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Full Length Research Paper

Protective effects of *Chrysanthemi Flos* extract against streptozotocin-induced oxidative damage in diabetic mice

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The present study was aimed to evaluate the protective role of *Chrysanthemi Flos* extract (CFE) against free radical-mediated oxidative damage in liver and kidney of streptozotocin (STZ) - induced diabetic mice. *Chrysanthemi Flos* was extracted with methanol. Male ICR mice (n = 24) were divided into four groups. Diabetes was induced by STZ injection (ip) at a dose of 100 mg/kg. Animals of treated groups were given CFE and gliclazide (1000 and 50 mg kg^{-1} body weight) for 30 days. The results revealed that oral administration of CFE and gliclazide inhibited the increase in lipid peroxidation, aspartate transaminase (AST), alanin transaminase (ALT) and alkaline phosphatase (ALP) activities caused by STZ. Besides, a significant amelioration in antioxidant enzymes like superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) was observed. On the other hand, non enzymic antioxidant such as reduced glutathione (GSH) and ascorbic acid level was significantly increased while α-tocopherol level was decreased. Furthermore, CFE caused significant restoration in DNA fragmentation induced by STZ. These findings suggest that CFE has protective effects against STZ induced oxidative damage in liver and kidney of diabetic mice.

Key words: Antioxidant, diabetes, DNA damage, *Chrysanthemi Flos*, lipid peroxidation.

INTRODUCTION

Diabetes mellitus is the most prevalent serious metabolic disorder that is considered to be one of the five leading causes of death in the world (Rahimi et al., 2005). This metabolic disorder affects approximately 4% of the population worldwide and is expected to rise by 5.4% in 2025 (Kim et al., 2006). Numerous studies have reported that diabetes mellitus is associated with oxidative stress, leading to an increased production of reactive oxygen species (ROS), including superoxide radical (O_2•−), hydrogen peroxide (H_2O_2), and hydroxyl radical (OH•) or diminution of antioxidant defense system (Vincent et al., 2004). Inference of oxidative stress in the pathogenesis of diabetes mellitus is suggested not only by oxygen free radical generation but also due to non-enzymatic protein glycosylation, auto-oxidation of glucose; weaken antioxidant enzyme, and formation of peroxides (Vincent et al., 2004; Pari and Latha, 2005). Lipid peroxidation (LPO) is a key marker of oxidative stress. It is a free radical-induced progression causing oxidative worsening of polyunsaturated fatty acids that eventually consequences in extensive membrane damage and dysfunction. The noteworthy extent of LPO products that was measured as thiobarbituric acid reactive substances (TBARS) has been reported in diabetes (Pari and Latha, 2005; Rajasekaran et al., 2005). Non-enzymatic and uncontrolled oxidation of biomolecules by ROS impairs the structural and functional integrity of DNA. ROS may play a major role as endogenous initiators and promoters of DNA damage and mutations that contribute to diabetes (Wiseman and Halliwell, 1996). Streptozotocin (STZ), a well-known genotoxic agent, was used as the ROS generating system. STZ caused oxidative damage may involve single and double-strand DNA breakages, base

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modifications, abasic sites, fragmentation of deoxyribose and formation of DNA–protein cross-links (Cooke et al., 2003). Severe DNA damage by STZ results in cell death by apoptosis or necrosis (Bolzan and Bianchi, 2002).

The formation of ROS was prevented by an antioxidant system that included non-enzymatic antioxidants (glutathione, vitamins C and E), enzymes rejuvenating the reduced forms of antioxidants, and ROS–scavenging enzymes such as superoxide dismutase, catalase, and glutathione peroxides (Zhang and Tan, 2000). Several plant products are known to exhibit potent antioxidant activities in the prevention and treatment of diabetes (Xie et al., 2003; El-Alfy et al., 2005). In this view, we planned to evaluate the protective effects of *Chrysanthemi Flos* against STZ-induced oxidative damage through its antioxidant potency.

