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Original Article

Influence of omega-3 fatty acids, soya isoflavones and their combination for abrogating carbon tetrachloride hazards in male rats

Influência de ácidos graxos ômega-3, isoflavonas de soja e sua combinação na anulação de riscos de tetracloreto de carbono em ratos albinos machos

E. S. Abdel-Baky[a]*, S. A. Radwan[a], M. F. Ibrahim[a] and O. N. Abdel-Rahman[a]

[a]Ain Shams University, Faculty of Education, Department of Biological and Geological Sciences, Cairo, Egypt

Abstract

Studies have shown that carbon tetrachloride (CCl_4) induces hepatic and renal damage arising from oxidative stress. The present study was undertaken to examine the effect of omega-3 fatty acids and/or soya isoflavones on CCl_4 induced toxicity in male albino rat liver and kidney. For this purpose, 42 rats were divided as follows: group 1, rats serves as the control without any treatment; group 2, rats were administered a single dose of CCl_4 intraperitoneally (1 mg/kg b. wt.); group 3, rats were supplemented daily with omega-300 orally (400 mg/kg b. wt.); group 4, rats were supplemented daily with pro-S orally (50 mg/kg b. wt.); group 5, rats were supplemented daily with omega-300 orally for four weeks, then after 24 hours treated with a single dose of CCl_4 at the same tested doses. group 6, rats were supplemented daily with pro- S orally for four weeks, then after 24 hours treated with a single dose of CCl_4 at the same tested doses; group 7, rats were supplemented daily with an oral combination of omega-300 and pro-S orally for four weeks, then after 24 hours treated with a single dose of CCl_4 at the same tested doses. Results showed that CCl_4 administration induces hepatic damage indicated by a significant increase in the activities of alkaline phosphatase (ALP), aspartate aminotransferase (AST) and Aalanine aminotransferase (ALT) enzymes and glucose level, with a significant increase in malondialdehyde (MDA) and nitric oxide (NO) levels and a significant decrease of reduced glutathione (GSH) level in liver tissue. Also, CCl_4 toxicity induce renal damage manifested in a significant increase in serum urea, creatinine, uric acid, and oxidative stress of kidney tissue reflected by increase of MDA, NO and the decrease of GSH levels. The pre-treatment with omega-3 fatty acids and/or soya isoflavones revealed ameliorative effect against deleterious effects of CCl_4 toxicity on hepatic and renal tissues and all tested parameters. Results of the current study revealed also that the pre-treatment with omega-3 fatty acids and/or soya isoflavones to rats improved liver and kidney function and produced high antioxidant activity.

Keywords: carbon tetrachloride, omega- 3 fatty acids, soya isoflavones, antioxidants.

Resumo

Estudos demonstram que o tetracloreto de carbono (CCl_4) induz danos hepáticos e renais decorrentes do estresse oxidativo. O presente estudo almejou examinar o efeito de ácidos graxos ômega-3 e/ou isoflavonas de soja na toxicidade induzida por CCl_4 no fígado e no rim de ratos albinos machos. Para tanto, 42 ratos foram divididos da seguinte forma: grupo 1, indivíduos que servem como controle sem nenhum tratamento; grupo 2, indivíduos que receberam uma dose única de CCl_4 intraperitonealmente (1 ml/kg do peso corporal); grupo 3, indivíduos que foram suplementados diariamente com ômega-300 por via oral (400 mg/kg do peso corporal); grupo 4, indivíduos que foram foram suplementados diariamente com pró-S por via oral (50 mg/kg do peso corporal); grupo 5, indivíduos que foram suplementados diariamente com ômega-300 por via oral por quatro semanas, depois de tratados por 24 horas com uma dose única de CCl_4 nas mesmas doses testadas; grupo 6, os indivíduos foram suplementados diariamente com pro-S por via oral por quatro semanas, depois de tratados por 24 horas com uma dose única de CCl_4 com as mesmas doses testadas; grupo 7, os indivíduos foram suplementados diariamente com uma combinação oral de ômega-300 e pró-S por via oral por quatro semanas, depois de tratados por 24 horas com uma dose única de CCl_4 com as mesmas doses testadas. Os resultados mostraram que a administração de CCl_4 induz dano hepático, indicado por um aumento significativo nas atividades das enzimas fosfatase alcalina (ALP), aspartato aminotransferase (AST) e Alanina aminotransferase (ALT) e nível de glicose, com aumento significativo de malondialdeído (MDA) e nítrico, e dos níveis de óxido (NO), além da diminuição significativa do nível de glutationa reduzida (GSH) no tecido hepático. Além disso, a toxicidade do CCl_4 induz dano renal manifestado em

