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## RESEARCH ARTICLE

### Alteration of $CCl_4$ -Induced Oxidative Stress and Nephrotoxicity by Omega-3 in Female Albino Rats.

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#### Abstract

In the present study, twenty one female albino rats were used and they were divided into three groups. This was carried out and designed to study the effect of omega 3 poly unsaturated fatty acid on carbon tetrachloride ( $CCl_4$ ) induced oxidative stress and kidney damage in female albino rats. However, the antioxidant activity of omega-3 was determined by assessing biochemical parameters (serum creatinine, urea, uric acid, and malondialdehyde (MDA)). Carbon tetrachloride caused significant increase in serum creatinine, urea, uric acid, and MDA level while, omega 3 significantly prevented the increase of the serum levels of creatinine, urea, uric acid, and MDA.

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#### Introduction

Free radical is an atom or ion possessing an unpaired electron that can be considered as fragments of molecules, these unpaired electrons make free radicals very reactive towards other substances, or even towards themselves: (Chesman and Slater, 1993). It was generally accepted that free radicals seek to pair with a new free electron, and free radicals would be generated and it is unstable and can react with another one to generate a new free radical. Thus, this chain reaction of free radicals subsequently damages biological systems and tissues (Ozbek, 2012).

However, reactive nitrogen species (RNS), reactive oxygen species (ROS) and their generation are two kinds of biologically active free radicals, their generation, chemical reactions, and in vivo effects are closely interrelated. Harman was the first person who recognized the pathogenic role of oxygen free radicals in diseased states (Jeng and Fan-fan, 2012). The studies of free radicals and antioxidants in biology demonstrated that free radicals played a role in chronic diseases including atherosclerosis, cancer (Lobo *et al.*, 2010) neurodegenerative (e.g. Parkinson's disease, Alzheimer's disease, multiple sclerosis) (Aruoma, 2003), asthma and diabetes (Florence, 2014). Exposure to heavy metals, ultraviolet (UV) radiation, environmental pollutants, drugs, cigarette smoking, alcohol consumption (Sahreem. *et al.*, 2012) and xenobiotics e.g.,  $CCl_4$  (Kim, *et al.* 1990) lead to metabolic activation of ROS, which in turn causes cellular injuries (Khan *et al.*, 2012).

Carbon tetrachloride intoxication in animals is an experimental model that generates oxidative stress in many pathophysiological states (Saber, *et al.* 2012). The long term  $CCl_4$  exposure generates reactive oxygen species and different free radicals in many tissues such as liver, heart, kidney, brain, lung and blood. Also, it defects antioxidant enzymes activities and increased thiobarbituric reactive substances concentrations (Théophile *et al.*, 2006). In addition, (Mohammed *et al.*, 2013) reported that  $CCl_4$  distributes at greater concentrations in the kidney than in the liver and it has a high affinity to the cortex part of kidney which contains cytochrome P-450 predominantly.

Epidemiological studies investigate that certain long chain highly unsaturated omega 3-fatty acids which derived from the tissues of oil fish are known to reduce the oxidant status of various tissues. Diet rich in long chain omega-3 fatty acids has been shown to help in the development of healthy brain, heart and immune system

(Meganathan, *et al* 2014). Omega-3 fatty acids are considered as strong antioxidant and have an anticancer role in most of the human malignancies (Abdou and Hassan, 2014). Furthermore, regular consumption of omega-3 fatty acids, particularly docosahexaenoic acid, has been reported to ameliorate cardiovascular disorders, including endothelial dysfunction, platelet aggregation, elevated blood pressure and triacylglycerols, and arrhythmia (Abdel-Moneim *et al.*, 2014).

The present investigation was conducted to study the evaluated mechanism of omega-3 fatty acids on kidney function test parameters in CCl<sub>4</sub> induced nephrotoxicity in female albino rats.

### Experimental animals:-

Twenty one adult female albino rats (*Rattus norvegicus*) were used in the present study. Animals bred in the animal house of Biology Department, College of Science, Salahaddin University. All rats were weighing about 150-250 gms and 9-12 weeks old. The experiments achieved under the standard laboratory conditions (12h light: 12h dark photoperiod) and  $22 \pm 4$  C°, the animals were supplied with standard rat diet and tap water *ad libitum*.