Chrysanthemi Flos (CF) is the flower of *Chrysanthemum indicum Linn.* or *Chrysanthemum morifolium Ramatuelle*. It is used for the treatment of a wide spectrum of ailments including antipyretic, anti-inflammatory, antihypertensive and neurasthenic head-ache in Korean traditional medicine (Yook, 1989). CF is also very common in traditional Chinese medicine, which is used as a heat-clearing and detoxication herb (Deng et al., 2006). It restrain the agglutination of blood platelet and enhance myocardial blood circulation and white cell phagocytosis, which has been used to cure diseases, such as furuncle and skin nodules (Zhang, 1997) Besides, CF has hypolipidemic, hepatoprotective, cardioprotective and inhibitory activity against nitric oxide (NO) production in lipopolysaccharide-activated macro-phages (Yoshikawa et al., 2000; Choo et al., 2004; Jiang et al., 2005). Earlier studies showed that the extract of CF had the antibacterial and dephlogisticate activity, in which three active phenolic compounds including chlorogenic acid, linarin, and luteolin were identified. Chlorogenic acid has antibacterial, antiphlogistic, antimutagenic, anti-oxidant and other biological activities (Nakamura et al., 1997). Linarin and luteolin belong to flavonoids, which are used as medicine because of their antiphlogistic, spasmolytic, good antioxidant and free radical scaveng-ing properties (Cao et al., 1997).

Dai et al. (2001) demonstrated the total flavonoids and main component of the flavonoids from CF, including luteolin-7-*O*-β-D-glucoside, apigenin-7-*O*-β-D-glucoside and acacetin-7-*O*-β-D-glucoside have hypotensive effect on rat under normal condition. CF also used for the treatment of prostatitis and chronic pelvic inflammation (National, 2005). Furthermore, although many aspects of CF induced protection are studied, its efficacy in protecting liver and kidney against STZ-induced free radical-mediated damage has not been demonstrated so far. We hypothesized that CF may protect liver and kidney cell against STZ-induced oxidative stress and resulting DNA damage and dysfunction. Possibly, CF would impart some protection against oxidative damage to liver and kidney cells, but it would be of interest to investigate the mode of CF persuaded cytoprotection. In this study, we have investigated the protective action of CFE on STZ-induced liver and kidney cell damage in diabetic mice. We showed here the CFE treatment for 30 days against STZ-induced liver and kidney cell damage and dysfunction by virtue of its free radical scavenging activity.

MATERIALS AND METHODS

Animals

Male ICR mice weighting 20 to 30 g were obtained from Dai-Han Experimental Animals, Seoul, South Korea. The mice were housed in an air-conditioned room at 25±1°C with a lighting schedule of 12 h light and 12 h dark. A standard pellet diet and tap water were supplied *ad libitum*. Throughout the experiments, animals were processed according to the suggested ethical guidelines for the care of laboratory animals. After one week of acclimation period, animals were used for the experiment.

Drugs and chemicals

All the drugs and biochemicals used in this experiment were purchased from Sigma Chemical Company Inc., St Louis, MO, USA. The chemicals were of analytical grade.

Plant material

Chrysanthemi Flos was purchased from Kyung-Dong Oriental Medicine Market, Seoul, South Korea. A voucher specimen (No. 4N-006) was deposited at the herbarium of the Department of Pharmacology, School of Dentistry, Kyung Hee University, Seoul, South Korea.

Preparation of plant extract

Extraction and fractionation of CF were performed as described by Harbone (1998). Briefly, CF (250 g) was cut into small pieces and extracted with 70% methanol (750 ml) for three hours (three times). The resulting methanol extract was concentrated by a vacuum evaporator (COSMOS-660, Kyung Seo Machine Co., Incheon, Korea) and dried in freeze-dryer and used for the present study. The extract was standardized for the content of total flavonoids using rutin as a reference using a colorimetric analysis (Miliauskas, 2004) and found to contain total flavonoids estimated as rutin equivalents (28 mg rutin /g extract).

Briefly, 1 ml of plant extract in methanol (10 mg/ml) was mixed with 1ml aluminum trichloride in ethanol (20 mg/ml) and diluted with ethanol to 25 ml. The absorption at 415 nm was read after 40 min at 20°C. Blank samples were prepared from 1 ml plant extract and one drop of acetic acid, and diluted to 25 ml. Rutin calibration curve was prepared in ethanolic solution with same procedure.

Induction of diabetes

A freshly prepared solution of STZ (100 mg/kg, body weight) in 0.1 M citrate buffer (pH 4.5) was injected intraperitonealy in a volume of 1 ml/kg. STZ injected animals exhibited massive glycosuria and hyperglycemia within 2 days (Siddique et al., 1989). Diabetes was confirmed in STZ mice by measuring the fasting blood glucose concentration 96 h after the injection of STZ. The mice with blood

Table 1. Effect of CFE on body weight in diabetic mice.

