Fish oil rich in eicosapentaenoic acid protects against oxidative stress-related renal dysfunction induced by TCDD in Wistar rats

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Abstract Humans are systemically exposed to persistent organic pollutants, of which 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) has become a major environmental concern. Exposure to TCDD results in a wide variety of adverse health effects which is mediated by oxidative stress through CYP1A1 activation and arachidonic acid metabolites. Eicosapentaenoic acid (EPA) exhibits antioxidant property and competes with arachidonic acid in membrane phospholipids and produces anti-inflammatory EPA derivatives. Since both EPA and its derivatives have been reported to enhance the antioxidant mechanism, the present study aimed at studying whether EPA could offer protection against TCDD-induced oxidative stress and nephrotoxicity in Wistar rats. Estimation of kidney markers (serum urea and creatinine) and histopathological studies revealed that EPA treatment significantly reduced TCDD-induced renal damage. TCDD-induced oxidative damage was reflected in a significant increase in CYP1A1 activity and lipid peroxide levels with a concomitant decline in non-enzymic antioxidant (GSH) and various enzymic antioxidants such catalase (CAT), superoxide dismutase (SOD), glutathione-*S*-transferase (GST), and glutathione peroxidase (GPx). In addition, TCDD-induced oxidative stress also resulted in decline in Na⁺-K⁺ and Mg²⁺ ATPases activities with increase in Ca²⁺ ATPases activity. Oral treatment with EPA showed a significant cytoprotection against TCDD-induced renal oxidative stress by decreased CYP1A1 activity and enhanced antioxidant

status. TCDD-induced alterations in ATPase enzyme activities were also prevented by EPA treatment. Our results show clear evidence that EPA ameliorates TCDD-induced oxidative stress and kidney damage; thus suggest the potential of EPA as an effective therapeutic agent against toxic effects mediated through redox imbalance.

Keywords CYP1A1 · EPA · Fish oil · Oxidative stress · TCDD

Introduction

Humans are systemically exposed to persistent organic pollutants (POPs). These environmental contaminants are chemicals that constitute health hazards because of persistence, toxicity levels and mobility. 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) is a potent congener among the halogenated aryl hydrocarbon. TCDD arises from a wide variety of sources, including municipal incinerators, sewage sludge and discharge from paper mills, forest fires, automobile exhaust and cigarettes (Whysner and Williams 1996). The hydrophobic structure of TCDD dictates it to be highly persistent, resistant to degradation, tending to accumulate in environment with an estimated half-life of 7.6 years (Alaluusua et al. 2004). As a result the International Agency for Research on Cancer's classification, upgraded TCDD from possible human carcinogen to human carcinogen (IARC 1997). TCDD exerts its toxicity by binding to the aryl hydrocarbon receptor (AhR) and results in activation of CYP1A1 (Alsharif et al. 1994). CYP1A1 is known to be the prototypical target gene of AhR (Yeager et al. 2009). The CYP1A1 during its effort to metabolize TCDD generates reactive oxygen species and mutagenic metabolites which cause

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increased oxidative stress conditions (Denison et al. 2011; Park et al. 1996). Most of the toxic effects caused by TCDD are known to be mediated through AhR. However, it cannot be ruled out that early increase in $[Ca^{2+}]_i$ levels also contribute to the toxic effects caused by TCDD. An early increase in $[Ca^{2+}]_i$ levels results in activation of phospholipase A2 with subsequent release of arachidonic acid (AA, n6 fatty acid) from membrane phospholipids (Kita et al. 2006; Kinehara et al. 2009; Kopec et al. 2010). The released arachidonic acid acts as a substrate for COX-2 and cytochrome P450s and results in the production of pro-inflammatory eicosanoids (Bui et al. 2012). Thus, both ROS and pro-inflammatory eicosanoids have a profound role in TCDD-induced oxidative stress and toxic responses (Capdevila et al. 1988, 1992; Rifkind et al. 1990; Lee et al. 1998).

