

**Ameliorative effect of olive leaf extract on carbon tetrachloride-induced nephrotoxicity in rats**Noorah S. Al-Sowayan<sup>1</sup> and H.M. Mousa<sup>2</sup><sup>1</sup>Department of Biology, Faculty of Science, Qassim University, Buraidah 51452, Saudi Arabia.<sup>2</sup>Department of food science and human nutrition, College of Agric. Vet. Science Qassim University, Saudi Arabia<sup>1</sup>[knaaj1@yahoo.com](mailto:knaaj1@yahoo.com), <sup>2</sup>[hasmousa@hotmail.com](mailto:hasmousa@hotmail.com)

**Abstract:** The present study was carried out to investigate the protective effect of olive leaf extract (OLE) on carbon tetrachloride (CCl<sub>4</sub>) induced nephrotoxicity in Wistar rats to validate its folklore use in kidney diseases. Treatment with CCl<sub>4</sub> (5 ml/kg body wt., i/p diluted in 9 volumes olive oil) significantly increased the level of serum creatinine, urea and significantly reduced the level of uric acid. In kidney homogenate, the activity of superoxide dismutase, catalase and reduced glutathione (GSH) were decreased while thiobarbituric acid reactive substances (TBARSs) were increased with CCl<sub>4</sub> treatment. Histopathological changes including glomerular atrophy, tubular necrosis, necrosis of epithelium, interstitial edema and congestion in capillary loops was observed after CCl<sub>4</sub> administration. Treatment with olive leaf extract (50 mg /kg body wt./d or 100 mg/kg body wt./d) significantly attenuated the biochemical and histopathological alterations induced by CCl<sub>4</sub> suggesting that OLE protected CCl<sub>4</sub>-induced nephrotoxicity through enhancement of renal antioxidant system.

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**1. Introduction**

Carbon tetrachloride (CCl<sub>4</sub>) is a potent hepatotoxin (Hanaa Wafay et al., 2012) and is rapidly biotransformed by cytochrome P-450 to trichloromethyl (CCl<sub>3</sub>) radical and Cl (Adewole et al., 2007). In the liver and kidneys after the initiation of lipid peroxidation, CCl<sub>3</sub> react with oxygen of cellular proteins and lipids producing a trichloromethylperoxyl radical which attacks rapidly lipid membrane of endoplasmic reticulum. Living cells continuously react with oxygen leading to the production of free radicals. The most common Reactive oxygen species (ROS) include superoxide (O<sub>2</sub><sup>•-</sup>) anion, hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), peroxyl (ROO<sup>•</sup>) radicals and reactive hydroxyl (OH) radicals. The reactive oxygen species are predisposing factors for many diseases including cancer, arthritis, aging and immunodeficiency diseases (Khalid et al., 2012). The damage caused by free radicals is partly ameliorated by a large group of protective agents called antioxidants that react with free radicals stopping the damage they cause. The antioxidants include vitamins (C and E), enzymes (Catalase, CAT; Super oxide dismutase, SOD; Glutathione peroxidase, GSHPx) and non-enzymes such as reduced glutathione (GSH) (Jing-Ying Xu, 2010).

A number of studies have postulated that herbal extracts and plant derived active ingredients can protect body against oxidative stress generated by CCl<sub>4</sub> and this can be achieved by altering the antioxidant enzymes activities (Tatiya et al., 2012; Ko et al., 1995). Some medicinal ingredients in plants may also act as antioxidants and protect the cell

against the damage caused by ROS (Kang et al., 2009, Kasdallah-Grissa et al., 2007).

Recently, the physiological effects of polyphenol-rich foods, such as olive oil, have been receiving much attention as dietary sources of antioxidants that are valuable for human health (Yokozawa, Kim, Kim, Lee, & Nonaka, 2007). Olive (*Olea europaea*) leaf extract is having high contents of polyphenols (free and total) and considered as one of the most important antioxidants (Djeridane et al., 2006; Mylonaki, et al., 2008). The antioxidant activity of phenolic compounds in olive leaf extract could be a result of the presence of hydroxyl groups in their structure such as oleuropein, hydroxytyrosol, and luteolin (Benavente-García, Castillo, Lorente, Ortuño Del-Río, 2000).

Olive Leaves have been widely used in traditional remedies in European and Mediterranean countries such as Greece, Spain, Italy, France, Turkey, Israel, Morocco, and Tunisia. Interest in the olive leaf beneficial effects has recently been increasing. It was reported that Ancient Egyptians used olive leaf for mummification and as a remedy against various diseases. The British used them to treat Malaria in Africa in the 1800s (Leila Abaza et al., 2007).

The present study was undertaken to investigate the possible nephroprotective effect of olive leaf extract (OLE) to validate its folklore use in kidney diseases.

**2. Material and Methods**

Green olive leaves (*Olea europaea*) were collected from nearby farms in Hael region; Saudi Arabia. Leaves were dried and stored at ambient

temperature in the dark. Before extraction processes, they were ground into particles with an average diameter between 0.9 and 2.0 mm. The extraction method of Mehmet Bilgin, Selin Sahin (2013) was followed. The obtained powder was stored in brown bottles at 4°C until use.