Parameter	Initial body weight (g)	Final body weight (g)
Control	23.08 ± 1.91	28.38 ± 3.39
Diabetic	24.03 ± 1.55^{NS}	24.92 ± 1.66*
Diabetic + CFE	24.02 ± 1.60^{NS}	27.95 ± 2.19*
Diabetic + Gliclazide	24.32 ± 1.11^{NS}	28.18 ± 1.51*

Values are expressed as mean ±SD. (n = 6). Diabetic mice were compared with control group. CFE and Gliclazide treated groups were compared with their respective diabetic group. $^{*}p < 0.05$ and NS: Non significant.

glucose level >200 mg/dl were considered to be diabetic and were used in the experiment.

Experimental procedure

The animals (n = 24) were randomly divided into four groups of six animals each as given subsequently. The gliclazide and CF extract were administered in aqueous solution (1%, v/v Tween 80 in water) once per day using an oral gavage: Group I, normal control (aqueous solution); Group II, diabetic control (aqueous solution); Group III, diabetic-CFE (1000 mg kg^{-1}); Group IV, diabetic gliclazide (50 mg kg^{-1}). All treatments continued for 30 days. On the evening of days 30, all mice were fasted overnight (16 h) and weighed the body weight.

Blood samples were collected via jugular vein under ether anesthesia. Blood samples were centrifuged at 4500×g for 10 min to obtain serum. Liver and kidney were dissected out and rinsed in ice-cold saline to remove the blood, patted dry, weighed immediately and stored at −80°C for various assays. Before analyzing, the liver and kidney was homogenized in 50 mM phosphate buffer solution (pH 7.4) using a tissue homogenizer at 4°C. The homogenates were centrifuged at 15,000 × g for 20 min and supernatant was used for analyses.

Biochemical analysis

Lipid peroxidation (LPO) was assessed by measuring the concentration of malondialdehyde (MDA). The pink chromogen produced by the reaction of MDA, a secondary product of LPO with thiobarbituric acid was estimated at 535 nm. The results were expressed as nmol MDA per mg protein (Beuge and Aust, 1978). The activities of AST, ALT and ALP were determined by commercially available kits as per the manufacturer's instructions.

SOD activity was assayed by the method of Kakkar et al. (1984). The assay was based on the 50% inhibition of the formation of NADH-phenazinemethosulphate-nitroblue-tetrazolim (NBT) formazan at 520 nm. The activity of CAT was assayed by the method of Sinha (1972). In this method, dichromate in acetic acid was reduced to chromic acetate when heated in the presence of H_2O_2, with the formation of perchloric acid as an unstable intermediate.

The chromic acetate thus produced was measured colourimetrically at 610 nm. The activity of GPx was assayed by the method of Rotruck et al. (1973). A known amount of enzyme preparation was incubated with H_2O_2 in the presence of GSH for a specified time period. The amount of H_2O_2 utilized was determined by the method of Ellman. The enzyme activity was expressed as µg of GSH consumed/min/mg protein.

Glutathione (GSH) in tissues and serum was assayed by the method of Ellman (1959). GSH estimation is based on the development of yellow color when 5,5'-dithiobis (2-nitro benzoic acid) di-nitrobisbenzoic acid was added to compounds containing sulphydryl group. Ascorbic acid was estimated by the method of Omaye et al. (1979). Ascorbic acid was oxidized by copper to form dehydroascorbic acid and diketoglutaric acid. These products were treated with 2,4-dinitrophenylhydrazine (DNPH) to form the derivative of bis-2,4-dinitrophenylhydrazine. This compound in strong sulphuric acid undergoes rearrangements to form a product with an absorption band that is measured at 520 nm. α-Tocopherol was estimated by the method of Baker et al. (1980). α–Tocopherol was extracted and mixed with 2,2^{-1}-dipyridyl and ferric chloride. This mixture was kept in dark for 5 min and added butanol. Then the absorbance was read at 520 nm. Proteins were estimated by the method of Lowry et al. (1951) using bovine serum albumin as the standard.

