, $p<0.05$ and $p<0.05$ respectively) (Table 2). The activity of GST in experimental rats tissues is shown in Table 2. There was a significant reduction in the GST activity in heart, liver and kidney ($p<0.001$) during diabetes. Administration of TFG and MC increased the activity of GST in heart, liver and kidney ($p<0.001$) to near normal.

TBARS levels

The levels of TBARS in different tissues are shown in Table 2. There was significant elevation of TBARS in diabetic rats, when compared to normal rats. It was found that TBARS levels were significantly decreased in the heart, liver and kidney ($p<0.001$) in TFG and MC-treated animals (groups III and IV).

Table 1—Effect of MC and TFG on fasting blood glucose level, urine glucose and body weight

[Values represent mean ± S.E.M., n = 5]

Groups	FBG (mg/dl)		Urine glucose		Body weight (g)		% of control at 0 day*
	0 day*	30th day	0 day*	30th day	0 day*	30th day	
Control	84.8 ± 3.9	88.6 ± 4.6	-	-	122.2 ± 3.5	137.5 ± 3.5	112.52
Diabetic	292.4 ± 6.5^{a}	322 ± 10.83^{a}	+	+	121.0 ± 1.3	106.0 ± 3.8^{a}	87.60
Diabetic + TFG	295.2 ± 4.7^{a}	90.4 ± 8.7^{a}	+	-	121.8 ± 2.7	126.0 ± 2.9^{a}	104.13
Diabetic + MC	289.2 ± 4.6^{a}	112.6 ± 8^{a}	+	-	122.6 ± 4.3	123.0 ± 2.9^{b}	100.81

Values are statistically significant at ($p<0.001^{a}$ and $p<0.01^{b}$). Diabetic control rats were compared normal control (group I) rats. Experimental groups (III and IV) were compared to diabetic control (group II). The experiments were repeated thrice. * = 4th day after alloxan injection, on the day when alloxan injection was given the blood glucose levels and body weight were same as on 0 day*.

Table 2—Effect of MC and TFG on anti-oxidant parameters in different tissues

[Values represent mean ± S.E.M. n = 5]

Tissue and enzyme	Control (Group I)	Diabetic (Group II)	Diabetic + TFG (Group III)	Diabetic + MC (Group IV)
Heart				
Catalase	88.37 ± 3.22	72.45 ± 2.46[a]	85.37 ± 2.69[a]	101.9 ± 5.7[a]
SOD	29.19 ± 1.6	6.52 ± 1.32[a]	19.75 ± 1.35[a]	14.3 ± 1.44[a]
GST	6.46 ± 0.38	3.69 ± 0.39[a]	5.56 ± 0.29[a]	4.53 ± 0.3[b]
GSH	0.93 ± 0.12	0.48 ± 0.03[a]	1.14 ± 0.09[a]	0.99 ± 0.1[a]
TBARS	34.22 ± 6.8	84.74 ± 16.9[a]	40.9 ± 8.15[a]	48.34 ± 3.47[a]
Liver				
Catalase	53.98 ± 3.8	31.23±3.02[a]	46.63 ± 1.59[a]	46.89 ± 2.21[c]
SOD	61.74 ± 3.86	21.13 ± 1.37[a]	39.61 ± 1.45[a]	35.27 ± 1.48[b]
GST	9.91 ± 0.4	4.96 ± 0.26[a]	8.14 ± 0.16[a]	7.08 ± 0.17[a]
GSH	0.71 ± 0.02	0.42 ± 0.06[a]	0.68 ± 0.03[a]	0.6 ± 0.02[b]
TBARS	161.60 ± 16.11	459.50 ± 27.82[a]	209.00 ± 23.44[a]	222.4 ± 25.06[a]
Kidney				
Catalase	46.4 ± 2.08	39.37 ± 1.57[a]	46.10 ± 2.5[a]	44.18 ± 2.09[b]
SOD	28.16 ± 2	9.51 ± 1.26[a]	23.12 ± 1.06[b]	20.33 ± 0.80[b]
GST	26.13 ± 2.01	9.62 ± 0.67[a]	22.34 ± 0.75[a]	20.03 ± 0.69[a]
GSH	0.72 ± 0.06	0.44 ± 0.04[a]	0.83 ± 0.06[a]	0.62 ± 0.03[a]
TBARS	30.96 ± 6.81	76.59 ± 4.46[a]	39.11 ± 1.62[a]	52.14 ± 4.46[a]

Values are statistically significant at ($p<0.001^a$, $p<0.01^b$ and $p<0.05^c$). Diabetic control rats were compared normal control (group I) rats. Experimental groups (III and IV) were compared with diabetic control (group II). The experiments were repeated thrice. The CAT activity was expressed as μmoles H_2O_2 decomposed/min/mg protein. One unit of SOD activity was defined as that causeed 50% inhibition of auto-oxidation of epinephrine by 1.0 ml of homogenate. The activity of GST was expressed as μmoles of GSH-CDNB conjugate formed/min/mg protein. CAT, SOD and GST activities as expressed as U/mg protein GSH as μmoles/mg protein and TBARS levels as nmoles/g tissue.

