Journal of
Biological Sciences

Protective Effect of Green Tea Extract on Cyclosporine A: Induced Nephrotoxicity in Rats

Samy Ali Hussein, Omayma A. Ragab and Mohammed A. El-Eshmawy
Department of Biochemistry, Faculty of Veterinary Medicine Moshtohor, Benha University, Egypt

Abstract: Cyclosporine A (CsA) is a potent and effective immunosuppressive agent, but its use is frequently accompanied by severe renal toxicity. CsA-induced nephrotoxicity results from increased production of free radical species in the kidney. The present study was designed to investigate the possible protective effect of Green Tea Extract (GTE) on CsA-induced nephrotoxicity in rats. Eighty male rats were divided into four equal groups. Group 1: Rats received no drugs and served as control, Group 2: Normal rats were treated with green tea extract (3% w/v) orally, Group 3: Rats treated with CsA (25 mg kg^{-1} b.wt., orally for 21 days) to induce nephrotoxicity, Group 4: Rats received green tea extract for 21 days before, 21 days concurrently during CsA administration and 21 days later after nephrotoxicity induction. Blood samples for serum separation and kidney tissue specimens were collected three times at weekly interval from the last dose of CsA administration. Serum glucose, total Protein, albumin, lipid profile (total cholesterol, triacylglycerols and phospholipids), renal function tests (urea, uric acid and creatinine), electrolytes (sodium and potassium), inorganic phosphorus and haptoglobin levels, lactate dehydrogenase (LDH) and Gamma Glutamyl Transferase (GGT) activities were determined. Moreover, kidney tissue malondialdehyde (MDA), reduced glutathione (GSH), nitric oxide (NO), Total Antioxidant Capacitys (TAO) levels, antioxidant enzymes (catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx) activities were also determined. The results revealed that, CsA-induced nephrotoxicity caused significant increase in serum glucose, renal functions tests, haptoglobin, lipid profiles and serum marker enzymes (LDH and GGT) with significant decrease in serum total protein, albumin and electrolytes concentrations which were reversed upon treatment with green tea extract. Also, CsA administration induced significant elevation in lipid peroxidation (MDA) along with significant decrease in antioxidant enzyme activities, non enzymatic antioxidant, total antioxidant capacity and nitric oxide level in the rat kidney. Meanwhile, Green tea extract administration improved renal function, by bringing about a significant decrease in peroxidative levels and increase in antioxidant status. These results indicate the renoprotective potential and usefulness of green tea extract, as an excellent source of antioxidants, in modulating CsA-induced nephrotoxicity.

Key words: Cyclosporine A, antioxidant enzymes, lipid peroxidation, renal function, nephrotoxicity, green tea extract

INTRODUCTION

Cyclosporine (Cs), a cyclic decapeptide obtained from extracts of soil fungus Tolypocladium inflatum gams, is the most effective and widely used first-line immunosuppressant in solid organ transplantation and autoimmune diseases (Padi and Chopra, 2002).

Nephrotoxicity is the main secondary effect of cyclosporine A (CsA) treatment. Although the mechanisms of nephrotoxicity are not completely defined, there is evidence that suggests the role of Reactive Oxygen Species (ROS) in its pathogenesis. It has been demonstrated in numerous *in vivo* and *in vitro*

experiments that CsA induced renal failure and increased the synthesis of ROS, thromboxane (TX) and lipid peroxidation products in the kidney. Furthermore, CsA modified the expression and activity of several renal enzymes (ciclooxygenase, superoxidedismutase, catalase and glutathione-peroxidase) (Cid *et al.*, 2003). It is reported that the level of free radicals in urine was increased significantly following CsA treatment. Reactive Oxygen Species (ROS) could also be derived either directly from CsA or during its metabolism by the cytochrome P450 system (Mohamadin *et al.*, 2005).

Moreover, acute CsA treatment induces reversible reduction of the Glomerular Filtration Rate (GFR) and renal

Corresponding Author: Samy Ali Hussein, Department of Biochemistry, Faculty of Veterinary Medcine, Moshtohor,
Benha University, Toukh, 13736, Kaliobia, Egypt Tel: 002-01060754457 Fax: 002-0132460640

blood flow that is related to afferent arteriolar vasoconstriction. This may be related to an increase in vasoconstrictors factors such as endothelin, thromboxane, angiotensin II and/or a decrease in vasodilators factors such as prostacyclin and Nitric Oxide (NO) (Capasso *et al.*, 2008). In addition, CsA has been reported to block mitochondrial calcium release, inducing an increase in intracellular free calcium that could account for the CsA vasoconstriction effect.

More attentions have been paid to the protective effects of natural antioxidants against drug-induced toxicities especially whenever free radical generations are involved. Tea has been one of the most commonly consumed beverages since ancient times and it is still the beverage most available to general populations in many countries all over the world (Bordoni *et al.*, 2002). As a naturally safe beverage, tea has many advantages over chemical preventive agents. It contains several antioxidants, including polyphenols of the catechin (green tea) and theaflavin (black tea) groups (Tsubono *et al.*, 2001).

In particular green tea catechins and their derivatives have been characterized as antioxidants that scavenge free radicals to protect cells in normal and pathological states (Khan and Mukhtar, 2007). Tea polyphenols scavenge harmful reactive nitrogen and oxygen species, such as superoxide radical, singlet oxygen, hydroxyl radical, peroxyl radical, nitric oxide, nitrogen dioxide and peroxynitrite (Costa *et al.*, 2009).

Epigallocatechin gallate (EGCG) is the most abundant component of polyphenol in green tea which exerts an antioxidative effect to protect cells from the damage by oxygen free radicals (Lin *et al.*, 1998). Thus, EGCG may have a protective effect on the impaired renal function resultant from oxygen free radicals in CsA-induced nephrotoxicity. To test this hypothesis, the present study was designed to investigate the possible protective effect of green tea extract on nephrotoxicity induced by CsA and the potential biochemical role by which green tea extract exerts its protective effect in ameliorating CsA nephrotoxicity.

MATERIALS AND METHODS

Experimental animals: Eighty white male albino rats of 12-16 weeks old and weighting 220-250 g were used in this study. Rats were housed in separated metal cages and kept at constant environmental and nutritional conditions throughout the period of experiment. The animals were fed on constant ration and water was supplied *ad-libitum*. The animals were left 14 days for acclimatization before the beginning of the experiment.