DNA fragmentation analysis

DNA fragmentation was assessed by the modified method of Wang et al. (2007). Briefly, the liver and kidney tissues were homogenized in lysis buffer (pH 8.0) containing 100 mM Tris, 20 mM EDTA, 0.8% SDS and incubated with proteinase K (0.4 µg/ml) at 50°C for 3 h. Then mixed with phenol: chloroform: isoamyl alcohol (25:24:1) and centrifuged at 13,000 rpm for 10 min. To the resulting aqueous phase, 2 volumes of ice-cold absolute ethanol and 1/10th volume of 3 M sodium acetate were added and incubated for 30 min on ice to precipitate DNA. DNA was pelleted by centrifuging at 13,000 rpm for 10 min at 4°C, the supernatant was aspirated and the pellet was washed with 1 ml of 70% ethanol. The extracted DNA was resuspended in Tris-EDTA buffer and 5 µg of DNA was electrophoretically separated on 1.5% agarose gel, stained with ethidium bromide then DNA pattern was examined by ultraviolet transillumination.

Statistical analyses

All data were expressed as mean±S.D. Statistical analysis was performed with one-way ANOVA followed by LSD post hoc test for multiple comparisons. $P < 0.05$ was considered as significant.

RESULTS

Effect of *Chrysanthemi Flos* extract (CFE) on body weight

Table 1 shows the average weight gained by the animals during the total experimental period of 30 days. The weight gain was significantly reduced in diabetic mice (p<0.05) as compared to the control mice. Exogenous CFE and gliclazide administration to the diabetic mice showed a significant gain (p<0.05) in weight during the

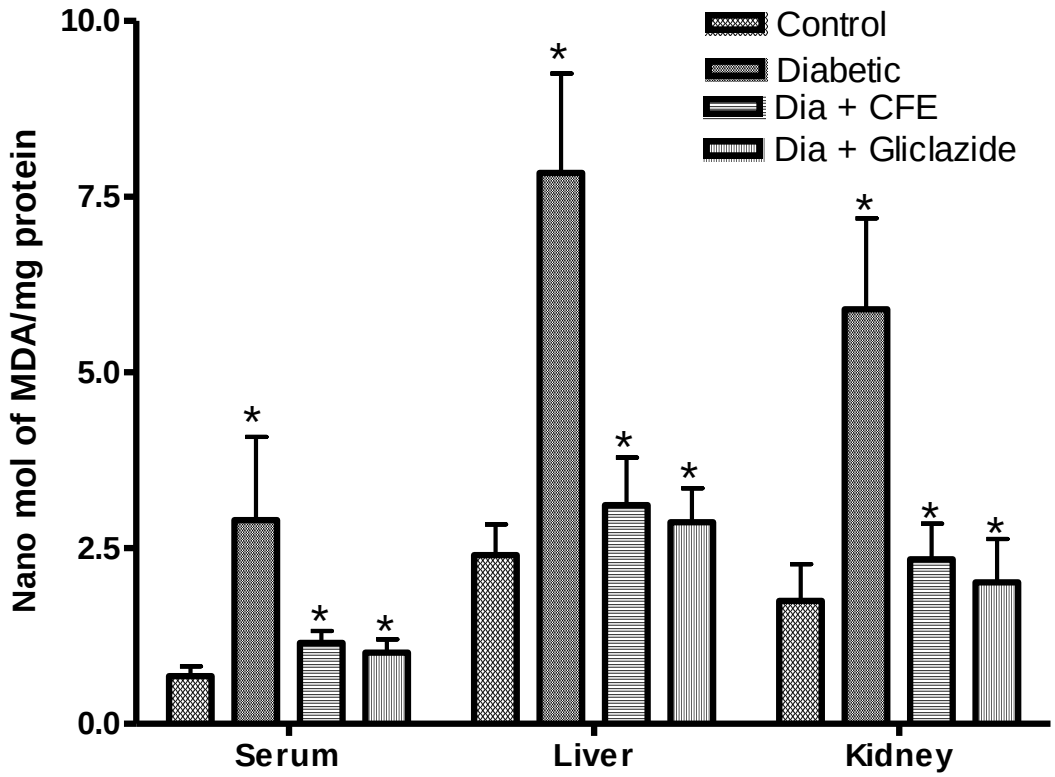

Figure 1. Effect of CFE on serum, liver and kidney MDA level in STZ-induced diabetic mice. Values are expressed as means ±SD. (n = 6). Diabetic mice were compared with control group. CFE and gliclazide treated groups were compared with their respective diabetic group. *p < 0.001.

experimental period compared with the untreated diabetic mice.

