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Renoprotective Effect of Dietary Fish Oil on Cyclosporine A: Induced Nephrotoxicity in Rats

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ABSTRACT

The immunosuppressive drug cyclosporine A (CsA) has been successfully used in several diseases with immunological basis and in transplant patients. Nephrotoxicity is the main secondary effect of CsA treatment. The present study was designed to investigate the possible protective effect of dietary fish oil (F.O.) 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 (dietary fish oil) omega-3 fatty acids 270 mg kg^{-1} b.wt. oral dose daily, Group 3: Rats treated with CsA (25 mg kg^{-1} b.wt., orally for 21 days) to induce nephrotoxicity, Groups 4: Rats received dietary fish oil 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 capacity (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 dietary fish oil. 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, Dietary fish oil 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 dietary fish oil, as an excellent source of antioxidants, in modulating CsA-induced nephrotoxicity.

Key words: Cyclosporine A, antioxidant enzymes, lipid peroxidation, renal function, nephrotoxicity, dietary fish oil

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 P-450 system (Mohamadin *et al*., 2005).

Moreover, 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 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.

In past few years much interest has been centered on the role of naturally occurring dietary substances for the control and management of various chronic diseases. A number of investigations have demonstrated that diet supplemented with fish oil (FO) enriched in omega-3 fatty acids has profound beneficial health effects against various pathologies (Simopoulos, 1991) including cardiovascular diseases, respiratory diseases, diabetes, depression, cancers, inflammatory and immune renal disorders (Thakkar *et al*., 2000). Reports showed that FO prevents gentamicin and cyclosporine-A-induced nephrotoxicity (Priyamvada *et al*., 2008).

Fish oil is rich sources of the essential fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Compared to saturated fats, PUFAs are more readily used for energy when initially ingested. Increasing the degree of unsaturation at a given carbon chain length increases the relative mobility of stored fat, making PUFAs more bioavailable (Storlien *et al*., 2000). Studies also showed that fish oil inhibited growth of breast cancer cells and hepatocarcinoma in rats (Rahman *et al*., 1999). Moreover, fish oil as n-3 polyunsaturated fatty acid, eicosapentaenoic and docosahexaenoic acid inhibited human lung carcinoma cell growth and prostate cancer (Han *et al*., 2009). Thakkar *et al*. (2000) showed that FO prevents gentamicin and cyclosporine-A-induced nephrotoxicity.

The most common dose-limiting effects of cyclosporin are hypertension and impaired renal function. These effects appear to be in part thromboxane mediated (Grieve *et al*., 1993). Fish oil inhibits TXA2 production and reduces both the hypertensive and nephrotoxic effects of cyclosporin (Stoof *et al*., 1989). An additional and important factor in the nephroprotective activity of any drug is the ability of its constituents to inhibit the aromatase activity of cytochrome P-450, thereby favoring liver regeneration. On that basis, it is suggested by Speck *et al*. (1990) which fish oil could be a factor contributing to its nephroprotective ability through inhibition of cytochrome P-450 aromatase. Accordingly, the present study was designed to investigate the possible protective effect of dietary fish oil on nephrotoxicity induced by CsA and the potential biochemical role by which dietary fish oil 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.

Drugs: The drugsused 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} body weight day^{-1} for 21 days

- **Fish oil (Omega-3 plus)^{R}:** Fish oil (Omega-3 plus) was obtained as soft gelatin capsule each capsule contains 1000 mg Omega-3 fatty acids. Fish oil (Omega-3 plus) was manufactured by South Egypt Drug Industries Co. (SEDICO),6 October City-Egypt. Omega-3 fatty acids was dissolved in propylene glycol and administered orally in a daily dose of 270 mg kg^{-1} b.w. The dosage of Omega-3 plus was chosen to be within the therapeutic range levels reported in the pamphlet

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 omega-3 fatty acids (270 mg kg^{-1} b.wt. $orally^{-1}$) once per day all over the experimental periods (9 weeks)
- **Group III:** Rats administered cyclosporine A (25 mg kg^{-1} b.wt.) start from the day 22 of experiment, once daily by oral gavage, for a period of 21 days
- **Group IV:** Rats received Omega-3 fatty acids at a dose of 270 mg kg^{-1} b.wt. orally and daily 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)

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 centrifuged at 6000 rpm for 15 min at 4°C then the supernatant was 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. Dietary fish oil 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, Dietary fish oil 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 fish oil administration on serum glucose, lipid profiles and renal function tests in normal and cyclosporine A-induced nephrotoxicity in rats