Drug and antioxidants: The drug and antioxidant compounds used in the present study were:

- **Cyclosporine (CsA):** Cyclosporine (CsA) presents in the form of soft gelatine capsules containing 50 mg cyclosporine under traditional name (Sandimmune®, Neoral®) was obtained from (Novartis Pharma AG,Basilea,Suiza) and freshly dissolved in propylene glycol. Nephrotoxicity was induced in rats after oral administration of cyclosporine (CsA) at a dose of 25 mg kg^{-1} b.wt. day^{-1} for 21 days
- **Green tea:** Green tea was obtained from Ahmad Tea Ltd,1 Wood Street, London EC2V 7WS. Green tea extract was freshly prepared daily at a concentration of (3% w/v), 30 g of dry tea was added to 1000 mL of boiled water for 20 min cooled to room temperature and filtered before administration to the rats in water bottles. All green tea extract bottles were cleaned, changed and administered orally and daily dose according to Vinson and Zhang (2005)

Experimental design: After acclimatization to the laboratory conditions, the animals were randomly divided into four groups (twenty rats each) as follows:

- **Group 1:** Rats received no drugs served as control for all experimental groups
- **Group 2:** Rats administered green tea extract in the concentration of (3% w/v) as their sole source of drinking water all over the experimental periods (9 weeks)
- **Group 3:** Rats were administered cyclosporine A (25 mg kg^{-1} body weight) start from the day 22 of experiment, once daily by oral gavage, for a period of 21 days
- **Group 4:** Rats received oral administration of green tea extract (3% w/v) in drinking water for 21 days before cyclosporine A, then for 21 days concomitant with cyclosporine A administration as in group 3 followed by 21 days later (end of experiment, 9 weeks)

Sampling: Blood samples and renal tissue specimens were collected from all animals groups, three times during the experiment at 1st, 2nd and 3rd weeks from the last dose of CsA administration:

- **Blood samples:** Blood samples were collected by ocular vein puncture in dry, clean and screw capped tubes and serum were separated by centrifugation at 2500 rpm for 15 min. The clean, clear serum was separated by Pasteur pipette and kept in a deep freeze at -20°C until used for subsequent biochemical analysis

- **Renal tissue specimens:** Rats killed by decapitation. The kidney specimen quickly removed, cleaned by rinsing with cold saline and stored at -20°C. Briefly, renal tissues was minced into small pieces, homogenized with ice cold 0.05 M potassium phosphate buffer (pH 7.4) to make 10% homogenates. The homogenates were be centrifuged at 6000 rpm for 15 min at 4°C until used for subsequent biochemical analysis

Biochemical analysis: Serum Glucose, Total protein, Albumin, Total cholesterol, Triacylglycerols, Phospholipids, Urea, Uric acid, Creatinine, Sodium, potassium, Inorganic phosphorus and Haptoglobin concentrations, Lactate dehydrogenase (LDH) and Gamma Glutamyl Transferase (GGT) activities were determined according to the methods described by Tietz (1995), Gornall *et al.* (1949), Young (1990, 1995), Allain *et al.* (1974), Stein (1987), Connerty *et al.* (1961), Kaplan (1984), Schultz (1984), Jaffe (1986), Henry and Marmion (1974), Gamst and Try (1980), Johnson *et al.* (1999) and Saw *et al.* (1983), respectively. Moreover, the supernatant of renal tissue homogenate were used for the determination of malondialdehyde (MDA), reduced glutathione (GSH), Nitric Oxide (NO), total antioxidant capacity (TAO) and antioxidant enzymes {catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (Gpx)} according to the methods described by Mesbah *et al.* (2004), Beutler *et al.* (1963), Montgomery and Dymock (1961), Koracevic *et al.* (2001), Xu *et al.* (1997), Paoletti and Macali (1990) and Gross *et al.* (1967), respectively.

Statistical analysis: The results were expressed as Mean±SE and statistical significance was evaluated by one way ANOVA using SPSS (version 10.0) program followed by the post hoc test, Least Significant Difference (LSD). Values were considered statistically significant when $p<0.05$.

RESULTS

The results presented in (Table 1 and 2) revealed that, CsA-induced nephrotoxicity caused significant increase in serum glucose, lipid profile (total cholesterol, triacylglycerols and phospholipids), renal function tests (urea, uric acid and creatinine), haptoglobin levels, lactate dehydrogenase (LDH) and Gamma Glutamyl Transferase (GGT) activities with significant decreased in serum total protein, albumin and electrolytes (sodium, potassium and inorganic phosphorus) concentrations. Green tea extract administration to CsA treated rats restore serum renal functions tests (urea, uric acid and creatinine), haptoglobin, lipid profiles and serum markers enzyme (LDH and GGT) activities and also reversed the increase in serum proteins and electrolytes to normal range.

The obtained results demonstrated in (Table 3) revealed that, CsA administration caused significant elevation in kidney tissue malondialdehyde (MDA) along with significant decrease in antioxidant enzymes (CAT, SOD and GPx) activities, non enzymatic antioxidant (GSH), total antioxidant capacity and nitric oxide level in the rat kidney. Meanwhile, Green tea extract administration to rats received oral dose of CsA improved renal function, by bringing about a significant decrease in peroxidative levels and increase renal tissue antioxidant status as revealed by enhanced renal tissue antioxidant enzymes activities (CAT, SOD and GPx), GSH and total antioxidant capacity levels.

Table 1: Effect of green tea extract administration on serum glucose, lipid profiles and renal function tests in normal and cyclosporine A-induced nephrotoxicity in rats

Groups	Parameters (mg dL^{-1})						
	Glucose	Total cholesterol	Triacylglycerols	Phospholipids	Creatinine	Uric acid	Urea
1st week							
C	72.00±2.27^c	97.23±9.71^b	114.18±4.99^b	134.39±13.19^b	0.86±0.02^b	2.51±0.13^b	28.71±2.99^c
GT	78.00±2.48^c	91.20±2.42^b	90.50±7.74^c	136.63±0.840^b	0.72±0.03^c	2.46±0.16^b	28.06±2.36^c
CsA	147.33±15.00^a	159.28±13.46^a	145.82±11.91^a	195.07±8.350^a	1.86±0.05^a	4.86±0.74^a	60.32±3.52^a
CsA+GT	92.50±6.14^{bc}	75.81±1.78^b	92.02±3.68^{bc}	127.23±7.480^b	0.80±0.01^{bc}	2.62±0.15^b	38.96±1.33^b
2nd week							
C	95.00±3.81^d	115.53±5.64^b	74.56±5.56^b	135.19±16.53^b	0.75±0.01^c	1.89±0.14^b	27.97±2.61^b
GT	113.00±2.38^{bc}	75.73±2.67^{cd}	73.93±5.06^b	135.99±2.630^b	0.70±0.01^c	2.42±0.15^b	26.28±0.38^b
CsA	187.50±2.60^a	259.32±17.04^a	137.98±9.79^a	232.81±2.760^a	1.37±0.04^a	4.30±0.72^a	48.88±0.28^a
CsA+GT	89.00±2.80^d	96.05±1.35^{bc}	74.31±3.00^b	143.63±8.170^b	0.76±0.01^c	2.52±0.23^b	26.24±1.26^b
3rd week							
C	104.75±4.59^{ab}	97.26±2.39^b	61.65±6.80^b	139.33±18.46^b	0.65±0.02^{bc}	1.84±0.14^b	26.38±1.46^b
GT	83.25±2.69^b	95.66±2.18^b	39.37±5.43^b	137.42±5.430^b	0.60±0.03^c	1.98±0.03^b	19.43±3.55^c
CsA	133.50±26.47^a	163.74±25.60^a	207.97±19.69^a	207.64±20.99^a	1.72±0.27^a	4.92±0.97^a	37.86±1.07^a
CsA+GT	118.25±3.75^{ab}	89.97±5.00^b	64.56±4.56^b	135.35±7.180^b	0.76±0.02^{bc}	1.90±0.12^b	33.57±0.71^a