Most of the reports on identifying the protective compound against TCDD-induced toxicity have been focused on inhibition of CYP1A1, since it has been well documented in mediating oxidative stress. However, an overall protection strategy against TCDD-induced toxicity can be achieved by targeting both CYP1A1 and AA metabolites. Nutrition can modulate the toxicity of environmental pollutants by influencing the lipid milieu and antioxidant status and thus protect from health and disease outcome (Hennig et al. 2007). The levels of n3 and n6 fatty acid content in the cell and organelle membrane and membrane micro domain strongly influence membrane function and numerous cellular processes such as cell death and survival (Ross et al. 1992). Of these, n-3 fatty acids have been in focus recent years since they had been linked in reduction of various diseases by virtue of their antioxidant potential and anti-inflammatory mechanisms (Simopoulos 2002; Jho et al. 2002). Previous studies have shown the protective effect of fish oil rich in n-3 fatty acids against various pathological conditions such as cancer (Aronson et al. 2001), cardiovascular risk (Psota et al. 2006), depression (Hibbel 1998), nephrotoxicity (Donadio 2001; Priyamvada et al. 2008; Naqshbandi et al. 2012).

The present study was designed to analyze TCDD-induced nephrotoxicity in Wistar rats, since the toxic effects of TCDD in kidney tissue are not well understood. In addition, an effort was made to analyze whether EPA might act as a potent antidote against the toxic effects induced by TCDD. The study was attempted based on the hypothesis that EPA competes with arachidonic acid in membrane phospholipids and alters the n3/n6 ratio and beneficially influences inflammatory conditions by producing anti-inflammatory eicosanoids from EPA. In addition, EPA by itself is a potent antioxidant, and recent studies have documented that anti-inflammatory eicosanoids formed from EPA acts a potent activator of Nrf-2 and thereby enhances expression of various antioxidant enzymes (Wang et al. 2010; Groeger et al. 2010). The present study evaluated the effect of fish oil rich in eicosapentaenoic acid

(EPA; n3 fatty acid) against TCDD-induced nephrotoxicity in Wistar rats. The results show that EPA treatment markedly ameliorated TCDD-induced nephrotoxicity by maintaining kidney morphology, ATPase enzyme activities and counteracted oxidative stress by enhancing the antioxidant defense mechanisms.

Materials and methods

Chemicals

TCDD was a kind gift from Dr. Howard G. Shertzer (Director, Environmental Genetics & Molecular Toxicology Division, University of Cincinnati). EPA concentrate with 70 % EPA was a kind gift from Dr. R. Anandan, Sr. Scientist, Central Institute of Fisheries Technology (Cochin, Kerala, India). 7-Ethoxy resorufin, dicumarol, resorufin were purchased from Sigma-Aldrich (Bangalore, India). All other chemicals were purchased from Himedia Laboratories, Mumbai, India.

Animals

Male albino Wistar rats weighing around 150 g was procured from Kerala Agricultural University Mannuthy, Kerala, India. The study protocol was approved by the Institutional Animal Ethics Committee constituted in accordance with the rules and guidelines of the CPCSEA (Committee for the Purpose of Control and Supervision of Experiments and Animals), India. The rats were maintained in a controlled environment with standard temperature (28 ± 2 °C) and humidity with an alternating light and dark cycle. The animals were fed with commercially available pelleted rat chow (Sai Durga Private Limited, Bangalore, India) and water ad libitum. After a week of acclimatization, animals were randomly divided into control and test groups. Six rats were maintained in each group.

Treatment schedule

Rats were given an intraperitoneal injection of TCDD (15 µg/kg body weight) dissolved in 0.15 ml of corn oil and 0.065 ml of EPA concentrate which is equivalent to 45 mg of EPA (300 mg/kg body weight) was administered orally.