	Parameters (mg dL^{-1})						
Groups	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}
FO	79.25±4.71^{c}	87.90±3.88^{b}	73.67±9.42^{c}	127.23±7.39^{b}	0.78±0.03^{bc}	2.61±0.17^{b}	29.46±2.77^{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+FO	104.25±1.89^{b}	94.07±6.22^{b}	68.73±2.72^{c}	127.23±4.43^{b}	0.84±0.02^{b}	2.66±0.18^{b}	29.68±2.68^{c}
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}
FO	109.75±5.85^{c}	111.16±4.05^{b}	90.51±4.49^{b}	119.91±16.11^{b}	0.87±0.05^{b}	2.07±0.06^{b}	13.67±1.16^{c}
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+FO	121.00±2.38^{b}	66.84±6.05^{d}	83.55±12.33^{b}	137.26±4.07^{b}	0.87±0.02^{b}	2.23±0.13^{b}	16.49±1.18^{c}
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}
FO	121.00±2.48^{a}	89.97±9.45^{b}	59.24±7.06^{b}	131.21±10.08^{b}	0.84±0.05^{bc}	1.60±0.07^{b}	20.45±1.83^{bc}
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+FO	120.75±5.22^{a}	78.29±3.87^{b}	67.60±8.30^{b}	131.21±9.39^{b}	1.01±0.08^{b}	1.89±0.17^{b}	20.79±1.51^{bc}

C: Control normal group, FO: Fish oil group, CsA: Cyclosporine A group, CsA+FO: Cyclosporine A+Fish oil 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 fish oil administration on serum electrolytes, proteins and Haptoglobin concentrations, LDH and GGT activities in normal and cyclosporine A-induced nephrotoxicity in rats

	Parameters							
Groups	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}
FO	143.78±1.10^{a}	6.72±0.10^{a}	2.53±0.74^{c}	6.38±0.10^{a}	3.82±0.15^{a}	66.97±24.14^{b}	781.17±246.43^{c}	15.29±0.71^{ab}
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+FO	139.43±0.81^{a}	6.09±0.50^{a}	1.40±0.07^{c}	6.43±0.15^{a}	3.75±0.08^{a}	88.97±15.70^{b}	1321.15±129.28^{bc}	12.57±0.74^{c}
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}
FO	143.43±1.10^{ab}	6.33±0.32^{a}	2.89±0.51^{a}	6.28±0.18^{a}	3.70±0.19^{a}	26.07±1.22^{a}	1584.0±117.65^{b}	14.96±0.76^{ab}
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+FO	140.05±0.46^{c}	5.54±0.26^{b}	1.89±0.17^{a}	6.03±0.17^{a}	3.20±0.03^{b}	47.88±15.93^{a}	594.98±81.65^{c}	16.85±0.36^{a}
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}
FO	142.68±1.40^{ab}	5.87±0.44^{a}	3.12±0.52^{b}	6.02±0.08^{ab}	3.28±0.07^{b}	43.80±4.54^{a}	720.46±80.75^{c}	15.50±0.35^{a}
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+FO	143.75±0.99^{ab}	6.32±0.34^{a}	1.61±0.37^{b}	6.68±0.10^{a}	3.11±0.06^{b}	44.70±20.35^{a}	716.41±17.95^{c}	14.46±0.71^{ab}

C: Control Normal group, FO: Fish oil group, CsA: Cyclosporine A group, CsA+ FO: Cyclosporine A+fish oil 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 $p<0.05$

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).

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

	Parameters						
Groups	L-MDA (nmole 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±8.47^{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}
FO	24.73±3.21^{c}	2.38±0.03^{a}	21.55±1.41^{c}	0.45±0.013^{a}	35.00±2.08^{b}	106.48±3.98^{ab}	0.64±0.03^{c}
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+FO	96.64±0.57^{a}	1.94±0.23^{a}	30.28±2.22^{abc}	0.42±0.003^{b}	32.00±3.51^{b}	82.67±28.01^{ab}	0.74±0.12^{bc}
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}
FO	19.16±2.82^{a}	1.85±0.18^{bc}	30.95±0.97^{a}	0.43±0.007^{a}	37.67±4.98^{a}	39.44±3.70^{ab}	0.68±0.13^{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+FO	57.05±12.15^{ab}	2.11±0.07^{b}	28.85±4.36^{ab}	0.44±0.019^{a}	44.50±8.37^{a}	52.59±6.58^{a}	0.74±0.03^{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}
FO	28.20±6.08^{b}	2.02±0.16^{a}	35.28±4.87^{a}	0.42±0.006^{ab}	33.33±4.70^{bc}	68.51±4.65^{a}	0.81±0.02^{abc}
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+FO	51.74±0.94^{ab}	1.42±0.23^{b}	29.28±0.58^{a}	0.41±0.016^{ab}	31.33±3.84^{bc}	58.37±3.18^{ab}	0.68±0.08^{cd}

C: Control Normal group, FO: Fish oil group, CsA: Cyclosporine A group, CsA+ FO: Cyclosporine A+fish oil 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 $p<0.05$

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). Dietary fish oil 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 Luo *et al.* (1996) who demonstrated that, feeding Sprague Dawley rats with a fish oil diet for 6 weeks increased the incorporation of n-3 fatty acids into the membrane phospholipid fraction of adipocytes. This was associated with increased insulin sensitivity in the adipocytes, as insulin-stimulated glucose uptake was positively correlated with the degree of unsaturation of membrane phospholipid fatty acids.

