PROTECTIVE EFFECT OF GREEN TEA EXTRACT ON CYCLOSPORINE A-INDUCED NEPHROTOXICITY IN RATS

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A B S T R A C T

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 not receive drugs or green tea and served as control, group 2 normal rats administrated with green tea extract (3% w/v) orally, group 3 rats supplied with CsA (25 mg/kg body weight, orally for 21 days) to induce nephrotoxicity, groups 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 cholesterol, triacylglycerols and phospholipids, urea, uric acid and creatinine, sodium and potassium, inorganic phosphorus, total Protein, albumin, and haptoglobin levels, lactate dehydrogenase (LDH) and gamma glutamyl transferase (GGT) activities were also determined. Moreover, kidney tissue malondialdehyde (MDA), reduced glutathione (GSH), nitric oxide (NO), total antioxidant capacity (TAO) levels, antioxidant enzymes catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GP_x) activities were also determined. The results revealed that CsA-induced nephrotoxicity caused asignificant increase in serum glucose, renal functions tests, lipid profiles and serum LDH and GGT activities with asignificant decrease in serum total protein, albumin and electrolytes concentrations which were reversed upon treatment with green tea extract. Also, CsA administration induced asignificant elevation in lipid peroxidation (MDA) along with a 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 functions, by bringing about a a significant decrease in peroxidative levels and increase in antioxidant status. These results indicate the nephroprotective potential effect and usefulness of green tea extract, as an excellent source of antioxidants, in modulating CsA-induced nephrotoxicity.

Keywords: Cyclosporine A; antioxidant enzymes, lipid peroxidation; Nephrotoxicity; Green tea extract.

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1. 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 [1]. Nephrotoxicity is the main effects of cyclosporine A (CsA) treatment. Although the mechanisms of nephrotoxicity are not completely defined, there is an 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, superoxide dismutase, catalase and glutathione-peroxidase) [2]. It is reported that the level of free radicals in urine was increased significantly following CsA treatment. ROS could also be derived either directly from CsA or during its metabolism by the cytochrome P450 system [3]. Acute CsA treatment induces reversible reduction of the glomerular filtration rate (GFR) and renal blood flow that is related to afferent arteriolar vasoconstriction. This may be referred 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) [4]. 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. 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 [5]. 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 [6]. 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 [7]. 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.

2. MATERIALS AND METHODS

2.1. Experimental animals:

Eighty white male albino rats of 12-16 weeks old and weighting 220 - 250 gm 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.

2.2. Drug and antioxidants:

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

1- 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 body weight/day for 21 days.

2- 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 1000ml 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 [8].

2.3. Experimental design:

After acclimatization to the laboratory conditions, the animals were randomly divided into four groups (twenty rats each) as follows: Group I : Rats received no drugs served as control for all experimental groups. Group II: 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 III: Rats were administered cyclosporine A (25 mg/kg body weight)

start from the day 22 of experiment, once daily by oral gavage, for a period of 21 days. Group IV : 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 III followed by 21 days later (end of experiment, 9 weeks).

2.4. 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.

2.4.1. 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 r.p.m for 15 minutes. The clean, clear serum was separated by Pasteur pipette and kept in a deep freeze at -20°C until used for subsequent biochemical analysis.

2.4.2. 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 r.p.m for 15 minute at 4°C until used for subsequent biochemical analysis.

2.5. Biochemical analysis:

Serum glucose, total protein, albumin, total cholesterol, triacylglycerols, phospho-lipids, 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 [9-22] 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 (GP_x) according to the methods described by [23-29] respectively.

2.6. 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 $p < 0.05$.

3. RESULTS:

The results presented in (Tables 1 and 2) revealed that, CsA-induced nephrotoxicity caused asignificant increase in serum glucose, lipid profiles (total cholesterol, triacylglycerol and phospholipids), renal functions 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 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 GP_x) activities, non-enzymatic antioxidant (GSH), total antioxidant capacity and nitric oxide level in the rat kidney.