Experimental procedure

The animals were divided into six groups with six rats in each group. The dosing protocol of TCDD (15 µg/kg body weight) was chosen from the previous reports of Shertzer et al. (1998). EPA (300 mg/kg body weight) dosage was fixed based on the

reports by Li et al. (2011) and our preliminary results also confirmed the same.

Groups	Concentration and volume of toxin/antioxidant/vehicle administered	Treatment period	Sacrifice
Group I (control)	—	—	On 41st day
Group II (corn oil — vehicle for TCDD)	0.15 ml of corn oil administered/day	From 31st to 33rd day	On 41st day
Group III (EPA concentrate)	300 mg EPA/kg body weight (0.065 ml of EPA concentrate) per day by oral administration	From 11th to 40th day	On 41st day
Group IV (TCDD)	15 µg/kg body weight dissolved in 0.15 ml corn oil per day by intraperitoneal injection	From 31st to 33rd day	On 41st day
Group V (pre-treatment with EPA followed by TCDD treatment)	300 mg EPA/kg body weight (0.065 ml of EPA concentrate) per day by oral administration	From 1st to 30th day	On 41st day
	15 µg TCDD/kg body weight dissolved in 0.15 ml corn oil per day by intraperitoneal injection	From 31st to 33rd day	
Group VI (co-treatment with EPA and TCDD)	300 mg EPA/kg body weight (0.065 ml of EPA concentrate) per day by oral administration	From 11th to 40th day	On 41st day
	15 µg TCDD/kg body weight dissolved in 0.15 ml corn oil per day by intraperitoneal injection	From 31st to 33rd day alone	

After the treatment period, the animals were fasted overnight, weighed and anesthetized by exposing to diethyl ether. Blood was collected from the jugular vein, and serum was separated and used for kidney marker enzyme assays. The kidney tissues were dissected out, washed in ice-cold physiological saline, patted dry and weighed. A small portion of the kidney tissue was stored in 10 % formalin for histopathological examination. The remaining tissue was stored at -80°C for further analysis. About 100 mg kidney tissue was weighed and homogenized in chilled 0.1 M Tris–HCl buffer in Potter–Elvehjem Teflon homogenizer. The homogenates were centrifuged at 3,000 rpm for 15 min, and the supernatant was aliquoted and used for assessing oxidative stress parameters and ATPase enzyme activities.

Serum kidney marker analysis

Blood samples were allowed to clot at room temperature and then centrifuged at $1,200\times g$ for 15 min. The clear serum was separated and used for biochemical assays. Renal function was assessed by measurement of serum urea (Natelson et al. 1951) and serum creatinine levels (Brod and Sirota 1948).

Histopathology

A small portion of kidney tissue from the experimental animals were fixed in 10 % neutral buffered formalin and processed by standard procedure for paraffin embedding and serial sections of about 5 µm were cut and were stained with hematoxylin and eosin (H&E) dye.

Assessment of oxidative stress status and ATPase activities

Enzymic antioxidants (CAT) (Aebi 1974), superoxide dismutase (SOD) (Marklund and Marklund 1974), glutathione-S-transferase (GST) (Habig et al. 1974), glutathione peroxidase (GPx) (Rotruck et al. 1973), non-enzymic antioxidant (GSH) (Moron et al. 1979), thiobarbituric acid reactive substances (TBARS) (Ohkawa et al. 1979), membrane bound ATPase, Na^+K^+ ATPases (Bonting 1970), Ca^{2+} ATPases (Hjerten and Pan 1983) and Mg^{2+} ATPases (Ohnishi et al. 1982) were carried out in tissue homogenate. LPO is expressed as nanomol TBA reactants/g of tissue, GSH in nanomol GSH/g of tissue. The specific activities of the enzymes were calculated as units/mg of protein, where CAT 1 U=the amount of enzyme that consumes 1 nanomol H_2O_2 /min; SOD 1 U=the amount of enzyme required to give 50 % inhibition of pyrogallol auto-oxidation; GPx 1 U=the amount of enzyme that converts 1 µmol GSH to GSSG in the presence of H_2O_2 /min; GST 1 U=amount of enzyme that conjugates 1 µmol CDNB/min. The ATPase enzyme activities were expressed as µM of Pi liberated/mg of protein.