Discussion

The present study was designed to evaluate beneficial effect of the two commonly used natural products i.e. MC and TFG in Indian population on anti-oxidant status and anti-hyperglycemic activity in alloxan-induced diabetic rats. Our preliminary studies showed non-toxic nature of MC pulp (13.33 and 65 g/kg body weight) and TFG seed extracts (9 and 45 g/kg body weight) on normal rats. The rats were fed with these doses via oral route for 60 days. There was no morbidity and all the rats showed normal growth (gain in body weight), similar to that of normal rats. Effect of these plant extracts were observed on kidney and liver function. No significant deviation from control values was observed in serum urea and creatinine values in all the groups. Similarly, no significant changes from normal control rats were observed in serum transaminases ALT and AST and alkaline phosphatase levels. It has been reported that alloxan-induced diabetic animals may exhibit most of the diabetic complication mediated by oxidative stress[35]. The mechanism of diabetes induction due to alloxan involves free radical-mediated destruction of pancreatic β-cells[36].

Alloxan-induced diabetes is characterized by severe loss in body weight[37] and the same was also observed in present study (Table 1). Treatment of MC (pulp) and TFG (seeds) extract could control this weight loss. The treatment of alloxan resulted in increase of FBG levels from 88.6 ± 4.6 mg/dl to 322 ± 10.83 mg/dl (on day 30th). The treatment of TFG and MC normalized the blood glucose levels to 90.4 ± 8.7 and 112.6 ± 8.0, respectively.

A significant decrease in the activities of antioxidant enzymes, such as SOD, CAT and GST in different tissues (heart, liver and kidney) from diabetic rats indicated the extent of oxidative stress or free radical-induced damage due to high blood glucose levels. The administration of MC and TFG in diabetic rats improved the status of the antioxidant enzymes in different tissues, suggesting thier role in improving the antioxidant activities in DM. The improved SOD and CAT activities have been reported in RBCs from diabetic rats treated with MC pulp extracts[24], in liver, pancreas and plasma of diabetic rats treated with MC[22,23] and TFG seed extracts[38].

Reduced glutathione (GSH) functions as a direct free radical scavenger, co-substrate for glutathione

peroxidase activity, co-factor of many enzymes and forms conjugates in endo- and xenobiotic reactions[39]. It is also involved in the protection of thiol disulfide in number of proteins[40]. The distinct decrease in GSH content in diabetic animals and its subsequent attainment of near normal values after administration of MC pulp and TFG seed extracts to diabetic rats were observed. This suggested that the protection was offered by MC and TFG in combating oxidative stress due to diabetes (Table 2). These observations were in accordance with previous reports in different tissues and plasma[22-24,38].

Free radical-mediated tissue damage occurs in the progression of DM[37]. Impaired insulin secretion may evoke lipid peroxidation[41,42]. Present study showed that TBARS levels in heart, liver and kidney were significantly lower in the MC pulp and TFG seed extracts-treated groups, compared to diabetic control group, suggesting that their administration might improve antioxidant activities and protect the tissues from lipid peroxidation (Table 2). The increased TBARS levels in erythrocytes[24], liver and kidney[22], plasma and pancreas have been reported in diabetic rats[23]. Earlier studies have shown that the treatment of MC pulp extract decreases TBARS levels in RBCs[24], and MC seed extract decreases TBARS levels in liver, kidney[22], plasma and pancreas[23]. Increased serum TBARS levels in alloxan induced-diabetic rats have been reported to be significantly decreased by TFG seed extracts[38]. The data of present study in heart, kidney and liver tissues suggested the protective role of MC and TFG seeds in complications resulted due to diabetes induced oxidative stress in these tissues.

In conclusion, the anti-oxidative potential of aqueous extracts of *Momordica charantia* (fruit pulp) and *Trigonella foenum graecum* (seeds) may be attributed mainly due to the amelioration of hyperglycemia-induced oxidative stress by their anti-hyperglycemic effect. An alteration in normal glycemic and antioxidant parameters may be responsible for diabetes-related complications (retinopathy, neuropathy and nephropathy). Therefore, it is suggested that these natural products may help not only in glycemic control and resisting oxidative insult due to diabetes, but also in minimizing/delaying the development of secondary complications associated with diabetes.