C: Control normal group, GT: Green tea group, CsA: Cyclosporine A group, CsA+GT: Cyclosporine A+green tea group. Data are presented as (Mean±SE). SE: Standard error. Mean values with different superscript letters in the same column are significantly different at ($p<0.05$)

Table 2: Effect of green tea extract administration on serum electrolytes, proteins and haptoglobin concentrations, LDH and GGT activities in normal and cyclosporine A-induced nephrotoxicity in rats

Groups	Parameters							
	Sodium (meq. L^{-1})	Potassium (meq. L^{-1})	Inorganic phosphorus (mg dL^{-1})	Total protein (g dL^{-1})	Albumin (g dL^{-1})	Haptoglobin (mg dL^{-1})	LDH (U L^{-1})	GGT (U L^{-1})
1st week								
C	142.30±2.02[a]	6.70±0.13[a]	7.80±0.34[a]	6.23±0.20[a]	3.62±0.23[a]	70.70±15.01[b]	1191.72±196.91[bc]	17.33±0.43[a]
GT	142.03±1.43[a]	6.29±0.28[a]	1.71±0.33[c]	6.35±0.10[a]	3.46±0.09[ab]	68.30±21.03[b]	1221.83±196.34[bc]	14.02±0.54[bc]
CsA	130.55±3.35[b]	3.95±0.40[b]	1.57±0.17[c]	4.07±0.09[b]	3.17±0.11[b]	173.17±4.19[a]	2475.04±85.37[a]	66.89±3.80[c]
CsA+GT	141.75±0.36[a]	6.86±0.35[a]	4.66±0.15[b]	6.68±0.19[a]	3.59±0.03[a]	85.17±26.39[b]	1673.65±137.39[b]	13.56±1.10[bc]
2nd week								
C	145.40±0.43[a]	6.64±0.23[a]	2.82±0.15[a]	6.24±0.22[a]	3.69±0.10[a]	55.25±3.32[a]	1542.10±113.74[b]	12.88±1.29[b]
GT	145.58±1.25[a]	6.35±0.19[a]	2.33±0.17[a]	6.24±0.11[a]	3.69±0.10[a]	58.83±26.82[a]	1355.91±157.26[b]	15.53±0.57[a]
CsA	131.50±0.87[d]	3.28±0.13[c]	1.81±0.15[a]	3.72±0.08[b]	3.12±0.03[b]	79.63±27.04[a]	2537.79±21.03[a]	69.16±1.10[c]
CsA+GT	142.35±0.65[bc]	6.53±0.19[a]	3.19±0.64[a]	6.41±0.10[a]	3.33±0.09[b]	34.53±7.57[a]	677.96±144.14[c]	14.74±0.73[ab]
3rd week								
C	146.05±0.72[a]	6.00±0.14[a]	4.64±0.26[a]	5.61±0.06[b]	3.61±0.21[a]	24.87±0.82[a]	1629.12±85.69[b]	15.36±0.33[a]
GT	143.50±0.76[ab]	6.23±0.33[a]	2.01±0.13[b]	5.90±0.33[b]	3.16±0.11[b]	66.32±19.78[a]	1641.27±133.20[b]	14.23±0.82[ab]
CsA	132.15±3.45[c]	4.66±0.29[b]	1.99±0.46[b]	4.02±0.36[c]	3.03±0.03[b]	64.25±12.64[a]	2329.34±47.93[a]	60.51±4.30[c]
CsA+GT	140.38±0.82[b]	6.11±0.12[a]	2.46±0.07[b]	5.51±0.29[b]	3.23±0.08[b]	34.17±5.42[a]	522.13±109.03[c]	13.67±0.22[b]

C: Control normal group, GT: Green tea group, CsA: Cyclosporine A group, CsA+GT: Cyclosporine A+green tea group. Data are presented as (Mean±SE). SE: Standard error.Mean values with different superscript letters in the same column are significantly different at (p<0.05)

Table 3: Effect of green tea extract administration on renal tissue L-MDA, CAT, SOD, GPx, GSH, NO and TAOC in normal and cyclosporine A-induced nephrotoxicity in rats

Groups	Parameters						
	(L-MDA) (U g^{-1} tissue)	CAT (U g^{-1} tissue)	(SOD) (U g^{-1} tissue)	GPx (GSH consumed min^{-1} mg^{-1} protein)	(GSH) (mg g^{-1} tissue)	(NO) (μmol g^{-1}tissue)	(TAO) (mmol g^{-1} tissue)
1st week							
C	31.23±8.470[c]	2.10±0.21[a]	36.36±3.22[a]	0.46±0.002[a]	64.33±2.330[a]	115.74±1.62[a]	1.11±0.04[a]
GT	25.97±10.96[c]	2.14±0.41[a]	27.77±3.70[abc]	0.46±0.005[a]	60.00±2.520[a]	84.86±13.36[ab]	0.66±0.05[bc]
CsA	106.27±9.210[a]	1.73±0.16[a]	24.38±4.12[bc]	0.42±0.009[b]	29.67±3.380[b]	66.66±6.41[b]	0.71±0.02[bc]
CsA+GT	63.45±10.71[b]	2.24±0.21[a]	33.71±5.16[ab]	0.45±0.003[a]	39.00±4.040[b]	72.94±11.64[ab]	0.86±0.08[b]
2nd week							
C	22.56±4.270[c]	1.60±0.24[bc]	26.71±2.72[abc]	0.43±0.011[a]	41.00±3.370[a]	44.44±0.32[a]	0.65±0.04[a]
GT	79.18±11.98[a]	2.88±0.33[a]	19.26±0.89[cd]	0.41±0.002[a]	47.67±4.060[a]	34.26±9.54[ab]	0.62±0.04[a]
CsA	67.68±2.630[ab]	1.43±0.06[c]	16.14±0.58[d]	0.40±0.011[a]	33.50±2.020[a]	23.34±4.21[b]	0.55±0.03[a]
CsA+GT	47.50±7.170[b]	1.45±0.08[c]	20.86±3.30[bcd]	0.42±0.002[a]	46.00±5.290[a]	47.28±4.88[a]	0.68±0.01[a]
3rd week							
C	29.07±7.100[b]	1.88±0.07[ab]	30.28±0.33[a]	0.43±0.012[a]	32.33±0.670[bc]	61.87±9.82[a]	0.74±0.08[bcd]
GT	35.47±5.820[ab]	2.08±0.23[a]	29.14±2.81[a]	0.40±0.004[b]	55.67±0.330[a]	39.81±9.38[b]	0.86±0.04[ab]
CsA	54.88±11.73[a]	1.63±0.06[ab]	20.71±0.25[b]	0.39±0.007[b]	27.50±1.440[c]	53.33±1.28[ab]	0.63±0.02[d]
CsA+GT	49.78±8.450[ab]	1.45±0.18[b]	30.56±1.62[a]	0.42±0.003[ab]	46.67±11.22[ab]	59.17±1.12[ab]	0.97±0.03[a]