Metabolic activation of TCDD by CYP1A1

Microsomes from kidney tissues were isolated by the method described by Walawalkar and Iyer (2006). CYP1A1 activity in the isolated microsomes was determined by the method of Peters et al. (2004). CYP1A1 activity was calculated as picomoles of resorufin formed/mg of protein and the results were expressed as percentage fold induction of CYP1A1 activity with control values considered as 100 %.

Statistical analysis

The data were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test. All

biochemical experiments were performed thrice in triplicates to ensure reproducibility.

Results

Effect of EPA on TCDD induced renal biomarkers serum urea and creatinine

Increased levels of kidney markers in the serum are indirect measure of nephrotoxicity. In the present study, the serum urea and creatinine the biomarkers of renal function were estimated (Fig. 1). TCDD-induced kidney toxicity was reflected in the significant increase ($p < 0.001$) in the levels of biomarkers when compared to control rats. Pre-treatment and co-treatment with EPA concentrate in combination with TCDD significantly decreased ($p < 0.001$) the levels of the biomarkers in the serum when compared to TCDD treated rats alone. Rats treated with corn oil/EPA concentrate alone did not show any change in the serum kidney markers when compared to control rats.

Effect of EPA on TCDD-induced histopathological alterations in rat kidney

To further examine the tissue damage caused by TCDD and the extent of protection offered by EPA concentrate, a histological study was performed. Figure 2 shows histopathological changes such as interstitial inflammation, dilation in proximal and distal convoluted tubules and glomerulus shows inflammation with fibrosis. Effect of EPA on TCDD-induced histological changes is shown in Fig. 3. The histopathological changes in control, solvent control and rats treated with EPA showed normal kidney architecture with prominent

glomerulus, proximal and distal convoluted tubules. However, TCDD-induced kidney damage was prevented by EPA treatment; the kidney shows normal morphology which is comparable to that of control rats.

Protective effect of EPA on TCDD-induced oxidative stress

TCDD-induced oxidative stress was measured by estimating the levels of lipid peroxidation and antioxidant status. Rats treated with TCDD alone showed a significant increase ($p < 0.001$) in TBARS levels with concomitant decrease ($p < 0.001$) in antioxidant status (GSH, SOD, CAT, GST and GPX) when compared to control rats. Rats treated with corn oil/EPA alone showed non-significant levels when compared to control rats. Pre-treatment and co-treatment with EPA showed a significant protective effect against oxidative stress induced by TCDD. Pre-treatment with EPA followed by TCDD treatment showed an increase in antioxidant status (GSH, CAT, GPx at $p < 0.01$; GST, SOD at $p < 0.001$) with a decrease in TBARS levels (at $p < 0.001$). Meanwhile, co-treatment with EPA and TCDD showed an increase in antioxidant status (GSH, GST, GPX and SOD at $p < 0.001$; CAT at $p < 0.01$) with a decrease in TBARS levels (at $p < 0.001$) when compared to TCDD treated rats (Fig. 4).

EPA decreases TCDD-induced CYP1A1 activity

EROD activity describes the rate of the CYP1A mediated de-ethylation of the substrate 7-ethoxyresorufin to form the product resorufin. The catalytic activity towards this substrate is an indication of the amount of enzyme present and is measured as the concentration of resorufin produced (Kennedy and Jones 1994). The results are expressed as percentage fold induction