References

1 Sexton W J & Jarow J P (1997) *Urology* 49, 508-513

2 Amos A F, McCarty D J & Zimmet P (1997) *Diabetes Med* 14 (Suppl 5), S1-S85

3 Atkinson M A & Maclaren N K (1994) *N Engl J Med* 331, 1428-1436

4 Yki-Jarvinen H (1994) *Lancet* 343, 91-95

5 Teixeira C C, Rava C A, Mallman da Silva P, Melchior R, Argenta R, Anselmi F, Almeida C R & Fuchs F D (2000) *J Ethnopharmacol* 71, 343-347

6 Kakkaar R, Kalra J, Mantha S V & Prasad K (1995) *Mol Cell Biochem* 151, 113-119

7 Devi G, Falco A & Patrono C (2005) *Antioxid Redox Signal* 7, 256-268

8 Lapolla A & Fedele D (1993) *Minerva Endocrinol* 18 (3), 99-108

9 Fridlyand L E & Philipson L H (2004) *Diabetes* 53, 1942-1948

10 Scott J A & King G L (2004) *Ann N Y Acad Sci* 1031, 204-213

11 Jabeen R & Saleemuddin M (2006) *Biotechnol Appl Biochem* 43, 49-53

12 Segal K R (2004) *Dis Manag* 7, S11-22.

13 Chandra A, Mahdi A A, Singh R K, Mahdi F & Chander R (2008) *J Med Food* 11, 506-512

14 Shih C C, Lin C H, Lin W L & Wu J B (2009) *J Ethnopharmacol* 123, 82-90

15 Nahas R & Moher M (2009) *Can Fam Physician* 55, 591-596

16 Kannappan S & Anuradha C V (2009) *Indian J Med Res* 129, 401-408

17 Grover J K & Yadav S P (2004) *J Ethnopharmacol* 93, 123-132

18 Alam S, Asad M, Asdaq S M & Prasad V S (2009) *J Ethnopharmacol* 123, 464-469

19 Batabval L, Sharma P, Mohan L, Maurya P & Srivastava C N (2009) *Parasitol Res* 105, 1205-1210

20 Abou El-Soud N H, Khalil M Y, Hussein J S, Oraby F S H & Farrag A R H (2007) *J App Sci Res* 3, 1073-1083

21 Tripathi U N, Jamal F & Chandra D (2007) *Nat J Life Sci* 4, 205-207

22 Sathishsekar D & Subramanian S (2005) *Asia Pac J Clin Nutr* 14, 153-158

23 Garg M, Dhar V J & Kalia A N (2008) *Phcog Mag* 4, 138-143

24 Sochor M, Baquer N Z & MCLean P (1985) *Mol Physiol* 7, 51-68

25 Karunanayake E H, Jeevathayaparan S & Tennekoon K H (1990) *J Ethnopharmacol* 30, 199-204

26 Xue W L, Li X S, Zhang J, Liu Y H, Wang Z L & Zhang R J (2007) *Asia Pac J Clin Nutr* 16, 422-426

27 Sinha A K (1972) *Anal Biochem* 47, 389-394

28 Misra H P & Fridovich I (1972) *J Biol Chem* 247, 3170-3175

29 Chandra D, Ramana K V, Wang L, Christensen B N, Bhatnagar A & Srivastava S K (2002) *Invest Ophthalmol Vis Sci* 43, 2285-2292

30 Habig W H, Pabst M J & Jakoby W B (1974) *J Biol Chem* 249, 7130-7139

31 Ohkawa H, Ohishi N & Yagi K (1979) *Anal Biochem* 95, 351-358

32 Lowry O H, Rosebrough N J, Farr A L & Randall R J (1951) *J Biol Chem* 193, 265-75
33 Ozturia Y, Altan V M & Yildizoglu A (1996) *Pharma Revi* 48, 69-112
34 Tomlinson KC, Gardiner S M, Hebden R A & Bennett T (1992) *Pharmacol Rev* 44, 103
35 Genet S, Kale R K & Baquer N Z (2002) *Mol Cell Biochem* 236, 7-12
36 Parinandi N L, Thomson E W & Schmid H H O (1990) *Biochem Biophys Acta* 1047, 63-69
37 Fernandes N P C, Lagishetty, Panda V S & Naik S R (2007) *BMC Complement Altern Med* 7, 1-8
38 Abou El-Soud N H, Khalil M Y, Hussein J S, Oraby F S H & Farrag A R H (2007) *J Appl Sci Res* 3, 1073-1083
39 Oberley L W (1988) *Free Radic Biol Med* 5, 113
40 Baynes J W (1995) *Mechanistic approach to diabetes*. In: Inoannides C, Flatt F R, eds., Eths Horwood Ltd, 203, pp 231-243
41 Chandra A, Mahdi A A, Ahmad S & Singh R K (2007) *Nutr Res* 27, 161-168
42 Sathishsekar D & Subramanian S (2005) *Biol Pharm Bull* 28, 978-983