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

Parameters / Groups		Glucose (mg/dl)	Total cholesterol (mg/dl)	Triacylglycerols (mg/dl)	Phospholipids (mg/dl)	Creatinine (mg/dl)	Uric acid (mg/dl)	Urea (mg/dl)
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 ± 0.84 ^{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± 8.35 ^{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± 7.48 ^{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 ± 2.63 ^{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± 2.76 ^{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± 8.17 ^{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± 5.43 ^{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± 7.18 ^{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 ± S.E). S.E = 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- induced nephrotoxicity in rats:

Parameters / Groups		Sodium (meq./L)	Potassium (meq./L)	Inorganic phosphorus (mg/dl)	Total protein (gm/dl)	Albumin (gm/dl)	Haptoglobin (mg/dl)	LDH (U/L)	GGT (U/L)
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 ± S.E). S.E = 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-induced nephrotoxicity in rats:

Parameters / Groups		(L-MDA) (nmole/g.tissue)	CAT (U/g. tissue)	(SOD) (U/g. tissue)	GPx (GSH consumed/min/mg protein)	(GSH) (mg/g.tissue)	(NO) (µmol/g.tissue)	(TAO) (mmol/g. tissue)
1st Week	C	31.23± 8.47 ^{c}	2.10± 0.21 ^{a}	36.36± 3.22 ^{a}	0.46± 0.002 ^{a}	64.33± 2.33 ^{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± 2.52 ^{a}	84.86± 13.36 ^{ab}	0.66± 0.05 ^{bc}
	CsA	106.27± 9.21 ^{a}	1.73± 0.16 ^{a}	24.38± 4.12 ^{bc}	0.42± 0.009 ^{b}	29.67± 3.38 ^{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± 4.04 ^{b}	72.94± 11.64 ^{ab}	0.86± 0.08 ^{b}
2nd Week	C	22.56± 4.27 ^{c}	1.60± 0.24 ^{bc}	26.71± 2.72 ^{abc}	0.43± 0.011^{a}	41.00± 3.37 ^{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± 4.06 ^{a}	34.26± 9.54 ^{ab}	0.62± 0.04 ^{a}
	CsA	67.68± 2.63 ^{ab}	1.43± 0.06 ^{c}	16.14± 0.58 ^{d}	0.40± 0.011 ^{a}	33.50± 2.02 ^{a}	23.34± 4.21 ^{b}	0.55± 0.03 ^{a}
	CsA+GT	47.50± 7.17 ^{b}	1.45± 0.08 ^{c}	20.86± 3.30 ^{bcd}	0.42± 0.002 ^{a}	46.00± 5.29 ^{a}	47.28± 4.88 ^{a}	0.68± 0.01 ^{a}
3rd Week	C	29.07± 7.10 ^{b}	1.88± 0.07 ^{ab}	30.28± 0.33 ^{a}	0.43± 0.012 ^{a}	32.33± 0.67 ^{bc}	61.87± 9.82 ^{a}	0.74± 0.08 ^{bcd}
	GT	35.47± 5.82 ^{ab}	2.08± 0.23 ^{a}	29.14± 2.81 ^{a}	0.40± 0.004 ^{b}	55.67± 0.33 ^{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± 1.44 ^{c}	53.33± 1.28 ^{ab}	0.63± 0.02 ^{d}
	CsA+GT	49.78± 8.45 ^{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 ± S.E). S.E = Standard error. Mean values with different superscript letters in the same column are significantly different at (P<0.05).

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 GP_x), GSH and total antioxidant capacity levels.