### Experimental design:-

One experiment was carried out and designed to study the effects of omega-3 fatty acid on malondialdehyde (MDA) and some renal function test parameters including serum creatinine, urea and uric acids) in CCl<sub>4</sub> induced nephrotoxicity in female albino rats.

The experimental rats were divided into three groups, each with seven individuals and the treatments were continued for 4 weeks.

The group one received standard rat chow and tap water (Maulood, 2005) and represents control rats. The rats in the second group received CCl<sub>4</sub> (0.25 mL/kg) body weight intraperitoneally and normal rat chow with tap water. The rats in third group received standard rat chow supplemented with omega-3 (1gm/Kg) body weight and intraperitoneally administration of CCl<sub>4</sub> (0.25 mL/kg) bodyweight.

### Samples collection:-

#### Collection of blood samples:-

The rats were anesthetized using a combination of ketamine hydrochloride (35 mg/kg) and xylazine (5mg/kg) (Liard *et al.*, 1996). Blood samples were taken by cardiac puncture into chilled tubes with EDTA (4.5 mM as an anticoagulant) and centrifuged at +4C° for 15 minutes; then plasma was stored at -80°C (Sony, Ultra low, Japan).

#### Determination of serum malondialdehyde (MDA):-

Serum malondialdehyde (MDA) concentration, one of the end products of lipid peroxidation, was determined by the method of Wallin *et al.* (1993). The level of serum MDA was determined spectrophotometrically. In brief: To 150  $\mu$ l serum sample the followings were added: 1ml of 17.5% trichloroacetic acid (TCA) and 1ml of 0.66% (TBA), mixed well by vortex, incubated in boiling water for 15 minutes, and then allowed to cool. One ml of 70% TCA was added and the mixture was allowed to stand at room temperature for 20 minutes, and then centrifuged at 2000 rpm for 15 minutes, and the supernatant was taken for scanning spectrophotometrically at 532nm.

The concentration of MDA calculated as follow:

$$\text{MDA } (\mu\text{mol/L}) = \frac{\text{Absorbance at } 532\text{nm} \times D}{L \times E_0}$$

L: light path (1cm)

E<sub>0</sub>: Extinction coefficient  $1.56 \times 10^5 \text{ M}^{-1} \cdot \text{cm}^{-1}$

D: Dilution factor = 1 ml Vol. Used in ref./0.15 = 6.7

### Biochemical determination:-

#### Determination of serum creatinine:-

Serum creatinine level was determined by spectrophotometer Creatinine kit (Biolab, France). Creatinine in alkaline solution reacts with picrate to form a coloured complex; the intensity of the colour is proportional to the concentration of the creatinine in the serum and urine. The absorbance was measured at 500 nm using spectrophotometer.

**Determination of serum uric acid:-**

Serum uric acid was determined by uricase method, using colorometric test kit and the absorbance was measured at 492 nm using spectrophotometer (Biolab,France).

**Determination of serum urea:-**

Serum urea was determined by using colorometric test kit and the absorbance was measured at 600 nm using spectrophotometer (Biolab,France).

**Statistical analysis:-**

Analysis of data was performed by using {statistical package for social science (SPSS) version 24}. Results are expressed as mean  $\pm$  standard error (mean  $\pm$  SE). Statistical differences were determined by Duncan's test for multiple comparisons after analysis of variance (ANOVA).

**Results:-****Serum MDA:-**

The serum MDA level of CCl<sub>4</sub> treated rats increased significantly and reached ( $10.939 \pm 1.3 \mu\text{mol/L}$ ) as compared with control rats ( $4.206 \pm 1.1 \mu\text{mol/L}$ ). While, serum MDA level decreased significantly in animals that were provided with diet supplemented with omega-3 ( $6.418 \pm 0.5 \mu\text{mol/L}$ ), compared with CCl<sub>4</sub> treated rats (Table1 and figure 1).

**Serum uric acid level:-**

Statistical analysis revealed significant increase in serum uric acid level ( $14.61 \pm 7.05 \text{mg/dL}$ ) of CCl<sub>4</sub> treated rats when compared with control group ( $9.99 \pm 0.55 \text{mg/dL}$ ). On the other hand, rats treated with (omega-3 and CCl<sub>4</sub>) showed significant decreases in serum uric acid ( $9.61 \pm 0.71 \text{mg/dL}$ ) compared to CCl<sub>4</sub> treated rats (Table.1 and figure 2 )

**Serum urea:-**

Carbon tetrachloride caused significant increase in serum urea level ( $31.12 \pm 0.76 \text{mg/dL}$ ) when compare with control rats ( $21.00 \pm 0.48 \text{mg/dL}$ ). on the other hand, rats treated with (omega-3 and CCl<sub>4</sub>) showed significant decreases in serum urea ( $23.57 \pm 0.64 \text{mg/dL}$ ) when compare with CCl<sub>4</sub> treated rats (Table. 1 and figure 3).