Fig. 1 Effect of EPA and TCDD on renal markers in Wistar rats. Group I — control; Group II — corn oil (vehicle for TCDD); Group III — EPA; Group IV — TCDD; Group V — pre-treatment with EPA followed by TCDD; Group VI — co-treatment with EPA and TCDD. **a** Serum creatinine; **b** Serum urea. Values are means $\pm$ standard error of mean of

each group. *** $p < 0.001$, significantly different when compared to control. ### $p < 0.001$, significantly different when compared to TCDD group. NS non-significant, when compared to control (one-way ANOVA followed by Tukey's multiple comparison)

Fig. 2 Histology of TCDD-induced renal damage. **a** Interstitial inflammation ($\times 400$). **b** Proximal convoluted tubules showing dilatation and shedding of epithelium ($\times 400$). **c** Glomeruli showing increased

interstitium suggesting fibrosis ($\times 400$). **d** Dilated distal convoluted epithelium ($\times 400$). **e** Histological changes induced by TCDD at $\times 100$. Scale bar for $\times 100$ is 100 μm , and for $\times 400$ it is 30 μm (H&E)

Fig. 3 Histopathological changes of rat kidney treated with EPA and TCDD. **a** Control rats; **b** rats treated with corn oil (vehicle for TCDD); **c** rats treated with EPA — showing typical kidney morphology with normal glomerulus and tubules. **d** Rats pre-treated with EPA concentrate

followed by TCDD; **e** rats co-treated with EPA and TCDD shows normal glomeruli, proximal and distal tubules with no fibrosis and inflammatory infiltration (H&E; magnification $\times 400$; scale bar = 50 μm)

Fig. 4 Effect of EPA and TCDD on TBARS and antioxidant status in experimental rats. Group I — control; Group II — corn oil (vehicle for TCDD); Group III — EPA; Group IV — TCDD; Group V — pre-treatment with EPA followed by TCDD; Group VI — co-treatment with EPA and TCDD. **a** Lipid peroxidation; **b** catalase (CAT); **c** superoxide dismutase (SOD); **d** reduced glutathione (GSH); **e** glutathione peroxidase (GPx); **f** glutathione-S-transferase (GST). LPO is expressed in nanomoles of TBA reactants/g of tissue, GSH in nanomoles of GSH/g of tissue. The specific activities of the enzymes were calculated as units/mg of protein.

CAT 1 U=amount of enzyme that consumes 1 nanomol H_2O_2 /min; SOD 1 U=amount of enzyme required to give 50 % inhibition of pyrogallol auto-oxidation; GPx 1 U=amount of enzyme that converts 1 μ mol GSH to GSSG in the presence of H_2O_2 /min; GST 1 U=amount of enzyme that conjugates 1 μ mol of CDNB/min. Each bar represents mean $\pm$ standard error of mean of each group. *** $p < 0.001$, significantly different when compared to control, NS non-significantly different from control. ### $p < 0.001$, ## $p < 0.01$ significantly different when compared to TCDD-treated group (one-way ANOVA followed by Tukey's multiple comparison)

of EROD activity with control values considered as 100 %. In the present study, TCDD-treated rats showed a significant increase ($p < 0.001$) in CYP1A1 activity when compared to control rats. Pre-treatment and co-treatment with EPA followed by TCDD treatment showed a significant decrease ($p < 0.001$) in CYP1A1 activity when compared to rats treated with TCDD only. Rats which received EPA/corn oil alone did not show significant change in the activity when compared to control rats (Fig. 5).

EPA maintained the ATPases enzyme activity

Rats treated with TCDD alone showed a significant decrease in both Na^+/K^+ ATPase ($p < 0.001$) and Mg^{2+} ATPase ($p < 0.01$) activities and significant increase ($p < 0.001$) in the Ca^{2+} ATPase activity when compared to control rats. In contrast, rats which received EPA/corn oil alone did not show any significant change in ATPases activity when compared to control rats. Pre-treatment and co-treatment with EPA followed by TCDD treatment showed a significant increase in Na^+/K^+ ATPase ($p < 0.01$) and Mg^{2+} ATPase activities ($p < 0.01$) with a significant

decrease ($p < 0.01$) in Ca^{2+} ATPases activity when compared to rats treated with TCDD only (Fig. 6).