C: Control normal group, GT: Green tea group, CsA: Cyclosporine A group, CsA+GT: Cyclosporine A+green tea group). Data are presented as (Mean±SE). SE: Standard error. Mean values with different superscript letters in the same column are significantly different at (p<0.05)

DISCUSSION AND CONCLUSION

Nephrotoxicity is the most common and clinically significant adverse effect of cyclosporine (Burdmann *et al.*, 2003). Oxidative stress is the main mechanism resulting in cyclosporine-induced nephrotoxicity because of its ability to stimulate endogenous melatonin production (Ghorbanihaghjo *et al.*, 2008).

Cyclosporine treatment to control rats resulted in significant increase in serum glucose concentration compared to control group. These results came in agreement with those recorded by Petkovska *et al.* (2008) who reported that, cyclosporine is believed to have a direct toxic effect on pancreatic beta cells, whereas a reversible suppression of insulin release has also been documented. Other studies have also demonstrated that greater cyclosporine dosages and trough levels were associated with higher insulin values and indices of Insulin Resistance (IR). Cyclosporine belongs to the family of calcineurin inhibitors and acts as a prodrug since it remains inactive until it connects with its cytoplasmic receptor known as cyclophilin (Bressan *et al.*, 2010). In insulin-secreting cells, calcineurin is involved in the stimulation of insulin gene transcription through the activation of the transcription factor Nuclear Factor of Activated T-cells (NFAT). Nevertheless, the degree and comparability of the calcineurin inhibitors in impairing beta-cell function is yet to be established (Lawrence *et al.*, 2002). Green tea extract administration to CsA treated rats resulted in significant decrease in serum glucose levels compared with CsA group. These results are in agreement with the study of Tsuneki *et al.* (2004) and Babu *et al.* (2006) who observed that, green tea reduced blood glucose level in both type 1 and 2 of diabetic rats models. The antihyperglycemic effect of

green tea constituents was ascribed to the activities of basal insulin (Wu *et al.*, 2004) and inhibition of intestinal glucose transporter (Kobayashi *et al.*, 2000) and decrease the expression of genes that control gluconeogenesis (Waltner-Law *et al.*, 2002). Additionally, Waltner-Law *et al.* (2002) reported that, green tea catechin epigallocatechin gallate was shown to repress hepatic glucose production by modulating the redox status of the cell. Black and green teas were shown to have *in vitro* insulin-enhancing activity, with the majority of the green tea activity due to epigallocatechin gallate. Intestinal glucose uptake is mainly accomplished by the sodium-dependent glucose transporter, SGLT1. The transport activity of SGLT1 was markedly inhibited by green tea polyphenols.

Administration of cyclosporine in normal rats exhibited a significant increase in serum lipid profile levels as compared with control group. Similarly, Hulzebos *et al.* (2004) showed that, hypertriglyceridemia and hypercholesterolemia are common side effects of cyclosporine A (CsA), to date, however, only limited data are available on the mechanism of CsA-associated hyperlipidemia in humans *in vivo*. Additionally, results from *in vivo* animal studies and from *in vitro* studies in human hepatoma cells indicated that CsA increases hepatic lipoprotein production and reduces lipoprotein clearance. Green tea extract administration to CsA treated rats caused significant decrease in serum lipid profile levels as compared with CsA group. These results are in agreement with the study of Shirai and Suzuki (2004) who recorded that, the high TG content in mice fed the vegetarian diet is significantly reduced when the diet is supplemented with GTE. This is consistent with reports that indicate that saponins and catechins, contained in GTE, suppress the absorption of fat from the intestine by inhibition of lipase activity. These findings show the possibility that the significant lowering of plasma TG concentration in the vegetarian food+GTE diet group may be resulted from GTE inhibit the absorption of fat. Catechins compounds derived from green tea, have been shown to reduce plasma cholesterol levels and the rate of cholesterol absorption (Lorenz *et al.*, 2004).

A significant increase in serum renal function tests (urea, uric acid and creatinine) concentrations were observed in CsA treated rats as compared with control group. These results are in agreement with the study of Tirkey *et al.* (2005) who reported that, chronic administration of CsA for 21 days caused a marked impairment of renal function along with significant oxidative stress in the kidneys. Oxidative stress can promote the formation of a variety of vasoactive mediators that can affect renal function directly by causing renal vasoconstriction or decreasing the glomerular capillary ultrafiltration coefficient and thus reducing glomerular filtration rate (Garcia-Cohen *et al.*,

2000). These findings were further evident from the marked elevation of serum urea, uric acid and creatinine concentrations, thereby suggesting a significant functional impairment of kidneys in cyclosporine A treated rats (Burdmann *et al.*, 2003). This suggestion was supported with the finding of Elshater *et al.* (2008) who observed that, plasma uric acid and creatinine can be used as a rough index of the glomerular filtration rate. High levels of uric acid and creatinine indicates several disturbances in kidney. Green tea administration to cyclosporine treated rats exhibited a significant decrease in serum renal function (urea, uric acid and creatinine) levels as compared with CsA group. Similarly, Elshater *et al.* (2008) reported that, administration of green tea extract normalized the levels of plasma creatinine and uric acid. Chronic administration of CsA also produced oxidative stress and increased the lipid peroxidation in kidneys (Skrzydlewska *et al.*, 2002). Oxidative stress is caused by oxygen free radicals in the kidney, including the superoxide anion, the hydrogen peroxide and the hydroxyl radical (Rao and Nammi, 2006). The antioxidant properties of flaavonoids are due to their ability to directly scavenge some radical species (Chander *et al.*, 2003).