*e-mail: enas_saleh@edu.asu.edu.eg
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um aumento significativo da ureia sérica, creatinina, ácido úrico e estresse oxidativo do tecido renal, refletindo no aumento de MDA, NO e diminuição dos níveis de GSH. O pré-tratamento com ácidos graxos como ômega-3 e/ou isoflavonas de soja revelou efeito melhorador contra os efeitos deletérios da toxicidade do CCl_4 nos tecidos hepático e renal e em todos os parâmetros testados. Os resultados do presente estudo demonstraram também que o pré-tratamento com ácidos graxos ômega-3 e/ou isoflavonas de soja em ratos melhorou a função hepática e renal e produziu alta atividade antioxidante.

Palavras-chave: tetracloreto de carbono, ácidos graxos ômega-3, isoflavonas de soja, antioxidantes.

1. Introduction

Carbon tetrachloride (CCl_4) is an industrial solvent that is used in the synthesis of chlorinated organic compounds including chlorofluorocarbon refrigerants, agricultural fumigant, the production of semiconductors, the processing of fats, rubber and in laboratory applications (Kauppinen et al., 2000). Xu et al. (2010) confirmed that each liver and kidney were the target organs of CCl_4. A wide variety of reports proven that similarly to hepatic and kidney toxicity, CCl_4 also causes disorders in lungs, testes as well as within the blood by producing free radicals (Ozturk et al., 2003). Rikans et al. (1994) concluded that CCl_4 toxicity is mediated by metabolites that react with antioxidant enzymes. CCl_4 elicits free radicals which might be leading to membrane lipid peroxidation (Basu, 2003).

Omega-3 fatty acids (O3FAs) are long chains, polyunsaturated fatty acids of plant and marine origin. These fatty acids must be derived from nutritional sources, as they cannot be synthesized by the human body. Flax seed, hemp, and walnuts are rich sources of O3FAs polyunsaturated fatty acids alphalinolenic acid (Attia and Nasr, 2009). O3FAs are strong antioxidants and considered as anticancer agent in most human malignancies (Calviello and Serini, 2010; Shaikh et al., 2010). O3FAs were determined to play defensive roles inside the cardiovascular system, liver, and kidney and they have been used in clinical preoperative overall parenteral nutrients (Fassett et al., 2010; Koletzko and Goulet, 2010). These fatty acids, such as docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), are included in many parts of the body and have a role in the anti-inflammatory and antioxidant processes and cell signaling (Batlle et al., 2012). Also, they are considered as the precursors of active metabolites that have many beneficial effects in treating several diseases (Swanson et al., 2012), most significantly, the cardiovascular diseases (Ebrahimi et al., 2009; Oscarsson and Hurt-Camejo, 2017). Moreover, the renoprotective effect of O3FAs has been also reported (El-Ashmawy et al., 2018). Clinical studies suggested that the administration of O3FAs improved renal function and lowered the chance of end-stage renal disease and death (Hassan and Gronert, 2009).