Discussion

Free radical-mediated oxidative stress plays a major role in TCDD-induced toxic responses in various organs (reviewed by Stohs 1990). The intracellular signals elicited by TCDD results in formation of arachidonic acid metabolites and ROS generation. These are the early toxic responses initiated by TCDD which ultimately leads to redox imbalance with inflammatory response and results in severe damage to the tissues. EPA is a potent antioxidant and competes for membrane incorporation with AA and thereby produces anti-inflammatory eicosanoids, thus EPA might act as a potent antidote against TCDD-induced toxicity. Since EPA and its metabolites improve antioxidant mechanisms, the protective effect of EPA can be seen by enhanced antioxidant defense mechanisms with cytoprotection. From the results of the present study we observed that renal oxidative stress and

Fig. 5 Effect of EPA on TCDD-induced CYP1A1 activity. Group I — control; Group II — corn oil (vehicle for TCDD); Group III — EPA; Group IV — TCDD; Group V — pre-treatment with EPA followed by TCDD; Group VI — co-treatment with EPA and TCDD. Each bar represents mean $\pm$ standard error of mean of each group. CYP1A1 activity was calculated as picomoles of resorufin formed/mg of protein

and the results were expressed as percentage fold induction of CYP1A1 activity with control values considered as 100 %. *** $p < 0.001$, significantly different when compared to control. NS non-significantly different when compared to control. ### $p < 0.001$, when compared to TCDD-treated group (one-way ANOVA followed by Tukey's multiple comparison)

histopathological changes induced by TCDD was effectively ameliorated by oral treatment with EPA.

The histopathological observations in rats treated with TCDD showed severe damage to the kidney tissue with dilation and shedding of epithelium in proximal and distal convoluted tubules and glomerulus showed increased interstitium with loss in shape. A significant increase in serum urea and creatinine levels along with damage to the renal tissue indicates TCDD causes renal dysfunction. A rise in levels of kidney markers in the serum may be due to their release from the damaged kidney tissue. These observations are in accordance with the earlier reports of Lu et al. (2009) and Ciftci et al. (2012). Treatment with EPA significantly maintained the kidney architecture to near normal and further reduced TCDD-induced increase in levels of serum markers. The maintenance of kidney architecture by EPA might be attributed to its membrane stabilizing activity through incorporation into membrane phospholipids (Kim et al. 2000). The protective effect of fish oil rich in EPA observed in the present study is consistent with the earlier reports on protection offered by fish oil against cisplatin-induced nephrotoxicity (Naqshbandi et al. 2012) and isoproterenol-induced myocardial infarction (Padma and Devi 2009).

It is well established that TCDD-induced CYP1A1 activation and subsequent generation of reactive oxygen species is the prime cause for the TCDD-induced toxic events (Okey et al. 1994; Nebert 1991). CYP1A1 is an enzyme involved in bioactivation of procarcinogens and during such process it produces reactive metabolites and ultimately leads to

oxidative stress (Whitlock 1999). CYP450 during its metabolic cycling results in the formation of H_2O_2 and hydroxyl radicals (Ingelman-Sundberg and Johansson 1984; Kuthan and Ullrich 1982). Thus it results in an atmosphere of oxidative stress, a state of imbalance between intracellular ROS and antioxidant status. Moreover, these reactive oxygen species play a major role in tissue damage by perturbing the levels of both lipid peroxides and antioxidant status. So, in the present study we have evaluated various oxidative stress parameters in different experimental groups treated with EPA and TCDD. Determination of CYP1A1 activity is a biomarker for TCDD-induced toxicity and levels of lipid peroxides is an index to evaluate the extent of tissue oxidative stress which results from free radical damage to membrane lipids (Yeager et al. 2009).