**Serum creatinine:-**

Carbon tetrachloride caused a significant elevation in serum creatinine level ( $147.43 \pm 24.2 \text{mg/dL}$ ) in CCl<sub>4</sub> treated rats when compared with control rats ( $42.52 \pm 10.5 \text{mg/dL}$ ), while, serum creatinine level significantly decreased when rats treated with CCl<sub>4</sub> and omega 3 ( $65.64 \pm 6.1 \text{mg/dL}$ ) when compared with CCl<sub>4</sub> treated rats (Table1 and figure 4).

**Discussion:-**

Treatment with CCl<sub>4</sub> for days elevated serum MDA level. MDA has been reported to one of the secondary products produced following exposure to reactive oxygen species and free radicals, and it can be used to check out oxidative damage through measuring serum levels of thiobarbituric acid reactive substance (Lee *et al.*, 2012). However our results confirm those of (Ganieet *al.*, 2011) as they concluded that, CCl<sub>4</sub> enhances lipid peroxidation through increases free radical formation. CCl<sub>4</sub> is metabolized by cytochrome p-450 and produce highly reactive free radical which initiates lipid peroxidation of the endoplasmic reticulum s cell membrane and causes a chain reaction.

Our results were correlated with (Attia and Nasr 2009) who reported that omega-3 caused significant reduction in lipid peroxidation induced by CCl<sub>4</sub> in different organs of rats. The decrease in the MDA level by fish oil may be due to its antioxidant properties. Omega-3 could maintain normal levels of Superoxide dismutase and catalase activities (Pauwels and Kostkiewicz, 2008) In addition, the anti-inflammatory and anti-oxidant properties of omega-3 may be through its ability to inhibit lipid peroxidation and scavenging of free radicals (Abdou and Hassan, 2014).

The present results reveal that, intoxicated rats with CCl<sub>4</sub> showed a significant increase in serum creatinine, urea and uric acid levels as compared to normal control rats. These pathological changes indicate the potential damage to kidney cells induced with CCl<sub>4</sub> treatment since the observed increase is indication of an impairment of glomerular function and renal disorder (El-bazet *al.*, 2015). Our results agree with earlier reports reported by (Lotfy, 2009) , who attributed these increases to the pathological changes observed in the kidney tissue and to the damage in kidney

glomeruli as a result of CCl<sub>4</sub> injection. Moreover (Diabet *et al.*, 2014) recorded that CCl<sub>4</sub> induced elevation in serum urea could be related to the impairment of renal blood flow as well as reduction in glomerular filtration rate. However, creatinine production, in fact, has a strong relationship with muscle mass and increased it is level indicates the muscular wasting occurred during CCl<sub>4</sub> intoxication (Banfi and Del Fabbro, 2006). Although, CCl<sub>4</sub> causes depletion and deamination of muscle proteins but associated renal disorder prevents normal excretion process and therefore, causes accumulation and elevation serum levels of creatinine and urea (Rahmat *et al.*, 2014).

Oral administration of omega-3 produces significant nephroprotective effects in CCl<sub>4</sub> intoxicated rats. Our results are in agreement with (Shaheen *et al.*, 2016) who suggest that Omega-3 may find clinical application in a different condition where cellular damage is a result of oxidative stress. The nephroprotective effects of omega-3 may be explained by the antioxidant effect of omega-3 fatty acids. The antioxidant or anti-inflammatory effects of omega-3 through inhibiting lipid peroxidation and scavenging of free radicals have been reported previously (Abdou and Hassan, 2014). However, omega-3 protects rat from CCl<sub>4</sub> induced kidney injury as evident from significant decrease in serum creatinine, urea and uric acid. Changizet *et al.*, (2012) suggested that the ameliorative effects of omega-3 on kidney function may be due to the improvement of glomerular filtration. Moreover, it was generally accepted that fish oil reduce hyperlipidemia by decreasing serum total cholesterol and low density lipoprotein cholesterol concentration, so, creatinine might be decreased due to glomerulosclerosis reduction (Lauretani *et al.*, 2009). In addition, in a study (Abdel-Moneim *et al.*, 2014) demonstrated that elevated serum level of creatinine, urea and uric acid were improved in the hypothyroid treated rats with fish oil, they concluded that the protective effects of omega-3 in the concentration of creatinine, uric acid and urea may be due to that omega-3 ameliorated concentration of thyroid hormones and improved glomerular filtration.