Soybean contains a high complex of protein, carbohydrates, oligosaccharides, dietary fiber, phytosterol, saponin, isoflavone, lecithin, trypsin inhibitor, phytic acid and minerals (Latif et al., 2014). Soybean isoflavone (SI) has been tested for its protective effects in animals and humans. Also, it considered as a selective receptor modulator for estrogen (Setchell, 2001), lower blood cholesterol levels (Hermansen et al., 2001) and possess potential antioxidant activity (Ruiz-Larrea et al., 1997; Genovese et al., 2005). Substantial data from scientific intervention trials concerning animals and human beings strongly guide the useful effect of isoflavone-rich soy protein in stopping diverse continual diseases such as, cancer (Zhang et al., 2004), cardiovascular disease (Teede et al., 2001), osteoporosis (Alekel et al., 2000), and symptoms of menopause (Alekel et al., 2000; Upmalis et al., 2000; Somekawa et al., 2001). Soy protein containing genistein that may save oxidative damage inside the liver by means of reducing plasma- free fatty acids and decreasing CYP2E1 expression (Yang et al., 2011). Also, genistein has been reported to prevent radical scavenging action, activation of antioxidant enzymes and LDL oxidation (Yoon and Park, 2014). Suppression of hepatic lipid synthesis can be accounted as one mechanism for the lipid-reducing action of genistein (Kim et al., 2004).

The aim of this work is to investigate whether Omega-3 fatty acids, soya isoflavone or coadministration of omega- 3 fatty acids with soya isoflavone have protective effects against hepato and renal toxicity induced by carbon-tetrachloride.

2. Materials and Methods

2.1. Chemicals

The chemical compounds used in the present study are:

Carbon tetrachloride (CCl_4) was purchased from Sigma-Aldrich (St. Louis, MO, USA).

Omega-300 capsules; each soft gelatin capsule contains fish oil 1000 mg, produced by the Arab Co. for gelatin and pharmaceutical products for montana pharmaceutical.

Pro– S tablets; each film coated tablet contains soya isoflavones 50 mg, produced by the Arab Company for pharmaceutical and medical plants.

The hepato and renal toxicity was produced by intraperitoneal administration of CCl_4 (1 mg/kg body weigh) (Moreno and Muriel, 2006).

The equivalent protective doses of omega- 300 and pro - S were calculated for the rats according to method of Paget and Barnes (1964). The dosage of omega-300 and pro -S for each rat was calculated depending on the factor's human-rat therapeutic dose.

2.2. Animals

Forty-two adult male rats were purchased from the animal house at National Research Centre, weighing (195±20 g). The rats were kept under a controlled temperature (25±5°C), humidity (50±10%), and acclimatized to 12 h light/dark. The experimental period was four weeks

on which water and food were supplied *ad libitum*. The guidelines of the institutional animal ethics committee were conducted on the animal experiment.

2.3. Experimental design

The animals were randomly assigned into seven experimental groups (each of six). All international and local rules and regulation for handling animals in experiments were followed.

Group 1: rats were fed the balanced diet without any treatment and served as normal control.
Group 2: rats were treated with a single dose of CCl_4 intraperitoneally (1 mg/kg b. wt.).
Group 3: rats were treated daily with omega-300 orally (400 mg/kg b. wt.) for four weeks.
Group 4: rats were treated daily with pro - S orally (50 mg/kg b. wt.) for four weeks.
Group 5: rats were treated daily with omega-300 orally for four weeks, then after 24 hours treated with a single dose of CCl_4 intraperitoneally at the same tested doses.
Group 6: rats were treated daily with pro - S orally for four weeks, then after 24 hours treated with a single dose of CCl_4 intraperitoneally at the same tested doses.
Group 7: rats were treated daily with an oral combination of omega-300 and pro-s for four weeks, then after 24 hours treated with a single dose of CCl_4 intraperitoneally at the same tested doses.

By the ending of the experiment, the animals have been anesthetized with diethyl ether after 12 h fasting, each experimental rat was decapitated, and the blood samples were collected. Blood samples left for 15 min at 37 °C for serum separation, then centrifuged at 3000 rpm for 20 min, then sera were separated and kept in plastic vials at − 20 °C until analyses. Liver and kidney organs were removed, rinsed with cold saline, and dried with filter paper. The liver and kidney of each rat was homogenized in phosphate buffer solution (pH 7.4) and centrifuged at 5000 rpm. The supernatant was used for measuring nitric oxide (NO), reduced glutathione (GSH), and lipid peroxidation (MDA).