Effect of CFE on lipid peroxidation

Streptozotocin (STZ) produced a significant elevation in serum, liver and kidney MDA levels in diabetic mice (p<0.001) as compared with control mice. Administration of CFE and gliclazide significantly decreased (p<0.001) the value of MDA production in diabetic mice as compared to untreated diabetic mice. The results are shown in Figure 1.

Effect of CFE on aspartate transaminase (AST), alanin transaminase (ALT) and alkaline phosphatase (ALP)

The activities of serum AST, ALT and ALP of control and experimental mice are given in Figure 2. AST, ALT and ALP activities were significantly elevated in STZ- induced diabetic mice (p<0.001) as compared with control mice. Treatment with CFE and gliclazide showed significant reduction (p<0.001) in the activities of AST, ALT and ALP in diabetic mice as compared with untreated diabetic mice.

Effect of CFE on enzymic antioxidant

Data are shown in Table 2. During diabetes, there was a significant reduction (p<0.001) in the activities of SOD, CAT and GPx were observed in the liver and kidney of diabetic mice as compared with the control mice. The decreased activity of SOD, CAT and GPx in the diabetic mice were significantly restored (p<0.001) by CFE and gliclazide treatment as compared with untreated diabetic mice.

Effect of CFE on non enzymic antioxidant

α-Tocopherol level was significantly elevated in diabetic mice (p < 0.001) as compared with that of the control. CFE and gliclazide treatment of diabetic mice caused a significant reduction (p<0.001) of α-tocopherol as compared with untreated diabetic mice. Ascorbic acid and GSH level was reduced markedly (p<0.001) in diabetic mice when compared with that of the control mice. CFE and gliclazide treatment showed significant amelioration (p<0.001) in ascorbic acid and GSH

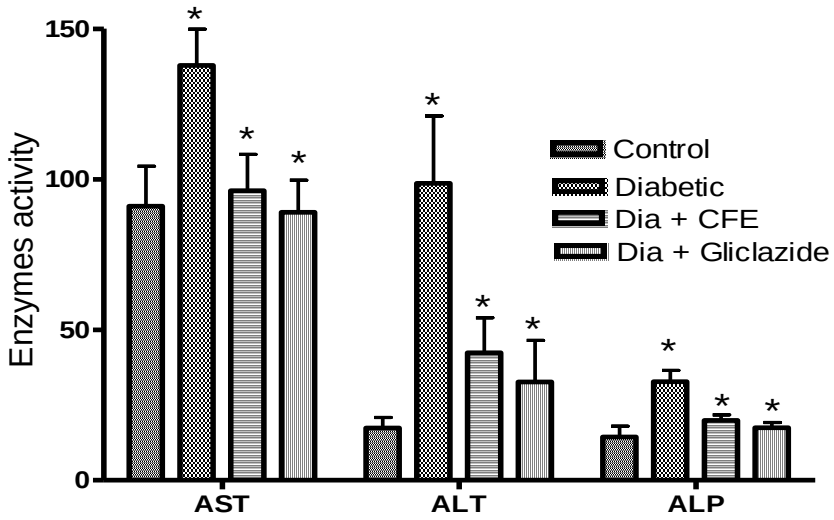

Figure 2. Effect of CFE on serum AST, ALT and ALP activities in STZ-induced diabetic mice. AST and ALT activities were expressed as IU/L; ALP activity was expressed as KA unit. Values are expressed as means ± SD. (n = 6). Diabetic mice were compared with control group. CFE and Gliclazide treated groups were compared with their respective diabetic group. *p < 0.001.

Table 2. Effect of CFE on enzymatic antioxidant.

Parameter	Control	Diabetic	Diabetic + CFE	Diabetic + Gliclazide
Liver				
SOD (units[a])	3.94 ± 0.37	2.05 ± 0.48***	3.06 ± 0.36 ***	3.07 ± 0.28***
CAT (units[b])	7.59 ± 0.77	4.47 ± 0.52***	6.49 ± 0.59***	6.63 ± 0.51***
GPx (units[c])	5.72 ± 0.38	3.31 ± 0.37***	4.77 ± 0.46***	5.10 ± 0.40***
Kidney				
SOD (units[a])	3.75 ± 0.55	2.08 ± 0.40***	3.08 ± 0.27***	3.41 ± 0.40***
CAT (units[b])	3.90 ± 0.47	2.63 ± 0.42***	3.26 ± 0.45*	3.32 ± 0.36**
GPx (units[c])	3.08 ± 0.40	1.34 ± 0.30***	2.24 ± 0.51***	2.42 ± 0.46***

Values are expressed as mean ±SD. (n = 6). Diabetic mice were compared with control group. CFE and gliclazide treated groups were compared with their respective diabetic group. ***p <0.001, **p < 0.01 and *p < 0.05. [a]50% inhibition of NBT reduction/min/mg protein; [b]µmol of H_2O_2 consumed/min/mg protein; [c]µmol of GSH consumed/min/mg protein.

concentration in diabetic mice as compared with untreated diabetic mice. The results are expressed in Table 3.