Administration of Cyclosporine A to normal rats resulted in significant decrease in serum electrolytes (sodium, potassium) as compared with control group. These results are nearly similar with the study of Chia *et al.* (2012) who found that, sodium depletion to be associated with CsA treatment. Also, Higgins *et al.* (2004) reported that, hypokalaemia was more frequent under cyclosporin treatment. CsA-induced nephrotoxicity has been characterized by 20-30% reduction in glomerular filtration rate and up to 40% reduction in renal blood flow resulting in elevated serum creatinine levels, decreased creatinine clearance and reduction in sodium and potassium (Mohamadin *et al.*, 2005). Administration of green tea to cyclosporine treated rats induced significant increase in serum electrolytes levels compared with CsA group. These results came in agreement with Petrussa *et al.* (2001) who shown that, serum potassium level was significantly higher in the CsA group than in the control group and significantly lower in the CsA-GTE group than in the CsA group. As to the potassium channel, it has been reported that CsA induces the opening of a potassium-selective channel in higher plant mitochondria. The decrease in N_a^+-K^+AT Pase activity caused by CsA is thought to be one of the mechanisms for the observed potassium ion secretion defects (Tumlin and Sands, 1993).

Administration of cyclosporine A to normal rats significantly decreased serum total protein and albumin concentrations when compared with control group. These results are in agreement with the study of Jeon and Kim (2011) who found that, CsA is hepatotoxic including inhibition of hepatic protein synthesis CsA administered

orally (100 mg kg^{-1} day^{-1}) to rats for 21 days causes marked decreases in total serum protein and albumin, accompanied by rises in alkaline phosphatase and total bilirubin. A morphological examination of the liver revealed moderate centrilobular fatty changes and minor dilatation of endoplasmic reticulum. The protein depression might be due to loss of protein either by reduced protein synthesis or increased proteolytic activity or degradation (Yeragi *et al.*, 2003). A significant increase in serum protein levels was observed after green tea administration in cyclosporine treated rats as compared with CsA group. Issabeagloo *et al.* (2012) reported that, the most common Liver function tests include serum aminotransferases, alkaline phosphatase, bilirubin and albumin. Hepatocellular damage causes release of these enzymes into circulation. Return of the above enzymes to normal serum values following Green tea extract treatment may be due to prevention of intracellular enzyme leakage resulting from cell membrane stability or cellular regeneration. Effective control of bilirubin and albumin shows early improvement of functional and secretory mechanism of hepatic cells.

Cyclosporine A administration in rats resulted in significant increase in serum markers enzymes (LDH and GGT) activities as compared with control group. Also, Heikal *et al.* (2013) demonstrated that, LDH can be used as an indicator for cellular damage and cytotoxicity of toxic agents. In fact, elevation in LDH activity indicates cell lysis and death as well as the switching over of anaerobic glycolysis to aerobic respiration. The change in LDH activity resulted from overproduction of superoxide anions and hydroxyl radicals which cause oxidative damage to the cell membrane and increase in membrane permeability. Cyclosporine is a calcineurin inhibitor, the most limiting side effects of calcineurin inhibitors is inhibition of NO production, through a calcinurin-regulating eNOS dephosphorylation (Kou *et al.*, 2002). Another side effect of calcineurin inhibitors is increased activity of LDH and lactate accumulation (Higginson *et al.*, 2002). Administration of green tea to cyclosporine treated rats resulted in significant decrease in serum enzymes (LDH and GGT) activities when compared with CsA group. Kumar *et al.* (2010) recorded that,increased activities of serum SGOT, SGPT, LDH and GGT are well known diagnostic indicators of hepatic injury. In cases such as liver damage with hepatocellular lesions, these enzymes are released from the liver into the blood stream. Pre-treatment with GTE significantly lowered the levels of these enzymes and the values were comparable with that of the control group.

Moreover, Co-administration of GTE significantly prevented the injury in CsA treated animals where the hepatocytes regained their normal appearance (Fetouh and Ibrahim, 2013).

Cyclosporine treatment to normal rats resulted in significant increase in renal tissue (L-MDA) levels as compared with control group. Similarly, Amudha *et al.* (2006) showed significant increase in lipid peroxidation during CsA administration which suggests the involvement of oxygen free radicals in the pathogenesis of renal injury. Cyclosporine A treatment has been shown to increase the production of free radicals and the formation of lipid peroxides *in vivo* and *in vitro*. Cyclosporine A increased malondialdehyde, a stable product of lipid hydroperoxide, in isolated hepatic and renal microsomes. An increase in superoxide radical and hydrogen peroxide following CsA has been demonstrated. Moreover, CsA administration results in excess local production of hydroxyl radical, leading to lipid peroxidation and nephrotoxicity (Hagar *et al.*, 2006). However, administration of green tea in cyclosporine treated rats significantly decreased renal tissue (L-MDA) levels compared with CsA group. These results came in agreement with Heikal *et al.* (2013) who reported that, Green Tea (GT) extract reversed the elevation of lipid peroxidation. Hence, it is possible that the mechanism of hepatoprotection of GT extract may be attributed to polyphenolic compounds (e.g., epicatechins) that scavenge a wide range of free radicals including the most active hydroxyl radical which initiate lipid peroxidation (Yang *et al.*, 2009). Therefore, it may decrease the concentration of lipid free radicals (Skrzydlewska *et al.*, 2002).

Administration of cyclosporine to normal rats exhibited a significant decrease in renal tissue antioxidant enzymes (CAT, SOD and GPX) activities as compared with control group. The presently observed decrease in the catalase activity in CsA treated rats is due to the decreased availability of NADPH which is required for catalase activity from its inactive form . Therefore, it is possible that depletion of NADPH production during CsA treated rats could decrease the catalase activity. Decrease in the activity of GPx during CsA administration indicates the reduction in the levels of GSH and increase in the levels of peroxides. The depletion of glutathione causes a proportional decrease in H$_2$O$_2$ detoxification by glutathione peroxidase (Amudha *et al.*, 2006). The decline in renal SOD activity after CsA administration was in agreement with the results reported by Mohamadin *et al.* (2005). It is well known that an efficient endogenous

anti-oxidant defence system operates to combat the production of free radicals. The antioxidant enzymes catalase, SOD, GPX and catalase constitute the major defence against ROS-induced oxidative damage. Superoxide dismutase is considered as the first line of defence against the deleterious effects of oxygen radicals in cells, where it scavenges ROS by catalysing the dismutation of superoxide to H_2O_2 and O_2. Green tea administration to cyclosporine treated rats resulted in significant increase in renal tissue antioxidant enzymes (CAT, SOD and GPX) activities as compared with CsA group. These results are in agreement with the study of Khan *et al.* (2007) who reported that, the biological defense system was perturbed by GT consumption as a result of free radical scavenging properties of its polyphenols and other active constituents. The profound lowering of LPO in the renal cortex and liver in GT compared with control rats suggests that oxidative damage even under normal physiologic conditions was significantly lowered by GT constituents. The reduction in LPO in the liver was associated with a profound increase in catalase activity, whereas in the renal cortex it appeared to be due to increases in catalase and SOD activities.