2.4. Biochemical assays

Liver functions were determined by measuring the activities of the following enzymes in the serum: Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) activities were estimated according to King (1965). Alkaline phosphatase (ALP) activity was estimated according to Englehardt (1970). Serum glucose was determined by an enzymatic colorimetric method using the kit obtained from Diamond Diagnosing Company according to Young (2001). Serum urea and uric acid were determined according to Young (2001). Creatinine was determined according to the method described by Bartels et al. (1972).

2.5. Determination of nitric oxide, reduced glutathione and lipid peroxidation in liver and kidney tissues homogenate

Nitric oxide was determined calorimetrically according to Montgomery and Dymock (1961), reduced glutathione was measured by the method of Beutler et al. (1963) and lipid peroxidation was determined using the method of Ruiz-Larrea et al. (1994).

2.6. Statistical analysis

The obtained data were presented as means ± SE. One-way analysis of variance (ANOVA) followed by post-hoc test significant difference analysis at ($p < 0.05$) was performed using the statistical package for social science (SPSS) version 16 to compare all treated groups (Glantz, 1992). Differences were significant when $p < 0.05$.

3. Results

Results showed in Tables 1, 2 demonstrated that CCl_4 administration induces liver and renal damage via oxidative stress indicated in a significant increase in MDA and NO in liver and renal tissues. This increase in the previously mentioned parameters was accompanied by a significant decrease in GSH level in liver and renal tissues. The results reported that the pre-treatment with omega- 3 fatty acids (O3FAs) and/or soya isoflavones (SI) showed the oxidative stress for both manifested in a significant amelioration for the previous mentioned parameters comparing with the CCl_4 treated group and control group.

Also, liver function results showed a significant increase in the activities of ALP, AST, and ALT enzymes in CCl_4 intoxicated group as compared to control group. Also, serum glucose level showed a significant increase comparing with the control group. However, the soly treatment and pre-treatment with O3FAs or SI showed to some extent a significant improvement in the liver functions and glucose level (Table 3).

Finally, results in Table 4 reported that CCl_4 toxicity induces renal dysfunction manifested in a significant increase in serum levels of urea, creatinine, and uric acid. The results also revealed that the soly treatment with O3FAs or SI showed a non-significant change comparing with control animals in the previous parameters. The pre-treatment with O3FAs or SI showed improvement in the levels of these parameters levels to some extent comparing with their levels in CCl_4 treated group. Whereas the pre-treatment with O3FAs or SI showed returned the level of these parameters to control like values.

4. Discussion

The current work was attempted to examine the protective role of pre-treatment with omega- 3 fatty acids (O3FAs) and /or soya isoflavones (SI), in attenuating oxidative stress induced by carbon tetrachloride (CCl_4) that leading to deleterious effects in the liver and kidney tissues of male albino rats. The use of omega- 3 fatty acids and soya isoflavones offers a great potential as they are normal diet. Carbon tetrachloride (CCl_4) has been known to be an environmental pollutant and its toxicity has also been associated with health hazards (Wu et al., 2018). Oxidative stress refers to altered cellular redox balance. Our results revealed a significant increase in the lipid

Table 1. Effects of O3FAs, SI and O3FAs + SI on the liver tissue levels lipid peroxidation (MDA), nitric oxide (NO) and glutathione (GSH) in CCl4-treated group.

	Control	CCl_4	% D	O3FAs	% D	SI	% D	CCl_4 + O3FAs	% D	CCL_4 + SI	% D	CCl_4 + O3FAs + SI	% D
MDA (nmol/g)	**0.74^{ae} ± 0.04**	**4.7^{b}**±0.19	**540.54**	**0.8^{ae}**±0.02	**8.1**	**0.70^{a} ± 0.03**	**-5.4**	**2.92^{c} ± 0.23**	**294.6**	**3.64^{d} ± 0.13**	**391.9**	**1.13^{e} ± 0.07**	**52.7**
NO (µmol/g)	**11.89^{a} ± 0.42**	**36.82^{b}**±2.02	**209.67**	**10.4^{a}**±0.6	**-12.5**	**9.52^{a} ± .47**	**-19.9**	**22.2^{c} ± 1.31**	**86.7**	**31.44^{d} ± 0.95**	**164.4**	**16.82^{e} ± 0.86**	**41.46**
GSH (mmol/g)	**4.83^{ad} ± 0.24**	**3.32^{b}**±0.36	**-31.26**	**5.64^{c}**±0.2	**16.8**	**5.43^{cd} ± 0.15**	**12.4**	**4.07^{e} ± 0.14**	**-15.7**	**3.45^{b} ± 0.15**	**-28.6**	**4.67^{ae} ± 0.15**	**-3.31**