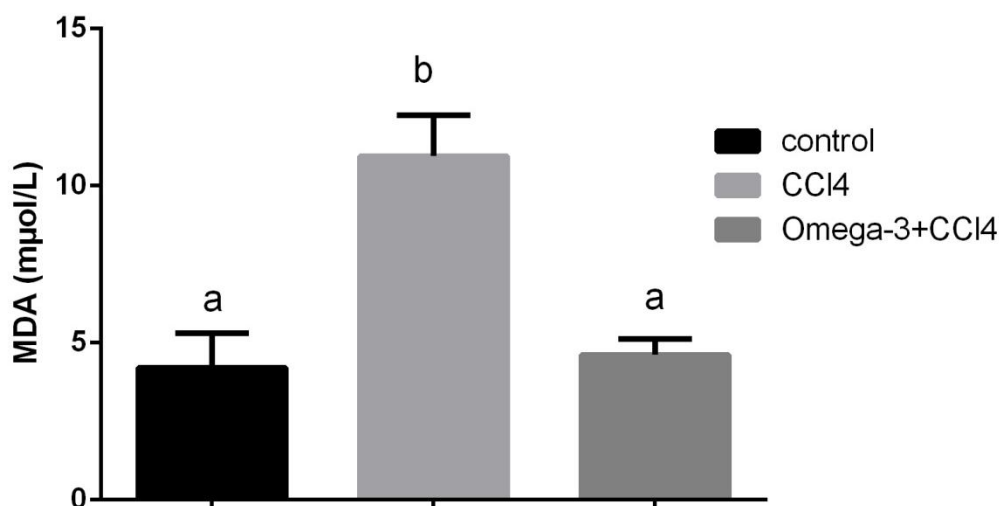
### Conclusion:-

In conclusion, from this study it was concluded that omega-3-fatty acids, protects the kidney against CCl<sub>4</sub> induced nephrotoxicity in female albino rats.

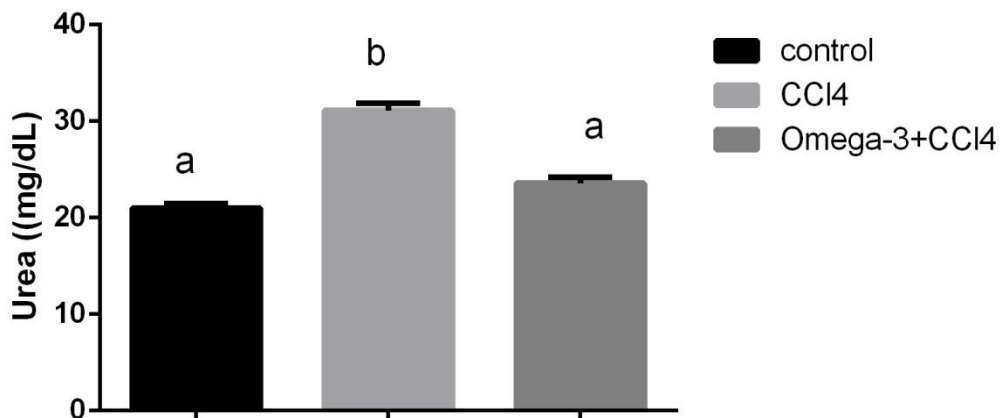
**Table 1:-** Effects of omega-3 on serum MDA, uric acid, urea and creatinine in CCl<sub>4</sub> induced oxidative stress and nephrotoxicity in female albino rats