Effect of CFE on DNA fragmentation

DNA fragmentation pattern was examined in the control and experimental groups of liver and kidney (Figures 3 and 4). Diabetic mice treated with CFE showed a significant decrease in DNA fragmentation compared with untreated diabetic mice, whereas there was no difference in its laddering pattern in the gliclazide treated diabetic mice as compared with control. These observations suggest that CFE treatment appears to significantly suppress the progression of apoptosis in the liver and kidney of diabetic mice.

DISCUSSION

Oxidative stress is suggested to be a potential donor to the progress of complication in diabetes. Elevated free radical production or diminished antioxidant defense responses, both of which occur in the diabetic state may give rise to increased oxidative stress. Consequences of oxidative stress are alteration or cell injury, that is, damage to DNA, proteins and lipids, disruption in cellular homeostasis and accumulation of damaged molecules (Jakus, 2000). Reduced oxidative stress in the

Table 3. Effect of CFE on non enzymatic antioxidant.

Parameter	Control	Diabetic	Diabetic + CFE	Diabetic + Gliclazide
Serum				
GSH (mg/dl)	30.75 ± 3.46	18.70 ± 1.49***	27.01± 3.56***	28.89 ± 2.09***
Ascorbic acid (mg/dl)	0.97 ± 0.22	0.31± 0.09***	0.76 ± 0.12***	0.82 ± 0.10***
α-Tocopherol (mg/dl)	4.68 ± 1.19	13.10 ± 1.84***	6.49 ± 1.22***	5.84 ± 0.99***
Liver				
GSH (nmol/mg protein)	3.99 ± 0.93	1.17 ± 0.44***	3.09 ± 0.34***	3.01 ± 0.63***
Ascorbic acid (mg/100g tissue)	0.67 ± 0.07	0.38 ± 0.07***	0.60 ± 0.09***	0.64 ± 0.10***
α-Tocopherol (mg/100g tissue)	0.72 ± 0.16	1.26 ± 0.31***	0.99 ± 0.14 *	0.95 ± 0.18*
Kidney				
GSH (nmol/mg protein) 0.39***	1.65 ± 0.41	0.74 ± 0.13***	1.25 ± 0.15**	1.46 ±
Ascorbic acid (mg/100 g tissue) 0.08***	0.49 ± 0.08	0.27 ± 0.06***	0.42 ± 0.08***	0.44 ±
α-Tocopherol (mg/100 g tissue) 0.09***	0.20 ± 0.09	0.80 ± 0.15***	0.30 ± 0.09***	0.27 ±

Values are expressed as mean ±SD. (n = 6). Diabetic mice were compared with control group. CFE and gliclazide treated groups were compared with their respective diabetic group, ***p <0.001, **p < 0.01 and *p <0.05.