#### Experimental design:

In this study, 40 adult male Wistar rats weighing between 180-200 g were purchased from the Animal house, College of pharmacy, King Saud University, Saudi Arabia. The animals were housed in clean plastic cages and allowed to acclimatize to the laboratory environment for two weeks under the same laboratory conditions of photoperiod (12-h light:12-h dark cycle), a minimum relative humidity of 40-45 % and room temperature  $23 \pm 2$  °C. The rats were provided ad libitum with tap water and fed with standard commercial rat chow.

Rats were divided equally into 4 groups: (1) Normal control; injected intraperitoneal (i.p) saline (0.5 ml) once a week for six weeks, Group (2) CCl<sub>4</sub> treated group; received only CCl<sub>4</sub> (injected i. p with 5ml CCl<sub>4</sub> /Kg body weight mixed with olive oil in ratio of 1:9 each week for six weeks), Group (3) treated similarly to group (2) but after 48h post CCl<sub>4</sub> administration each rat received OLE, 50 mg/kg body weight. OLE was administered orally by gavage every day, and Group (4) treated similarly to group 3 but the OLE dose was 100 mg/kg.

The experiment continued for six weeks and at the end of which the animals were anesthetized by exposure to an atmosphere of diethyl ether and then killed by decapitation. Blood was collected from the heart in dry tubes and left for 30 minutes at room temperature to clot and then centrifuged at 10,000rpm for 10 min. Serum was separated and stored.

**Preparation of kidney homogenate:** Both kidneys were isolated after dissecting rats. The left kidney was dried in liquid nitrogen and stored at -70 °C for further investigations and the right kidney was stored at 10% formalin for histological studies. About 150 mg of kidney tissue was homogenized in 10 volumes of 100 mM KH<sub>2</sub>PO<sub>4</sub> buffer containing 1 mM EDTA, pH 7.4 and centrifuged at 12,000 xg for 30 min at 40°C. The supernatant was harvested and used for the following estimations.

Estimation of Serum Creatinine, uric acid and urea serum Creatinine, uric acid and urea were determined using commercial kits (Bio-Merieux Laboratory Reagents and Products, France).

Estimation of protein and antioxidant enzymes activity in kidney homogenate.

Protein concentration of the supernatant was determined by the method of Bradford (1970). SOD activity in kidney homogenate was performed using Randox kits (Randox Lab-Ltd., Ardmore Diamond

Road, Crumlin, Co Antrim, UK). Catalase activity was estimated, after proper dilution, spectrophotometrically according to the method of Aebi (1984). CAT activity was determined according to the method of Aebi (1983), briefly, 10 ml of kidney supernatant was added to 2.99 ml of phosphate-buffered saline and the absorbance was read at 240 nm using UV spectrophotometer. Reduced glutathione (GSH) level in kidney homogenate was determined according to the method of Ellman (1959). Lipid peroxidation products in kidney homogenates were estimated in terms of thiobarbituric acid reactive substances (TBARSs) according to the method of Esterbauer and Cheesman (1990).

**Histopathological examination:** Kidney samples were taken and quickly fixed in 10% formalin for 24 hours. Paraffin sections, 4 µm thick, were prepared and stained with hematoxylin and eosin (H & E). Sections were studied under light microscope at 40 and 100 magnifications. Slides of all groups were studied.

**Statistical analysis:** Data were analyzed statistically using student t-test to find the significant difference between means.

### 3. Results

**Table 1: Effect of OLE on creatinine, uric acid and Urea in serum of rats**

Parameter	Group 1	Group 2	Group 3	Group 4
Serum creatinine (mg/dl)	0.68±0.03a	1.32±0.07b	0.72±0.02a	0.66±0.01a
Serum uric acid (mg/dl)	2.75±0.09a	1.4±0.21b	4.22±0.15C	4.66±0.19C
Urea (mg/dl)	16.64±0.48a	26.43±0.64b	18.12±0.42a	17.62±1.0a

\* Means having different superscript within the same row are significantly different ( $p \leq 0.05$ )

As presented in table 1, in the normal control rats, the levels of serum creatinine, uric acid and urea were found to be 0.68±0.03; 2.75±0.09 and 16.64±0.48 mg/dl, respectively while in CCl<sub>4</sub> group, levels of creatinine and urea increased ( $p \leq 0.05$ ) significantly to 1.32±0.07 and 26.43±0.64 mg/dl, respectively, while the level of uric acid decreased significantly in CCl<sub>4</sub> group to 1.4±0.21. When rats were treated with OLE, there was a significant ( $p \leq 0.05$ ) decrease in the level of creatinine and urea with both concentrations of OLE (50, 100 mg) when compared with CCl<sub>4</sub> group and there was a significant increase in the level of uric acid in both OLE treated groups.

The effect of OLE on CCl<sub>4</sub>- induced changes in the antioxidant profile and lipid peroxidation is shown in table 2.