In the present study, TCDD-induced oxidative stress was reflected in a significant increase in CYP1A1 activity and lipid peroxide level. TCDD-induced lipid peroxidation and oxidative stress have been indicated as factors which lead to acute toxicity (Pohjanvirta et al. 1989; Slezak et al. 2000). TCDD-induced generation of lipid peroxides has been reported earlier in C57BL/6 J and DBA/2 J mice by Hassoun et al. (1996), in mouse by Shertzer et al. (1998), and in rat epidymal sperm by Latchoumycandane et al. (2002). In addition, the present study also showed depletion in both enzymic and non-enzymic antioxidant status during TCDD treatment. The reduction in antioxidant status during TCDD-induced nephrotoxicity is consistent with the earlier report of Ciftci et al. (2012). Cells contain antioxidant defense mechanisms to protect themselves

Fig. 6 Effect of EPA on TCDD-induced alterations in membrane bound ATPase enzyme activities. Group I — control; Group II — corn oil (vehicle for TCDD); Group III — EPA; Group IV — TCDD; Group V — pre-treatment of EPA followed by TCDD treatment; Group VI — co-treatment with EPA and TCDD. **a** Na⁺K⁺-ATPases; **b** Mg²⁺ATPases; **c**

Ca²⁺ ATPases. Each bar represents mean $\pm$ standard error of mean of each group. *** $p < 0.001$, ** $p < 0.01$, significantly different when compared to control. NS non-significantly different when compared to control. ## $p < 0.01$, significantly different when compared to TCDD-treated group (one-way ANOVA followed by Tukey's multiple comparison)

from the oxidative stress by scavenging the ROS. Optimum levels and proper balance among these enzymes may be critical for the prevention of cellular oxidative stress. However, when the oxidative stress induced by toxins is stronger with increased sustainability, then antioxidant defense mechanisms fail to counteract the oxidative stress. Thiol status is indicative of susceptibility of the cells to oxidative stress conditions. GSH is a non-protein thiol which directly reacts with reactive oxygen species and helps in their efficient removal from the cells. It was observed in the present study that the glutathione content in the kidney tissues after TCDD treatment was decreased significantly when compared to control rats. While rats treated with EPA showed significant protection from oxidative stress by maintaining the GSH content. Decrease in thiol level and an increase in LPO level observed in the present study is accordance with the earlier report of Stohs (1990). Along with the reduction in GSH content, activities of various enzymic antioxidants such as SOD, CAT, GST and GPx were also found to be significantly decreased during TCDD treatment. These results are in line with the earlier reports where TCDD-induced oxidative stress is mediated through an imbalance in

antioxidant status (Stohs 1990; Hassoun et al. 2004; Jin et al. 2008). From the results it is evident that oxidative stress acts as a major contributor in the TCDD-induced nephrotoxic effects. However, treatment with EPA significantly prevented TCDD-induced oxidative stress by decreasing LPO levels and augmenting antioxidant defense mechanisms. In addition, the EPA-mediated decline in CYP1A1 activity might also contribute to the significant nephroprotective effect. Thus, the free radical scavenging potential and enhancement of antioxidant defense mechanisms through Nrf-2 activation has a significant role to play in EPA's protective effect (Richard et al. 2008; Wang et al. 2010). Moreover the mechanism of Nrf-2-dependent antioxidant response was reported to be mediated by EFOX (electrophilic fatty acid oxo-derivatives) derivatives from EPA and DHA (Groeger et al. 2010). However, a recent study by Turkez et al. (2012) has shown that EPA provided cytoprotection against TCDD-induced stress by enhancing the total antioxidant capacity in rat hepatocytes. The protective effect of fish oil rich in n-3 fatty acids against various nephrotoxic conditions (Goksu Erol et al. 2013; Priyamvada et al. 2008; Naqshbandi et al. 2012; Thakkar et al. 2000; Xi and

Chen 2000) and results from the present study strongly support the ability of EPA as a potent nephroprotectant.