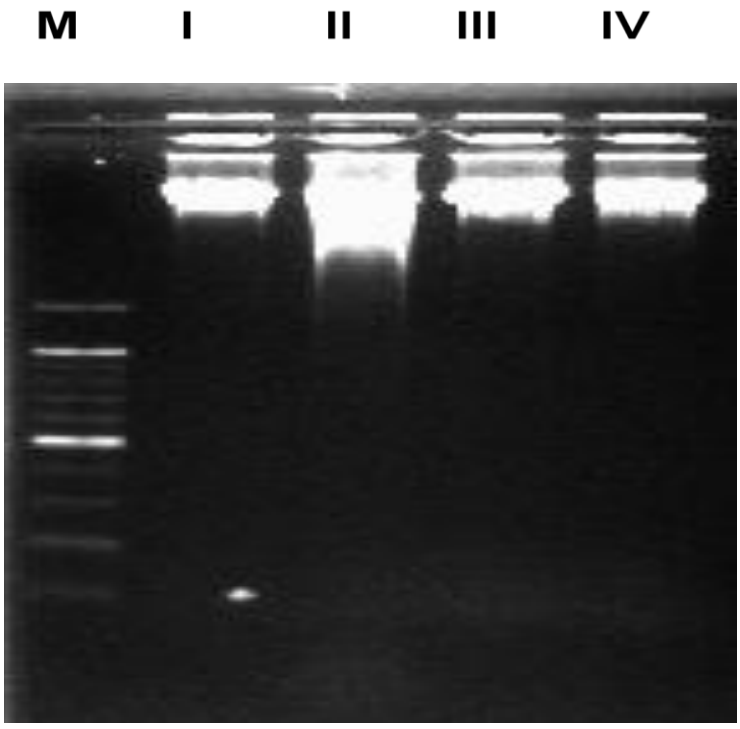

Figure 3. Effects of CFE on STZ-induced liver DNA fragmentation. DNA samples were electrophoresed on 1.5% agarose in TBE buffer, ethidium bromide stained gel represent as lane M is a DNA size marker, lane I showing intact DNA (control), lane II showing fragmented DNA (STZ-induced diabetic mice), Lane III and IV showing fragmentation reverted to similar control DNA (mice administered with CFE and gliclazide).

diabetic condition has been observed in experimental animals after the administration of certain plant extract and active compounds (Coskun et al., 2005; Sarkhail et al., 2007). STZ-induced diabetes is characterized by a severe loss in body weight and may exhibit most of the diabetic complications such as myocardial,

cardiovascular, gastrointestinal, nervous, liver, kidney and urinary bladder dysfunction through oxidative stress (Rajasekaran et al., 2005). In the present study, decreased body weight was observed in STZ-induced diabetic mice. The decrease in body weight in diabetic mice shows that the loss or degradation of structural proteins is due to diabetes and these structural proteins are known to contribute to the body weight (Rajkumar and Govindarajulu, 1991). When diabetic mice were treated with CFE and gliclazide, the weight loss was recovered. The capability of CFE to protect body weight loss seems to be related to its ability to reduce oxidative damage.

Oxidative stress that leads to an increased production of ROS and finally cellular lipid peroxidation has been found to play an important role in the development of diabetes mellitus (Rajasekaran et al., 2005). Several studies have shown increased LPO in the diabetic state (Yazdanparast et al., 2007; Emekli-Alturfan et al., 2008). Lipid peroxide-mediated tissue damage has been observed in the development of all types of diabetes mellitus (Feillet-Coudray et al., 1999). Diabetes mellitus is associated with generation of ROS leading to oxidative damage particularly in liver and kidney (Jin et al., 2008)]. In our study, there were significant increases in MDA, which was in agreement with others (Vijayakumar et al., 2006; Jin et al., 2008). The concentration of MDA was significantly decreased in the serum, liver and kidney of CFE and gliclazide treated diabetic mice. This indicates a powerful antiperoxidative effect of CFE.

LPO is one of the cellular features of chronic diabetes. Increased LPO impairs membrane function by decreasing membrane fluidity and changing the activity of membrane-bound enzymes and receptors (Soon and Tan, 2002). The activities of AST, ALT and ALP are the most sensitive tests employed in the diagnosis of organs damages. We have observed increased activities of serum AST, ALT and ALP in STZ-induced diabetic mice. Our results are consistent with earlier report (Bopanna et al., 1997; Heo et al., 2007). The increase in serum AST, ALT and ALP activities is an indicator of liver and kidney destruction. This is probably due to lipid peroxidation subsequent to free radical production. Administration of CFE and gliclazide to diabetic mice showed decreased activities of serum AST, ALT and ALP. This proves that CFE, to an extent preserves the structural integrity of the liver and kidney from the adverse effects of STZ-induced diabetes.