Values represent the mean ± S.E. % D: Percentage difference [(treated value − control value)/control value] × 100. The groups that showed statistically a non-significant change between each other take the same letter, but the group that showed statistically a significant change compared to the other groups take a different letter. Different letters indicate significantly different means p-value < 0.05; Same letters indicate non-significant changes.

Table 2. Effects of O3FAs, SI and O3FAs + SI on the kidney tissue levels lipid peroxidation (MDA), nitric oxide (NO) and glutathione (GSH) in CCl_4-treated group.

	Control	CCl_4	% D	O3FAs	% D	SI	% D	CCl_4 + O3FAs	% D	CCL_4 + SI	% D	CCl_4 + O3FAs + SI	% D
MDA (nmol/g)	**0.39^{a} ± 0.02**	**3.63^{b}**±0.24	**830.77**	**0.37^{a}**±0.03	**-5.13**	**0.34^{a} ±0.02**	**12.82**	**1.91^{c}**± 0.06	**389.7**	**3.27^{b}**± 0.24	**738.5**	**1.06^{d} ±0.08**	**171.79**
NO (µmol/g)	**7.07^{a} ± 0. 46**	**17.9^{b}**±0.5	**153.18**	**6.11^{a}**±0. 3	**-13.6**	**6.31^{a} ± 0.28**	**-10.8**	**10.41^{c} ± 0.3**	**47.2**	**14.21^{d} ± 0.43**	**100.9**	**9.02^{e} ± 0.25**	**27.58**
GSH (mmol/g)	**4.01^{a} ± 0.24**	**1.77^{b}**±0.08	**-55.86**	**4.74^{c}**±0.15	**15.4**	**4.65^{c} ± 0.27**	**15.9**	**2.66^{d} ± 0.1**	**-33.7**	**1.92^{b} ± 0.06**	**-52.1**	**3.2^{e} ± 0.14**	**-20.2**

Values represent the mean ± S.E. % D: Percentage difference [(treated value − control value)/control value] × 100. The groups that showed statistically a non-significant change between each other take the same letter, but the group that showed statistically a significant change compared to the other groups take a different letter. Different letters indicate significantly different means p-value < 0.05; Same letters indicate non-significant changes.

Table 3. Effects of O3FAs, SI and O3FAs + SI on ALP, AST and ALT activities and serum glucose levels in CCl4-treated group.

	Control	CCl_4	% D	O3FAs	% D	SI	% D	CCl_4 + O3FAs	% D	CCL_4 + SI	% D	CCl_4 + O3FAs + SI	% D
ALP (IU/L)	**77.79^a** ±3.2	**133.91^b**±0.8	**72.1**	**82.59^a**±1.3	**6.17**	**82.59± 1.2**	**6.2**	**104.8^c ± 1.6**	**34.8**	**115.06^d**±1.5	**47.91**	**94.03^e**±1.65	**20.88**
AST (IU/L)	**46.87^a ±1.2**	**95.19^b**±1.3	**103.1**	**45.71^a**±1.3	**-2.47**	**43.09^a**± 1.1	**-8.1**	**67.17^c ± 1.4**	**43.3**	**82.35^d**± 2	**75.70**	**56.30^e**± 1.44	**20.12**
ALT (IU/L)	**36.20^a ±1.1**	**79.67^b**±1.2	**120.1**	**35.92^a ±0.5**	**-0.77**	**34.64^a**± 1.1	**-4.3**	**53.89^c ± 1.4**	**48.9**	**65.88^d**± 3.5	**81.99**	**45.06^e**± 1.63	**24.48**
Glucose (mg/dl)	**104.81^a ±6.3**	**326.12^b**±10.1	**211.2**	**96.21^a**±4.4	**-8.2**	**93.92^a ± 2.97**	**-10.4**	**165.4^c**± 5.5	**57.8**	**194.88^d**±10.3	**85.94**	**135.08^e**± 1.71	**28.88**