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. Dietary fish oil 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 Balk *et al.* (2006) stated that, reductions in triglyceride (TG) levels following consumption of n-3 PUFAs have been demonstrated as well as increases in levels of high-density lipoprotein (HDL) cholesterol.

Dietary fish oils, rich in long-chain n-3 fatty acids, lower plasma triglyceride and Very Low Density Lipoprotein (VLDL) levels in both normo- and hyperlipidemic subjects. VLDL-triglyceride kinetic studies have been performed in humans and they suggested that the hypotriglyceridemic effect of fish oil is primarily caused by inhibition of VLDL-triglyceride synthesis (Van Vlijmen *et al.*, 1998). Omega-3 fatty acids have been shown to have a cholesterol-reducing effect in hypercholesterolemic animals, most likely through reduction in liver synthesis of cholesterol or increased clearance of triglyceride-richVLDLcholesterol (O'Connor *et al.*, 2007). Moreover, the reduction in lipidemia of animals supplemented with high EPA and DHA doses is well established (Gaiva *et al.*, 2003). Some mechanisms involve increase of fatty acid oxidation in liver, inhibition of de novo fatty acid synthesis, higher binding affinity of LDL to membrane (Castro *et al.*, 2005).

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. Similarly, Lanese and Conger (1993) found that, cyclosporine causes vasoconstriction of afferent and efferent glomerular arterioles, decreased glomerular filtration rate and reduced renal blood flow. The most common dose-limiting effects of cyclosporin are hypertension and impaired renal function. These effects appear to be in part thromboxane mediated (Grieve *et al.*, 1993).

Dietary fish oil administration to cyclosporine treated rats exhibited a significant decrease in serum renal function (urea, uric acid and creatinine) levels as compared with CsA group. The n-3 PUFAs present in fish oil (DHA and EPA) enhance the renal production and excretion of the trienoic series of eicosanoids: PGI3, PGE3 and TXA3. PGI3 and PGE3 are potent renal vasodilators, whereas TXA3 has little effect on vascular smooth muscle tone. Moreover, production of the dienoic prostaglandins (PGI2, PGE2) and the potent vasoconstrictor TXA2 is reduced by dietary supplementation with fish oil (De Caterina *et al.*, 1994).

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 Benkoel *et al.* (1999) who reported that, cyclosporine A may also exert its cytotoxic effects by altering the activity of different plasma membrane transport systems. Membrane ATPases play a key role in the production and maintenance of gradients that aid in the ion distribution in cells. The decline in the activities of ATPases in cyclosporine A treated rats may be due to enhanced oxidation of membrane lipids and proteins. Inhibition of membrane bound Na^+, K^+-ATPase which is present in both basolateral and apical domain of the rat plasma membrane will

cause an increase in intracellular Na^+ and loss of K^+ that leads to membrane depolarization. It is well known that cyclosporine A treatment induces a decrease in Na^+-, K^+ ATPases (Mardini *et al*., 2001) which involves a specific interaction between the drug and the enzyme catalytic subunit (You *et al*., 2002). In fact, it was shown in humans and rats that cyclosporine A directly interferes with the membrane permeability of Na^+ and K^+ by disturbing the transmembrane potential and cellular ionic gradients (Fricker and Fahr, 1997). Increasing evidence supports the point that free radicals are involved in the inactivation of Na^+, K^+ -ATPase and supplementation of antioxidant results in abolishing the inhibitory effect on the enzyme (Streck *et al*., 2001).

In contrast, these results came in disagreement with Prodanovic and Korman (2008) who reported that, electrolyte disturbances can be seen with cyclosporine use. Reduced efficiency of urinary potassium excretion by directly impairing the function of potassium secreting cells in the collecting tubule can lead to hyperkalemia that can be further exacerbated with use of medications that diminish aldosterone release, such as the ACE inhibitors. Administration of dietary fish oil to cyclosporine treated rats induced significant increase in serum electrolytes levels compared with CsA group. These results came in agreement with Mannaa *et al*. (2011) who found that, the dietary fish liver oil rich in eicosapentanoic and docosahexaenoic fatty acids may prevent the membrane alteration and by this mechanism prevent the changes in Na^+/K^+-ATPase activity.