In addition to the depletion in cellular antioxidant status, TCDD-mediated oxidative stress was associated with perturbation in the activities of ATPase enzymes. ATPases are membrane-bound enzymatic proteins which regulate ionic concentrations and it is vital for the normal functioning of the cells. These enzymes are sensitive to the alterations in the membrane lipid composition and oxidative stress (Carageorgiou et al. 2004). In the present study, a significant decrease in Na^+K^+ -ATPase and Mg^{2+} -ATPase activities with concomitant increase in Ca^{2+} ATPase activity was observed in rats treated with TCDD. Earlier reports suggest that the eicosanoids formed from arachidonic acid during oxidative stress have profound inhibitory action on Na^+K^+ -ATPase and Mg^{2+} -ATPase activities (Jabs et al. 1989; Schwartzman et al. 1985; Lear et al. 1990; Therien and Blostein 2000). In addition, altered expression of Na^+K^+ -ATPase in hepatic tissues during TCDD treatment has been reported earlier by Fletcher et al. (2005) and Peterson et al. (1979).

Thus, the alterations in the ATPase activities observed in the present study might be explained to be mediated through TCDD-induced perturbation in Ca^{2+} levels. Since it is a well-documented tenet that TCDD mediates its toxic response by increasing the intracellular Ca^{2+} levels and results in subsequent downstream events (Puga et al. 1997; Biswas et al. 2008), it is very important for cells to maintain low concentrations of Ca^{2+} for proper cellular signaling. TCDD-induced increase in intracellular Ca^{2+} levels mobilizes arachidonic acid from membrane phospholipids through activation of cPLA2. Thus, the released arachidonic acid acts as substrates for COX-2 and cytochrome P450s and results in formation of pro-inflammatory eicosanoids. Thus, the formed arachidonic acid metabolites might decrease the activities of Na^+K^+ -ATPase and Mg^{2+} -ATPases. Moreover, a significant rise Ca^{2+} ATPase during TCDD treatment may be due to the cells adaptive mechanism to overcome Ca^{2+} overloading and thereby prevent TCDD-induced toxic events. However, in spite of the increase in activity of Ca^{2+} ATPase, a significant decline in the Na^+K^+ -ATPase and Mg^{2+} -ATPase activities clearly suggest the inability of the cells to counteract against TCDD-induced disturbances in Ca^{2+} homeostasis. But during EPA treatment, the activities of Na^+K^+ -ATPase and Mg^{2+} -ATPase and Ca^{2+} ATPase were significantly maintained to near normal values to that of control rats. Thus the ability to maintain Ca^{2+} homeostasis by EPA might be responsible for its protective role against TCDD-induced alterations in ATPase enzyme activities. Maintenance of calcium homeostasis by EPA against H_2O_2 challenge was reported earlier by Jahangiri et al. (2000). The study shows that the EPA under normal conditions does not alter Ca^{2+} dynamics; however, during free radical stress, it prevents cells from overloading with Ca^{2+} influx, thereby offering a protective effect. Ca^{2+} overload in

response to Ouabain has also been prevented by EPA treatment (Hallaq et al. 1990). In addition to the above-mentioned property of EPA's role in the maintenance of Ca^{2+} homeostasis, a study by Kumosani (2011) has shown that EPA treatment resulted in activation of Na^+K^+ -ATPase activity in rat brain.

In conclusion, the present study shows that TCDD causes severe damage to renal tissue with decline in antioxidant defense system and exacerbated oxidative stress. The overall nephroprotective effect by EPA might be related to its ability to modulate to CYP1A1 activity, antioxidant defense mechanisms and ATPase enzyme activities, and thereby prevented TCDD-induced nephrotoxicity by counteracting oxidative stress. Thus, dietary consumption of EPA might offer protection against various environmental toxins which primarily mediates its effect through oxidative stress.

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Conflict of interest The authors declare that they have no conflict of interest.

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