Values represent the mean ± S.E. % D: Percentage difference [(treated value − control value)/control value] × 100. The groups that showed statistically a non-significant change between each other take the same letter, but the group that showed statistically a significant change compared to the other groups take a different letter. Different letters indicate significantly different means p-value < 0.05; Same letters indicate non-significant changes. IU/L: International unit/ Liter.

Table 4. Effects of O3FAs, SI and O3FAs + SI on the serum level of urea, creatinine and uric acid in CCl4-treated group.

	Control	CCl_4	% D	O3FAs	% D	SI	% D	CCl_4 + O3FAs	% D	CCL_4 + SI	% D	CCl_4 + O3FAs + SI	% D
Urea (mg/dl)	**3.45^a ± 0.3**	**19.46^b**±0.58	**464.1**	**2.90^a**±0.29	**-15.94**	**2.75^a**± 0.44	**-20.3**	**10.30^c**± 0.4	**198.6**	**14.58^d**± 0.43	**322.6**	**5.68^e**± 0.23	**64.64**
Creatinine (mg/dl)	**47.53^a ± 1.9**	**85.38^b**±1.43	**79.6**	**46.82^a**±2.58	**-1.49**	**46.61^a ± 2.0**	**-1.9**	**73.10^c**± 0.8	**53.8**	**65.01^d**±1.33	**36.8**	**54.35^e ± 1.30**	**14.35**
Uric acid (mg/dl)	**0.65^a ± 0.2**	**1.68^b**±0.03	**158.5**	**0.62^a**±0.03	**-4.62**	**0.63^a ± 0.28**	**-3.1**	**1.16^c ± 0.1**	**78.5**	**1.51^d**± 0.68	**132.3**	**0.91^e**± 0.3	**40**

Values represent the mean ± S.E. % D: Percentage difference [(treated value − control value)/control value] × 100. The groups that showed statistically a non-significant change between each other take the same letter, but the group that showed statistically a significant change compared to the other groups take a different letter. Different letters indicate significantly different means p-value < 0.05; Same letters indicate non-significant changes.

peroxidation (LPO) marker (i.e., MDA) and nitric oxide (NO) and a significant decrease in glutathione (GSH) enzyme activities in the liver and kidney tissues of rats following CCl_4 application. This is in agreement with previous researches Jan and Khan (2016) and Noureen et al. (2017). CCl_4 intoxication is associated with high free radical production in several organs, including the liver and kidney (Ozturk et al., 2003; Preethi and Kuttan, 2009). Lipid peroxidation is a process that damages the cell structure and function. Peroxidation of lipids of cell membrane initiates a loss of membrane integrity; membrane bound enzyme activity and causes cell lyses. However, the decreased activity of tissue antioxidant enzymes is likely to cause tissue damage by lipid peroxides or protein carbonyls (Pryor and Squadrito, 1995). CCl_4 binds to liver cytochrome P450 to form trichloromethyl (CCl_3) free radicals, which initiate membrane lipid peroxidation (Abdel-Kader et al., 2018). Secondary metabolic radicals of CCl_4 such as trichloromethylperoxy radical (CCl_3O_2), react with proteins or lipids leading alteration the permeability of membranes resulting in cell damage (Rahman et al., 2017). Also, CCl_4-induced altering the endogenous antioxidants in tissues (Alshammari et al., 2017), which is manifested by histopathological lesions. The increased levels of MDA are associated with a reduced level of GSH and increased the level of nitric oxide in the present study which indicated the occurrence of an oxidative insult that caused hepatic and renal damage. A decrease in GSH levels might be lead to oxidative stress and a consequent lipid peroxidation increasing (El-Nekeety et al., 2009).