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 dietary fish oil administration in cyclosporine treated rats as compared with CsA group. Natarajan *et al*. (2006) who showed a decrease of total protein content due to destruction of protein synthesizing subcellular structures due to several reasons like increased free radical production. Treatment with fish oil and purslane shows their ability to restore the normal functional status of the poisoned liver (Hozayen *et al*., 2011).

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 dietary fish oil to cyclosporine treated rats resulted in significant decrease in serum enzymes (LDH and GGT) activities when compared with CsA group. Fish oils are the main source of human dietary long chain Omega-3 polyunsaturated fatty acids. A fish oil rich diet, by replacing arachidonic acid by

eicosapentanoic acid (EPA) and docosahexanoic acid (DHA) in plasma and in phospholipid membranes, impairs the release of free radicals by different types of cells and induces changes in eicosanoid metabolites (Lopez *et al.*, 2001). 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 dietary fish oil in cyclosporine treated rats significantly decreased renal tissue (L-MDA) levels compared with CsA group. These results came in agreement with Gopal *et al.* (2011) who administrated that, pretreatment with fish oil, (both 5, 10% V/W of diet) there was significant reduction in the levels of lipid peroxides indicating that fish oil inhibit the lipid oxidation. Oxygen free radical attack objects on the polyunsaturated components of membranes and may cause a serious organizational dysfunction within cells and tissues (Devasagayam *et al.*, 2004). It has been suggested that the use of omega-3 PUFAs may have ameliorating effect on such damage by two possible ways: First, omega-3 PUFA may increase the levels of catalase within the peroxisome and in the cytoplasm resulting in enhanced defense against free oxygen radicals. Second,omega-3 PUFAs, which has been supplemented, may be replaced with Polyunsaturated fatty acid components of the membranes that had been attacked by oxygen free radicals such as superoxide anions, hydrogen peroxide and hydroxyl radicals (Ozgocmen *et al.*, 2000). High oxidative stress due to hyperglycaemia promotes free radicals generation evidence based mainly on increased lipid peroxidation (Hong *et al.*, 2004). Moreover, Delattre *et al.* (2010) reported that the possible mechanisms of DHA in decreasing lipid peroxidation as DHA associated with vinyl ether bonds of plasmalogens (glycerophospholipids) in the combat of free radicals.

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_2O_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 antioxidant 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. Dietary fish oil 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, Attia *et al.* (2011) who reported that, under normal

physiological conditions, a delicate balance exists between the rate of formation of H_2O_2 via dismutation of O^2- by SOD activity and the rate of removal of H_2O_2 by CAT and glutathione peroxidase. Therefore, any impairment in this pathway will affect the activities of other enzymes in the cascade. n-3 PUFA may stimulate α-tocopherol incorporation into membranes, increasing the level of CAT within both peroxisomes and cytoplasm, resulting in an enhanced defense against Reactive Oxygen Species (ROS).

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 dietary fish oil administration in cyclosporine A treated rats as compared with CsA group. These results are harmony with these reported by Xi and Chen (2000) who suggested that, GSH is often the first line of defense against oxidative stress. GSH levels can be increased due to an adaptive mechanism to slight oxidative stress through an increase in its synthesis; however, a severe oxidative stress may suppress GSH levels due to the loss of adaptive mechanisms and the oxidation of GSH to its oxidized form, GSSG. Fish oil may maintain or increase GSH levels through an adaptive mechanism by either increasing its synthesis or regeneration through increased GR activity.

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 dietary fish oil to cyclosporine treated rats caused significant increase in renal tissue (NO) level when compared with CsA group. These results disagreement with El-Saeed *et al.* (2013) who suggested that, NO, a lipid soluble ROS, is generated by the action of nitric oxide syntheses (NOS). Because NO is formed by the stoichiometric conversion of l-arginine to l-citrulline, decreased NO level in omega-3 EFA supplementation may suggest the decreased entrance of l-arginine.

A significant decrease in renal tissue total antioxidant 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 dietary fish oil to cyclosporine treated rats caused significant increase in renal tissue (TAOC) level as compared with CsA group. These results came in agreement with Sgro *et al.* (2002) who found that, fish oil which is rich in n-3PUFAs i.e., EPA and DHA interfere with arachidonic acid cascade by inhibiting 5-lipooxygenase (5-LOX). Incorporation of the n-3 PUFAs with biological membrane, increased antioxidant status normalizes the excited state, controls the physical status of membrane lipids and prevents rises in intracellular Ca in response to oxidative stress.

In view of these findings, it is possible to conclude that CsA administration results in pronounced oxidative stress and renal damage. Fish oil 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 Fish oil is effective in preventing functional impairment in CsA-induced nephrotoxicity in a rat model.

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