4. DISCUSSION

Nephrotoxicity is the most common and clinically significant adverse effect of cyclosporine [30].Oxidative stress is the main mechanism resulting in cyclosporine-induced nephrotoxicity because of its ability to stimulate endogenous melatonin production [31]. Cyclosporine treatment to control rats resulted in a significant increase in serum glucose concentration compared to control group. Cyclosporine had 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) [32]. 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 [33]. 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. Nevertheless, the degree and comparability of the calcineurin inhibitors in impairing beta-cell function is yet to be established [34]. Green tea extract administration to CsA treated rats resulted in significant decrease in serum glucose levels compared with CsA group. Green tea reduced blood glucose level in both type 1 and type 2 of diabetic rats models [35,36]. The antihyperglycemic effect of green tea constituents was ascribed to the activities of basal insulin [37], inhibition of intestinal glucose transporter [38] and decrease the expression of genes that control gluconeogenesis. Additionally, 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, $SGLT_1$. The transport activity of $SGLT_1$ was markedly inhibited by green tea polyphenols [39]. A significant increase in serum renal functions tests (urea, uric acid and creatinine) concentrations were observed in CsA-treated rats as compared with control group. Chronic administration of CsA for 21 days caused a marked impairment of renal function along with significant oxidative stress in the kidneys [40]. Oxidative stress can promote the formation of a variety of vasoactive mediators that can affect renal functions directly by causing renal vasoconstriction or decreasing the glomerular capillary ultrafiltration coefficient; and thus reducing glomerular filtration rate [41]. These findings were further evident from the marked elevation of serum urea, uric acid and creatinine concentrations, there by suggesting a significant functional impairment of kidneys in cyclosporine A-treated rats [30]. 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 [42]. Green tea administration to cyclosporine-treated rats exhibited a significant decrease in serum renal functions (urea, uric acid and creatinine) levels as compared with CsA group. Administration of green tea extract normalized the levels of plasma creatinine and uric acid [42]. Chronic administration of CsA also produced oxidative stress and increased the lipid peroxidation in kidneys [43]. Oxidative stress is caused by oxygen free radicals in the kidney, including the superoxide anion, the hydrogen peroxide

and the hydroxyl radical [44]. The antioxidant properties of flavonoids are due to their ability to directly scavenge some radical species [45]. Administration of Cyclosporine A to normal rats resulted in asignificant decrease in serum electrolytes (sodium, potassium) as compared with control group. Sodium depletion to be associated with CsA treatment [46]. Also, hypokalaemia was more frequent under cyclosporin treatment [47]. CsA-induced nephrotoxicity has been characterized by 20–30% reduction in glomerular filtiration 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 [3]. Administration of green tea to cyclosporine-treated rats induced asignificant increase in serum electrolytes levels compared with CsA group. 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 [48]. The decrease in Na^+-K^+ ATPase activity caused by CsA is thought to be one of the mechanisms for the observed potassium ion secretion defects [49]. Cyclosporine treatment to normal rats resulted in a significant increase in renal tissue (L-MDA) levels as compared with control group. Similarly, a 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 [51]. 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 [52]. However, administration of green tea in cyclosporine treated rats significantly decreased renal tissue (L-MDA) levels compared with CsA group. Green tea (GT) extract reversed the elevation of lipid peroxidation [50]. 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 [53]. Therefore, it may decrease the concentration of lipid free radicals [43]. Administration of cyclosporine to normal rats exhibited a significant decrease in renal tissue antioxidant enzymes (CAT, SOD and GP_x) 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 GP_x 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_2O_2 detoxification by glutathione peroxidase [51]. The decline in renal SOD activity after CsA administration [3]. It is well known that an efficient endogenous antioxidant defense system operates to combat the production of free radicals. The antioxidant enzymes catalase, SOD, GP_x 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 catalyzing the dismutation of superoxide to H_2O_2 and O_2. Green tea administration to cyclosporine A-treated rats resulted in a significant increase in renal tissue antioxidant enzymes (CAT,

SOD and GP_x) activities as compared with CsA group. The biological defense system was perturbed by GT consumption because 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 [54]. A significant decrease in renal tissue total antioxidant capacity (TAOC) level was observed in cyclosporine A-treated normal rats compared with control group. CsA therapy induces overproduction of reactive oxygen species (ROS) in hepatocytes and lowers their antioxidant capacity [55]. However, administration of green tea to cyclosporine A- treated rats caused a significant increase in renal tissue (TAOC) level as compared with CsA group. 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 [56]. 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.