Moreover, increased LPO under diabetic conditions can be due to increased oxidative stress in the cell as a result of depletion of antioxidant protective systems. Antioxidant protective systems against ROS and the breakdown products of peroxidized lipids, oxidized protein and DNA are provided by many enzyme systems such as SOD, CAT and GPx. Oxidative stress in diabetes is coupled to a decrease in the antioxidant status, which can increase the deleterious effects of free radicals (Picton et al., 2001). SOD and CAT are the two major scavenging enzymes that remove radicals in vivo. In the present study, SOD and CAT activities were significantly decreased in the liver and kidney of diabetic mice. A decrease in the activity of these antioxidants can lead to an excess availability of superoxide anion ($O_2^{\bullet-}$) and hydrogen peroxide (H_2O_2), which in turn generate hydroxyl radicals ($^{\bullet}OH$), resulting in initiation and propagation of LPO. SOD can catalyze dismutation of $O2^{\bullet-}$ into H_2O_2, which is then deactivated to H_2O by CAT or GPx (Aebi, 1984; Kumuhekar and Katyane, 1992). Normally, SOD works in parallel with selenium dependent GPx, which plays an important role in the reduction of H_2O_2 in the presence of reduced glutathione forming oxidized glutathione (GSSG) and thus, it protects cell proteins and cell membranes against oxidative stress. GPx has a key role in enzymatic defense systems and reduces organic peroxides into their corresponding alcohols. In our study, GPx activity was significantly decreased in the liver and kidney of diabetic mice. The significant decrease in GPx activity could suggest inactivation by ROS, which are increased in diabetic mice. The decrease may also be due to the decreased availability of its substrate, GSH, which has been shown to be depleted during diabetes (Jain, 1998; Ugochukwu et al., 2004). Treatment with CFE and gliclazide ameliorated SOD, CAT and GPx activities in diabetic mice. These results suggest that CFE has free radical scavenging activity, which may exert a beneficial effect against pathological changes caused by ROS. In addition, CFE reactivates the antioxidant defense system.

Apart from the enzymatic antioxidants, non-enzymatic antioxidants such as ascorbic acid, α-tocopherol and GSH play an important role in preventing the cells from oxidative damages. α-Tocopherol is the most ancient antioxidant in the lipid phase (Ingold et al., 1987). In our study, α-tocopherol was increased in diabetic mice as reported earlier (Ramesh and Pugalendi, 2006). Elevated α-tocopherol content observed in the diabetic state is compatible with the hypothesis that α-tocopherol excess in the serum, liver and kidney of diabetics plays a protective role against increased peroxidation. An increase in α-tocopherol level may also be due to the increase in total lipid levels. Administration of CFE and gliclazide restored the α-tocopherol levels which could be as a result of decreased membrane damage as evidenced by decreased lipid peroxidation.

Ascorbic acid and α-tocopherol are interrelated by recycling process (Packer et al., 1997). Recycling of tocopheroxyl radicals to tocopherol is achieved with ascorbic acid (Freisleben and Packer, 1993). In our study, ascorbic acid was decreased in diabetic mice as reported earlier (Ramesh and Pugalendi, 2006). Reduction in serum, liver and kidney ascorbic acid levels may occur due to excessive oxidation and lack of regeneration from their radical form to reduced form. Treatment with CFE and gliclazide brought back ascorbic

acid to near normal levels which could be due to decreased utilization.

GSH is important in the circumvention of cellular oxidative stress, detoxification of electrophiles and maintenance of intracellular thiol redox state. GSH is reported to be essential for recycling of other antioxidants like vitamin E and vitamin C (Constantinescu et al., 1993) and acts as a substrate for GPx and glutathione-S-transferace (GST) that are involved in preventing the deleterious effect of oxygen radicals (Levine, 1990). In our study, serum, liver and kidney of diabetic mice exhibited a decreased level of GSH which might be due to increased utilization for scavenging free radicals and increased consumption by GPx and GST. Treatment with CFE and gliclazide reversed GSH level in serum, liver and kidney of diabetic mice could be due to decreased utilization of GSH to reduce the lipid peroxidation.

Oxidative stress is also known to cause massive DNA fragmentation. STZ was shown to induce DNA strand breaks and mutations in the liver and kidney of mice (Schmezer et al., 1994). We observed significant increase in DNA fragmentation in the liver and kidney of STZ-induced diabetic mice. Imaeda et al. (2002) also reported that DNA damage in the liver and kidney were significantly induced after STZ injection. Increased DNA fragmentation may enhance apoptotic cell death in liver and kidney. CFE and gliclazide treatment significantly reduced DNA fragmentation in liver and kidney of diabetic mice. Free radical scavenging activity of CFE may be attributed to the reduction in DNA fragmentation.

In conclusion, the present investigation shows CFE possesses antioxidant effect that may contribute to its protective action against lipid peroxidation, DNA fragmentation and enhancing effect on cellular antioxidant defense. This activity contributes to the protection against oxidative damage in STZ induced diabetes.

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