Cyclosporine A treatment to normal rats exhibited a significant decrease in renal tissue (GSH) level compared to control group. Similarly, Shakiba *et al.* (2009) reported that, CsA ingestion leads to a meaningful decrease in hepatic reduced glutathione GSH content.The total amount of hepatocytes glutathione significantly decreased in rats treated with CsA. In addition they notified that CsA increases oxidized glutathione concentrations which can modulate the activity of various regulatory enzymes and might be a cause of the impaired hepatocellular functions induced by CsA. A significant increase in renal tissue (GSH) level was observed after green tea administration in cyclosporine A treated rats as compared with CsA group. These results are harmony with these reported by Yumei *et al.* (2006) who suggested that, EGCG, one of the major constituents of GTE, attenuates oxidative stress by increasing the level of cellular GSH in passaged rat hepatic stellate cells. It has been reported that pure flavonoids stimulate glutamate cysteine lyase catalytic subunit (a key rate-limiting enzyme in GSH synthesis) gene expression through antioxidant response elements in the gene promoter.

Administration of cyclosporine A to normal rats exhibited a significant decrease in renal tissue (NO) level compared with control group. Similarly, Wilcox (2002) reported that, the overproduction of free radicals induced by CsA may lead to the inhibition of NO synthesis with the consequent appearance of hypertension. However, recent studies suggest an important role of endothelin in CsA-induced increase in vascular resistance (Bobadilla and Gamba, 2007). Endothelin has also been shown to affect rennin-angiotensin system and inhibit NO and prostaglandin production leading to vasoconstriction (Shihab *et al.*, 2003). However, administration of green tea to cyclosporine treated rats caused significant increase in renal tissue (NO) level when compared with CsA group. These results came in agreement with Curin and Andriantsitohaina (2005) who demonstrate the therapeutically relevant effect of flavonoids may be their ability to induce nitric oxide production and vasodilatation as well as the expression of genes that protect cardiovascular system.

A significant decrease in renal tissue Total Anti Oxidant Capacity (TAOC) level was observed in cyclosporine treated normal rats compared with control group. Similar results were reported by Shakiba *et al.* (2009) who demonstrated that, CsA therapy induces overproduction of Reactive Oxygen Species (ROS) in hepatocytes and lowers their antioxidant capacity. However, administration of green tea to cyclosporine treated rats caused significant increase in renal tissue (TAOC) level as compared with CsA group. These results came in agreement with Rietveld and Wiseman (2003) who found that, tea flavonoids are potent antioxidants that are absorbed from the gut after consumption and significantly increase the antioxidant capacity of the blood. Beneficial effects of increased antioxidant capacity in the body may be the reduction of oxidative damage to important molecules.

In view of these findings, it is possible to conclude that, CsA administration results in pronounced oxidative stress and renal damage. GTE treatment significantly ameliorated the renal dysfunction and protected renal function from free radical-mediated injury from CsA by protecting the marker enzymes and further strengthened the antioxidant status of the cell. The results suggest that GTE is effective in preventing functional impairment in CsA-induced nephrotoxicity in a rat model.

REFERENCES

Allain, C.C., L.S. Poon, C.S. Chan, W. Richmond and P.C. Fu, 1974. Enzymatic determination of total serum cholesterol. Clin. Chem., 20: 470-475.

Amudha, G., A. Josephine and P. Varalakshmi, 2006. Role of lipoic acid in reducing the oxidative stress induced by cyclosporine A. Clin. Chem. Acta, 372: 134-139.

Babu, P.V.A., K.E. Sabitha and C.S. Shyamaladevi, 2006. Therapeutic effect of green tea extract on advanced glycation and cross-linking of collagen in the aorta of streptozotocin diabetic rats. Clin. Exp. Pharmacol. Physiol., 33: 351-357.

Beutler, E., O. Duron and B.M. Kelly, 1963. Improved method for the determination of blood glutathione. J. Lab. Clin. Med., 61: 882-888.

Bobadilla, N.A. and G. Gamba, 2007. New insights into the pathophysiology of cyclosporine nephrotoxicity: A role of aldosterone. Am. J. Physiol. Renal Physiol., 293: F2-F9.

Bordoni, A., S. Hrelia, C. Angeloni, E. Giordano, C. Guarnieri, C.M. Caldarera and P.L. Biagi, 2002. Green tea protection of hypoxia/reoxygenation injury in cultured cardiac cells. J. Nutr. Biochem., 13: 103-111.

Bressan, A.L., R.S. da Silva, E. Fontenelle and A.C. Gripp, 2010. Imunossupressores na Dermatologia. An. Bras. Dermatol., 85: 9-22.

Burdmann, E.A., T.F. Andoh, L. Yu and W.M. Bennet, 2003. Cyclosporine nephrotoxicity. Semin. Nephrol., 23: 465-476.

Capasso, G., C.I. Di Gennaro, F.D. Ragione, C. Manna and R. Ciarcia et al., 2008. In vivo effect of the natural antioxidant hydroxytyrosol on cyclosporine nephrotoxicity in rats. Nephrol. Dial. Transplant, 23: 1186-1195.

Chander, V., D. Singh and K. Chopra, 2003. Catechin, a natural antioxidant protects against rhabdomyolysis-induced myoglobinuric acute renal failure. Pharmacol. Res., 48: 503-509.

Chia, T.Y., M.A. Sattar, M.H. Abdullah, F.D. Ahmad and Z.O. Ibraheem et al., 2012. Cyclosporine A-induced nephrotoxic Sprague-dawley rats are more susceptable to altered vascular function and haemodynamics. Int. J. Pharmacy Pharma. Sci., 4: 431-439.

Cid, T.P., J.R.C. Garcia, F.C. Alvarez and G. de Arriba, 2003. Antioxidant nutrients protect against cyclosporine a nephrotoxicity. Toxicology, 189: 99-111.

Connerty, H.V., A.R. Briggs and E.H. Eaton Jr., 1961. Determination of Serum, Phospholipids, Lipid Phosphorous. In: Practical Clinical Biochemistry, Varley, H. (Ed.). 4th Edn., CBS Publishers, India, pp: 319-320.

Costa, R.M., A.S. Magalhaes, J.A. Pereira, P.B. Andrade, P. Valentao, M. Carvalho and B.M. Silva, 2009. Evaluation of free radical-scavenging and antihemolytic activities of quince (Cydonia oblonga) leaf: A comparative study with green tea (Camellia sinensis). Food Chem. Toxicol., 47: 860-865.

Curin, Y. and R. Andriantsitohaina, 2005. Polyphenols as potential therapeutical agents against cardiovascular diseases. Pharmacol. Rep., 57: 97-107.

Elshater, A.E.A., M.M.A. Salman and M.M.A. Moussa, 2008. Effect of green tea consumption on level of glucose, lipid profile and kidney functions in alloxan induced-diabetic rats. Egypt. Acad. J. Biolo. Sci., 1: 125-134.