The liver is one of the target organs affected by CCl_4 toxicity due to its storage in the liver. The present data also demonstrated that treatment with CCl_4 lead to a significant elevation in the activities of liver enzymes AST, ALT, and ALP. Liver enzymes such as ALT, AST and ALP were generally used to evaluate the hepatic dysfunction. The increased liver enzyme activities significantly reflect liver hepatocytes necrosis, and the high level of transaminases causes hepatocellular disorders (Ali et al., 2005). These findings indicated impaired function and damage of liver cells, cellular leakage and loss of functional integrity of the cell membrane in the liver by CCl_4 (Khan et al., 2012). Oxidative stress damages the integrity of biological membranes and increases permeability, resulting in the outflow of cytoplasm enzymes such as ALT, ALP, and AST into the blood (Ebaid et al., 2021). Thus, the ALT, ALP and AST activities in the serum are essential indices for evaluating liver injury. Also, CCl_4 -administered rats showed a very highly significant increase in the serum level of glucose concentration. The increasing level of glucose in CCl_4-treated rats may be due to hepatotoxicity which affect glucose metabolism.

The liver and kidney work in synergy to maintain homeostasis in the body. This ensures the right excretion of waste products and reabsorption of the useful substances by the kidney. Urea, uric acid, and creatinine are essential catabolic products of protein metabolism, and the elevation in their serum levels may indicate impairment of the kidney (Renugadevi and Prabu, 2010). Urea is the end product of protein catabolism and is mainly produced in the liver and secreted by the kidney. It is the primary vehicle for elimination of poisonous ammonia from the body. The determination of urea level is very important for the medical clinician to estimate renal function of patients (Harlalka et al., 2007). In general, the elevation of urea level is strongly related to nephritis, urinary tract obstruction, renal ischemia, and certain extrarenal diseases. The increase in uric acid level also may be owing to degradation of pyrimidines and purines associated with the increasing in the activity of xanthine oxidase causing overproduction of uric acid and generation of ROS. Creatinine is a break-down waste product of creatine phosphate in the muscles and is excreted out of the body by the kidneys with little or no tubular reabsorption (Rhodes et al., 1995). Creatinine level elevate when renal filtering capacity is deficient. Assessment serum creatinine level is the most generally used indicator of kidney function. A rise in blood creatinine level related with the damage of nephrons (Al-Qarawi et al., 2008). So, the renal injury due to CCl_4 intoxication could be evaluated by assessment of serum creatinine, urea, and uric acid, which were used as early markers for altered renal functions. The significant increase in serum creatinine, urea, and uric acid in the CCl_4 treated rats refer reduced glomerular filtration rate and the development of kidney dysfunction, this may be attributed to the significant reduction in glutathione antioxidant and elevation of MDA and NO that indicates its prooxidant effects in rat kidney. The ability of the body to produce these antioxidants influenced by exposure to environmental factors which includes chemicals and diet and controlled by genetic makeup (Halliwell, 1999).

Results of the current study revealed that the pre-treatment with O3FAs and /or SI, to rats improved the liver and kidney functions and produced good antioxidant activity. The hepatoprotective action of O3FAs on liver tissue reported herein was similar to the results reported by Attia et al. (2011) who recorded that the mode of action of O3FAs can be intercepted pharmacologically at different levels with agents that scavenge free reactive oxygen, block their generation, or heighten endogenous antioxidant capabilities. Meganathan et al. (2011) revealed that O3FAs improved AST, ALT, ALP, and LDH levels, and decreased the production of pro-inflammatory cytokines because of its anti-inflammatory effect. Fish oil O3FAs have antioxidants, anti-inflammatory, and antiapoptotic properties (Zararsiz et al., 2011). Also, O3FAs augment adiponectin hormone function which is responsible for reduction in fasting glucose. Adiponectin hormone increases peripheral insulin sensitivity through enhancing of lipid oxidation process and lead towards reduces hepatic glucose output (Ravussin, 2002). The reduction in the level of blood glucose might also owing to substituting of fuel with increasing of glucose utilization and reduction of fatty acid accessibility and improving insulin effect, the cycle concerned with glucose-fatty acids might also be the reason (Lam et al., 2003). Shariati et al. (2011) revealed that fish oil O3FAs reduced the glucose level by 50.09%.