Treatments	Parameters			
	MDA (μmol/L)	Uric acid (mg/dL)	Urea (mg/dL)	Creatinine (mg/dL)
Control	4.206 ± 1.1 <sup>a</sup>	9.99 ± 0.55 <sup>a</sup>	21.00 ± 0.48 <sup>a</sup>	42.52 ± 10.5 <sup>a</sup>
CCl <sub>4</sub> (0.25 mL/kg)	10.939 ± 1.3 <sup>b</sup>	14.61 ± 0.5 <sup>b</sup>	31.12 ± 0.76 <sup>b</sup>	147.43 ± 24.2 <sup>b</sup>
Omega-3 (1gm/Kg) + CCl <sub>4</sub> (0.25 mL/kg)	6.418 ± 0.5 <sup>a</sup>	9.6196 ± 0.71 <sup>a</sup>	23.57 ± 0.64 <sup>a</sup>	65.64 ± 6.1 <sup>a</sup>

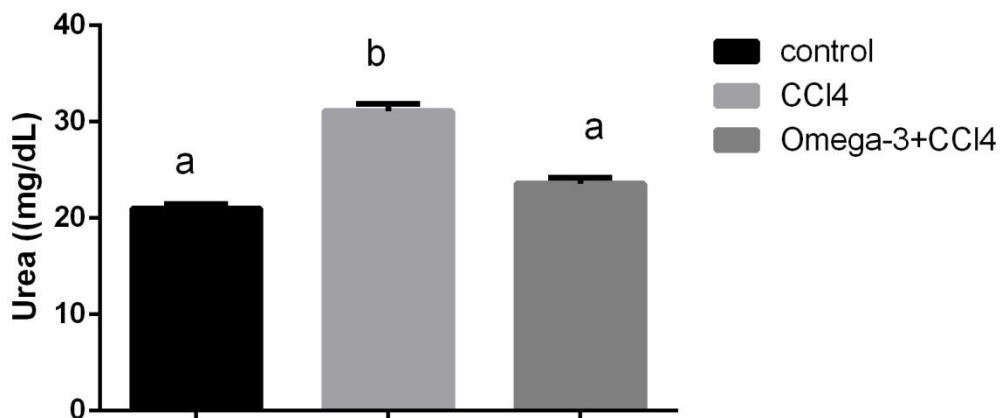
The same letters mean non significant differences while the different letters mean significant differences



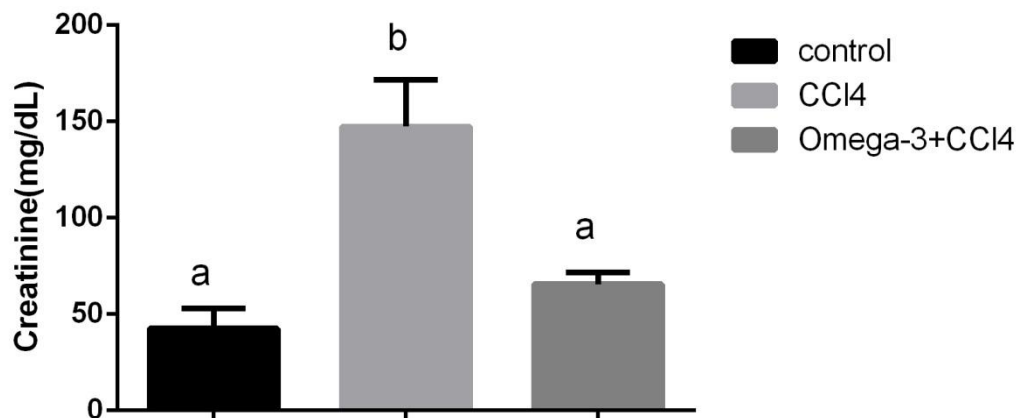
**Figure 1:-** Effects of omega-3 on MDA in CCl<sub>4</sub> induced nephrotoxicity. The CCl<sub>4</sub> treated rat group was compared with control while omega-3 treated rat group was compared with CCl<sub>4</sub> group.



**Figure 2:**-Effects of omega-3 on uric acid in CCl4 induced nephrotoxicity. The CCl4 treated rat group was compared with control while omega-3 treated rat group was compared with CCl4 group.



**Figure 3:**-Effects of omega-3 on urea in CCl4 induced nephrotoxicity. The CCl4 treated rat group was compared with control while omega-3 treated rat group was compared with CCl4 group.



**Figure 4:**-Effects of omega-3 on creatinine in CCl4 induced nephrotoxicity. The CCl4 treated rat group was compared with control while omega-3 treated rat group was compared with CCl4 group.

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