Fetouh, F.A. and A.A. Ibrahim, 2013. Hepato-protective effect of green tea extract on cyclosporine a treated rabbits: Histological and ultrastructural study. Life Sci. J., 10: 1924-1932.

Gamst, O. and K. Try, 1980. Determination of serum-phosphate without deproteinization by ultraviolet spectrophotometry of the phosphpmolybdic acd complex. Scand. J. Clin. Lab. Invest., 40: 483-486.

Garcia-Cohen, E.C., J. Marin, L.D. Diez-Picazo, A.B. Baena, M. Salaices and M.A. Rodriguez-Martinez, 2000. Oxidative stress induced by tert-butyl hydroperoxide causes vasoconstriction in the aorta from hypertensive and aged rats: Role of cyclooxygenase-2 isoform. J. Pharmacol. Exp. Ther., 293: 75-81.

Ghorbanihaghjo, A., H. Argani, H. Foroughimoghaddam, J. Safa, N. Rashtchizadeh and M. Mesgari, 2008. Effect of isoproterenol on Cyclosporine-induced nephrotoxicity in rat. Transplant. Proc., 40: 3737-3741.

Gornall, A.G., C.J. Bardawill and M.M. David, 1949. Determination of serum proteins by means of the biuret reaction. J. Biol. Chem., 177: 751-766.

Gross, R.T., R. Bracci, N. Rudolph, E. Schroeder and J.A. Kochen, 1967. Hydrogen peroxide toxicity and detoxification in the erythrocytes of newborn infants. Blood, 29: 481-493.

Hagar, H.H., E. El Etter and M. Arafa, 2006. Taurine attenuates hypertension and renal dysfunction induced by cyclosporine A in rats. Clin. Exp. Pharmacol. Physiol., 33: 189-196.

Heikal, T.M., A.T.H. Mossa, M.A. Abdel Rasoul and G.I.K. Marei, 2013. The ameliorating effects of green tea extract against cyromazine and chlorpyrifos induced liver toxicity in male rats. Asian J. Pharm. Clin. Res., 6: 48-55.

Henry, R.I. and O.P. Marmion, 1974. Clinical Chemistry Principle and Techniques. 2nd Edn., Harper Publishing Co., London, pp: 25.

Higgins, R., K. Ramaiyan, T. Dasgupta, H. Kanji, S. Fletcher, F. Lam and H. Kashi, 2004. Hyponatraemia and hyperkalaemia are more frequent in renal transplant recipients treated with tacrolimus than with cyclosporin. Further evidence for differences between cyclosporin and tacrolimus nephrotoxicities. Nephrol. Dial. Transplant., 19: 444-450.

Higginson, J., H. Wackerhage, N. Woods, P. Schjerling, A. Ratkevicius, N. Grunnet and B. Quistoff, 2002. Blockades of mitoge-activated protein kinase and calcineurin both change fiber-type markers inn skeletal muscle culture. Pflugers Arch., 445: 437-443.

Hulzebos, C.V., C.M.A. Bijleveld, F. Stellaard, F. Kuipers and V. Fidler *et al.*, 2004. Cyclosporine A-induced reduction of bile salt synthesis associated with increased plasma lipids in children after liver transplantation. Liver Transplant., 10: 872-880.

Issabeagloo, E., F. Ahmadpoor, P. Kermanizadeh and M. Taghizadieh, 2012. Hepatoprotective effect of green tea on hepatic injury due to leflunomide in rat. Asian J. EXP. Biol. Sci., 3: 136-141.

Jaffe, A.B., 1986. Technological opportunity and spillovers of R & D: Evidence from firms' patents, profits and market value. Am. Econ. Rev., 76: 984-1001.

Jeon, Y.J. and Y.S. Kim, 2011. Cyclosporin a inhibits albumin synthesis in Huh7 cells. Korean J. Int. Med., 26: 314-319.

Johnson, P.T.J., K.B. Lunde, E.G. Ritchie and A.E. Launer, 1999. The effect of trematode infection on amphibian limb development and survivorship. Science, 284: 802-804.

Kaplan, L.A., 1984. Glucose. In: Clinical Chemistry: Theory, Methods and Practice, Kaplan, L.A. and A.J. Pesce (Eds.). C.V. Mosby Co., St. Louis, MO., pp: 1032-1036.

Khan, N. and H. Mukhtar, 2007. Tea polyphenols for health promotion. Life Sci., 81: 519-533.

Khan, S.A., S. Priyamvada, N.A. Arivarasu, S. Khan and A.N. Yusufi, 2007. Influence of green tea on enzymes of carbohydrate metabolism, antioxidant defense and plasma membrane in rat tissues. Nutrition, 23: 687-695.

Kobayashi, Y., M. Suzuki, H. Satsu, S. Arai and Y. Hara *et al.*, 2000. Green tea polyphenols inhibit the sodium-dependent glucose transporter of intestinal epithelial cells by a competitive mechanism. J. Agric. Food Chem., 48: 5618-5623.

Koracevic, D., G. Koracevic, V. Djordjevic, S. Andrejevic and V. Cosic, 2001. Method for the measurement of antioxidant activity in human fluids. J. Clin. Pathol., 54: 356-361.

Kou, R., D. Greif and T. Michel, 2002. Dephosphorylation of endothelial nitric-oxide synthase by vascular endothelial growth factor. Implications for the vascular responses to cyclosporin A. J. Biol. Chem., 277: 29669-29673.

Kumar, P.V., A.A. Pricy, C.S. Kumar and G.K.K. Goud, 2010. Hepatoprotective effect of green tea (Camellia sinesis) on Cadmium chloride induced toxicity in rats. J. Chem. Pharm. Res., 2: 125-128.

Lawrence, M.C., H.S. Bhatt and R.A. Easom, 2002. NFAT regulates insulin gene promoter activity in response to synergistic pathways induced by glucose and glucagon-like peptide-1. Diabetes, 51: 691-698.

Lin, A.M., B.Y. Chyi, L.Y. Wu, L.S. Hwang and L.T. Ho, 1998. The antioxidative property of green tea against iron-induced oxidative stress in rat brain. Chin. J. Physiol., 41: 189-194.

Lorenz, M., S. Wessler, E. Follmann, W. Michaelis and T. Dusterhoft *et al.*, 2004. A constituent of green tea, epigallocatechin-3-gallate, activates endothelial nitric oxide synthase by a phosphatidylinositol-3-OH-kinase-,cAMP-dependent protein kinase- and Akt-dependent pathway and leads to endothelial-dependent vasorelaxation. J. Biol. Chem., 279: 6190-6619.

Mesbah, L., B. Soraya, S. Narimane and P.F. Jean, 2004. Protective effect of flavonides against the toxicity of vinblastine cyclophosphamide and paracetamol by inhibition of lipid-peroxydation and increase of liver glutathione. Haematology, 7: 59-67.