Also, kidney dysfunction induced by CCl_4 in the present investigation are mediated through oxidative stress. The rise in kidney biochemical parameters could be attributed to toxic effect of CCl_4 on kidney and the administration of

O3FAs to rats modulates the kidney function through its antioxidant properties (Pauwels and Kostkiewicz, 2008).

On the other hand, our results indicated that soya isoflavones has a protective effect against oxidative stress induced by CCl_4 in the liver and kidney and this is mediated by its antioxidant activities. So, the modulation of liver enzymes and glucose level that disrupted by CCl_4 in the present study can be attributed to the antioxidant effect of soya isloflavones (Genovese et al., 2005). Bartke et al. (2004), reported that the isoflavone intake reinforce glucose tolerance capacity in normal mice and can prolong lifespan. Hermansen et al. (2001) recorded the beneficial effect of soybean and its isoflavones on carbohydrate and lipid metabolism in diabetic animals. Also, Ali et al. (2004) revealed a wide range of soybean isoflavones benefits in diabetes including favorable altering of glycemic control, insulin resistance, and serum lipid control. Soybean phytochemical extract displayed a number of properties which can be useful for diabetes, particularly as an estrogenic agent, as an inhibitor of intestinal glucose-uptake and a preventive agent for glucose-induced lipid peroxidation (Vedavanam et al., 1999). The effect of soy isoflavone on blood glucose level in streptozotocin-induced diabetic rats have been reported (Lee, 2006) but the results were conflicting. Hsu et al. (2003) supplemented a diet with isoflavone (240~1920 mg/100g diet) for 24 days and found no effect on glucose level. Lee (2006) tested soy protein (200g/kg diet)-supplemented diets for 3 weeks and reported hyperglycemia correcting in diabetic animals. In both studies, the dosages of isoflavones were rather high and the feeding durations were relatively short. The results support the possibility of soya isoflavones in preventing renal dysfunction in CCl_4 treated animals. Also, there are many studies which have reported on the benefit effect of dietary soybean and soy protein regarding with improving kidney function in Type II diabetes with the nephropathy (Azadbakht et al., 2003; Teixeira et al., 2004) or delaying the development of chronic renal disease in animals and human (Anderson et al., 1999).

It can be concluded that oxidative damages may be the primary cause of CCl_4 toxicity leading to lipid peroxidation and cellular damage. Thus, the obvious change in liver and kidney functions depending on cellular damage intensity. The significant recovery of hepatic and renal antioxidant content and reversal in the enhancement of liver enzymes, glucose, urea, uric acid, and creatinine by omega- 3 and/or isoflavones suggest that they are potent chemopreventive agents against oxidative stress and may suppress CCl_4-mediated renal oxidative damage in rats. These results may be due to the containing fish oil on polyunsaturated EPA and DHA. Similar results were obtained by Garrel et al. (2012), who reported that feeding enriched EPA and DHA diet increased enzyme antioxidant. The antioxidant and anti-inflammatory effects of O3FAs through scavenging of free radicals and inhibiting lipid peroxidation have been reported previously by Pauwels and Kostkiewicz (2008) and the potential antioxidant activity for soya isoflavones (Ruiz-Larrea et al., 1997; Genovese et al., 2005). This oxidant/antioxidant theory may explain the protective role of omega-3 fatty acids and soya isoflavones against the hepatotoxicity and nephrotoxicity of CCl_4.

5. Conclusion

In conclusion, the pre-treatment with omega-3 fatty acids and/or soya isoflavones showed a significant improvment against deleterious alterations associated with CCl_4 toxicity that induced hepatic and renal damage arising from oxidative stress. Moreover, the recorded improvement in the studied parameters of rats pre-treated with the combination of omega-3 fatty acids and soya isoflavones proves their synergistic effect. Therefore, the intake of omega-3 fatty acids and soya isoflavones produces high antioxidant activity and may be useful for reducing the oxidative stress.

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