5. REFERENCES

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التأثير الوقائي لمستخلص الشاي الأخضر في التسمم الكلوي المحدث بالسيكلوسبورين في الفئران

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تساعد مضادات في الأغذية، على حماية الجسم من العديد من الأمراض المختلفة التي تنجم عما يسمى الجذور الحرة او الذرات الطليقة، وتشمل تلك الأمراض السرطان وامراض الكبد وامراض الحساسية وامراض القلب وامراض الكلى وغيرها من الأمراض الضارة، أما الجذور الحرة فهي ذرات تسبب أذية الخلايا، ومن ضمنها خلايا الكلى مما يساهم في التسمم الكلوي ويقوم الجسم عادة بصنع إنزيمات تسمى إنزيمات الأكسدة تقوم بتعديل الجذور الحرة، وبالإضافة لهذه الأنزيمات هناك العديد من المواد الكيميائية التي تعمل كمضادات للأكسدة مثل مستخلص الشاي الأخضر ويعتبر مستخلص الشاي الأخضر من مضادات الأكسدة المهمة والقوية. وقد أجريت هذه الدراسة لأمد قصير مدته ستة اسابيع على ثمانون من ذكور الفئران البيضاء والتي تتراوح أعمارهم من 8-10 اسابيع وأوزانهم من 220 -250 جرام وقد تم تقسيمهم بالتساوي الى اربعة مجموعات كل مجموعة اشتملت على 20 فأر كالاتي : المجموعة الأولى (المجموعة الضابطة) : اشتملت على20 فأر لم تعطى أي أدوية واستخدمت كمجموعة ضابطة للمجموعات الأخرى .المجموعة الثانية (مجموعة مستخلص الشاي الأخضر) : اشتملت على 20 فأر وقد تجرعت المستخلص المائي لشاي الأخضر عن طريق الفم بتركيز مقداره 3 % كمصدر لماء الشرب طول فترة التجربة . المجموعة الثالثة (مجموعة السيكلوسبورين المحدث بها التسمم الكلوي): اشتملت على 20 فأر تم تجرعهم السيكلوسبورين يوميا عن طريق الفم بجرعة مقدارها 25 مليجرام لكل كيلوجرام من وزن الجسم لمدة 21 يوم وذلك بعد ثلاث اسابيع من بداية التجربة. المجموعة الرابعة (مجموعة مستخلص الشاي الأخضرالمحدث بها التسمم الكلوي): اشتملت على 20 فأر وقد أعطيت المستخلص المائي لشاي الأخضر عن طريق الفم بتركيز مقداره 3 % كمصدر لماء الشرب طول فترة التجربة ثم تجرعهم السيكلوسبورين يوميا عن طريق الفم بجرعة مقدارها 25 مليجرام لكل كيلوجرام من وزن الجسم لمدة 21 يوم وذلك بعد ثلاث اسابيع من بداية التجربة. وخلصت الدراسة على أن التسمم الكلوي له تأثير ضار على المكونات الكيميائية الحيوية للسيرم وأنسجة الكلى وذلك من خلال اضطراب وخلل فى تركيب غشاء الخلية الذي يرجع أساسا إلى تكوين الجذور الحرة التي تهاجم غشاء الخلايا وأن المستخلص المائي لشاي الأخضر أدى إلى الحد من الأثار الضارة والناجمة للتسمم الكلوي المسببة لتلف وتدمير خلايا الكلى وذلك من خلال منع الأكسدة الفوقية للدهون والإجهاد التأكسدي والتقليل من استجابة الالتهابات نتيجة التجريع بالسيكلوسبورين. لذلك ينصح بتناول الشاي الأخضر في حدود الجرعة الطبية الموصي بها وذلك للوقاية من تلف الخلايا التي تنجم عما يسمى الجذور الحرة أو الذرات الطليقة المسببة للعديد من الأمراض الضارة للجسم.

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