Mohamadin, A.M., H.A. El-Beshbishy and M.A. El-Mahdy, 2005. Green tea extract attenuates cyclosporine A-induced oxidative stress in rats. Pharmacol. Res., 51: 51-57.

Montgomery, H.A.C. and J.F. Dymock, 1961. Colorimetric determination of nitric oxide. Analyst, 86: 414-417.

Padi, S.S. and K. Chopra, 2002. Salvage of cyclosporine A-induced oxidative stress and renal dysfunction by carvedilol. Nephron, 92: 685-692.

Paoletti, F. and A. Mocali, 1990. Determination of superoxide dismutase activity by purely chemical system based on NAD(P)H oOxidation. Meth. Enzymol., 186: 209-220.

Petkovska, L.,N. Ivanovski, C. Dimitrovski and V. Serafimoski, 2008. Clinical importance of insulin resistance after renal transplantation in patients on triple immunosuppressive therapy with cyclosporine, corticosteroids and mycophenolate mofetil. Prilozi, 29: 129-139.

Petrussa, E., V. Casolo, E. Braidot, E. Chiandussi, F. Macri and A. Vianello, 2001. Cyclosporin A induces the opening of a potassium-selective channel in higher plant mitochondria. J. Bioenerg. Biomembr., 33: 107-117.

Rao, N.K. and S. Nammi, 2006. Antidiabetic and renoprotective effects of the chloroform extract of Terminalia chebula Retz. seeds in streptozotocin-induced diabetic rats. BMC Complement. Altern. Med., Vol. 6. 10.1186/1472-6882-6-17

Rietveld, A. and S. Wiseman, 2003. Antioxidant effects of tea: Evidence from human clinical trials. J. Nutr., 133: 3285S-3292S.

Saw, M., J.H. Stromme, J.L. Iondon and L. Theodorsen, 1983. IFCC method for g-glutamyletransferase (g-glutamyl)-peptide: Aminoacid g-glutamyletransferase. Clin. Chem. Acta., 135: 315F-338F.

Schultz, A., 1984. Uric Acid. In: Clinical Chemistry: Theory, Methods and Practice, Kaplan, L.A. and A.J. Pesce (Eds.). C.V. Mosby Co., St. Louis, MO., pp: 1261-1266.

Shakiba, Y., A. Mostafaie, D. Arshadi and B. Sabayan, 2009. Application of garlic organosulfur compounds in prevention of cyclosporine A-induced hepatotoxicity. Iran J. Med. Hypotheses Ideas, 3: 1-4.

Shihab, F.S., W.M. Bennett, J. Isaac, H. Yi and T.F. Andoh, 2003. Nitric oxide modulates vascular endothelial growth factor and receptors in chronic cyclosporine nephrotoxicity. Kidney Int., 63: 522-533.

Shirai, N. and H. Suzuki, 2004. Effects of Western, vegetarian and Japanese dietary fat model diets with or without green tea extract on the plasma lipids and glucose and liver lipids in mice. A long-term feeding experiment. Ann. Nutr. Metab., 48: 95-102.

Skrzydlewska, E., J. Ostrowska, R. Farbiszewski and K. Michalak, 2002. Protective effect of green tea against lipid peroxidation in the rat liver, blood serum and the brain. Phytomedicine, 9: 232-238.

Stein, E.A., 1987. Lipids, Lipoproteins and Apolipoproteins. In: Fundementals of Clinical Chemistry, Tietz, N.W. (Ed.). 3rd Edn., WB Saunders, Philadelphia, Pages: 448.

Tietz, N.W., 1995. Clinical Guide to Laboratory Tests. 3rd Edn., W.B. Saunders, Philadelphia, pp: 306-309.

Tirkey, N., G. Kaur, G. Vij and K. Chopra, 2005. Curcumin, a diferuloylmethane, attenuates Cyclosporine-induced renal dysfunction and oxidative stress in rat kidneys. BMC Pharmacol., Vol. 5. 10.1186/1471-2210-5-15.

Tsubono, Y., Y. Nishino, S. Komatsu, C.C. Hsieh and S. Kanemura *et al.*, 2001. Green tea and the risk of gastric cancer in Japan. N. Engl. J. Med., 344: 632-636.

Tsuneki, H., M. Ishizuka, M. Terasawa, J.B. Wu, T. Sasaoka and I. Kimura, 2004. Effect of Green tea on blood glucose levels and serum proteomic patterns in diabetic (db/db) mice and on glucose metabolism in healthy humans. BMC Pharmacol., Vol. 4.

Tumlin, J.A. and J.M. Sands, 1993. Nephron segment-specific inhibition of Na^+/K^+-ATPase activity by cyclosporin A. Kidney Int., 43: 246-251.

Vinson, J.A. and J. Zhang, 2005. Black and green teas equally inhibit diabetic cataracts in a streptozotocin-induced rat model of diabetes. J. Agric. Food Chem., 53: 3710-3713.

Waltner-Law, M.E., L.X. Wang, B.K. Law, R. Hal, M. Nawano and D.K. Granner, 2002. Epigallocatechin gallate, a constituent of green tea, represses hepatic glucose production. J. Biol. Chem., 277: 34933-34940.

Wilcox, C.S., 2002. Reactive oxygen species: Roles in blood pressure and kidney function. Curr. Hypertens. Rep., 4: 160-166.

Wu, L.Y., C.C. Juan, L.T. Ho, Y.P. Hsu and L.S. Hwang, 2004. Effect of green tea supplementation on insulin sensitivity in Sprague-Dawley rats. J. Agric. Food. Chem., 52: 643-648.

Xu, J.B., X.F. Yuan and P.Z. Lang, 1997. Determination of catalase activity and catalase inhibition by ultraviolet spectrophtometry. Chin. Environ. Chem., 16: 73-76.

Yang, C.S., J.D. Lambert and S. Sang, 2009. Antioxidative and anti-carcinogenic activities of tea polyphenols. Arch. Toxicol., 83: 11-21.

Yeragi, S.G., A.M. Rama and V.A. Koli, 2003. Effect of pesticides on protein metabolism of mud skipper Boleophthalmus dussumieri. J. Ecotoxicol. Environ. Monit., 13: 211-214.

Young, D.S., 1990. Effects of Drugs on Clinical Laboratory Tests. AAGG Press, Washington, D.C.

Young, D.S., 1995. Effects of Drugs on Clinical Laboratory Tests. 4th Edn., AACC Press, Washington, DC.

Yumei, F., Y. Zhou, S. Zheng and A. Chen, 2006. The antifibrogenic effect of (-)-epigallocatechin gallate results from the induction of de novo synthesis of glutathione in passaged rat hepatic stellate cells. Lab. Invest., 86: 697-709.