The concentrations of SOD and CAT in normal control were 11.42±2.32 unit /mg protein and 2.21±0.12 µg/sec/g tissue, respectively. These

concentrations were significantly ( $p \leq 0.05$ ) reduced in CCl<sub>4</sub> group to  $0.82 \pm 0.22$  unit /mg protein and  $1.2 \pm 0.08$   $\mu\text{g}/\text{sec}/\text{g}$  tissue, respectively. However, these concentrations increased significantly ( $p \leq 0.05$ ) after administration of both concentrations of OLE (50, 100 mg).

Regarding the concentration of GSH, in the normal group the concentration was  $3.82 \pm 0.42$   $\mu\text{g}/\text{g}$  tissue whereas as in CCl<sub>4</sub> group there was a significant ( $p \leq 0.05$ ) decrease to  $0.86 \pm 0.12$   $\mu\text{g}/\text{g}$  tissue. In group 3 and 4, that received OLE, there was a significant increase in the level of GSH to  $2.82 \pm 0.24$   $\mu\text{g}/\text{g}$  tissue in the group that received 50 mg OLE and to  $4.62 \pm 0.45$   $\mu\text{g}/\text{g}$  tissue in the group that received 100 mg dose of OLE. The concentration of TBARS ( $\mu\text{g}/\text{mg}$  protein) in normal rats was  $38.68 \pm 2.42$  and it increased significantly ( $p \leq 0.05$ ) to  $110.64 \pm 12.8$  in group 2 that received CCl<sub>4</sub> only. Administration of OLE at 50 and 100 mg concentration reduced significantly the levels of TBARS to  $86.82 \pm 4.64$  and  $66.68 \pm 4.86$   $\mu\text{g}/\text{mg}$  protein respectively.

**Table 2. Effect of OLE on CCL4- induced changes in the antioxidant profile and lipid peroxidation.**

Parameter	Group 1	Group 2	Group 3	Group 4
SOD; unit /mg protein	$11.42 \pm 2.32a$	$0.82 \pm 0.22b$	$5.96 \pm 1.8c$	$9.48 \pm 2.24a$
CAT; $\mu\text{g}/\text{sec}/\text{g}$ tissue	$2.21 \pm 0.12a$	$1.2 \pm 0.08b$	$2.82 \pm 0.24a$	$2.10 \pm 0.22a$
GSH ; $\mu\text{g}/\text{g}$ tissue	$3.82 \pm 0.42a$	$0.86 \pm 0.12b$	$4.48 \pm 0.18a$	$4.62 \pm 0.45a$
TBARS; $\mu\text{g}/\text{mg}$ protein	$38.68 \pm 2.42a$	$110.64 \pm 12.8b$	$86.82 \pm 4.64c$	$66.68 \pm 4.86d$

\*Means having different superscript within the same row are significantly different ( $p \leq 0.05$ )

Effect of OLE on CCL<sub>4</sub>-induced alterations on renal morphology

The morphological changes associated with CCL<sub>4</sub> are shown in table 3. Sections of the control group showed normal morphology of glomeruli, tubules and capillaries. CCL<sub>4</sub> sections showed glomerular atrophy, Tubular Necrosis, Necrosis of epithelium, interstitial edema and Congestion in capillary loops. Treatment with OLE maintained the normal morphology of the kidney; however the higher dose of OLE preserved the morphology of the kidney to the extent of resemblance of the control group.

**Table 3. Effect of OLE on CCL4- induced alterations on kidney morphology**

	Glomerular atrophy	Tubular Necrosis	Necrosis of epithelium	Interstitial edema	Congestion in capillary loops
Group	-	-	-	-	-
Group 2	+++	+++	+++	+++	+++
Group 3	+	+	+	+	+
Group 4	-	-	-	-	-

\*- normal; + mild; +++ severe disruption

#### 4. Discussions

The literature survey revealed that some halogenated alkanes such as carbon tetrachloride (CCl<sub>4</sub>) are widely used as a model for the study of agents that cause liver damage by free-radical mechanism in experimental animals (Anonymous, 2013).

However, few investigations studied the nephrotoxicity that can be generated by CCl<sub>4</sub> which is an environmental toxin (Doughri et al., 2014; Adewole et al., 2007; Bhattacharya et al., 2005; Abraham et al., 1999).

Recently, interest has grown on the role and usage of fruits and vegetables as antioxidants to prevent and protect against oxidative damage generated by free radicals. Among these suggested natural antioxidant, olive leaf were nominated and found to be rich in biophenols such as oleuropein, verbascoside, ligstroside, tyrosol and hydroxytyrosol and these compounds have shown antioxidant activities (Visioli et al., 1998; Benavente-García et al., 2000). Thus, the present investigation was carried out to study the protective effect of OLE during nephrotoxicity induced by CCl<sub>4</sub> in rats.

In the present study it has been noticed that administration of CCl<sub>4</sub> caused a significant increase in serum creatinine and urea with concomitant decrease in uric acid. In the kidney homogenate administration of CCl<sub>4</sub> resulted in a significant decrease in SOD, CAT and GSH and increase in the level of TBARS. The decrease in the enzymes activity in the kidney may be due to the enhanced lipid peroxidation during CCl<sub>4</sub> toxicity. Results indicate that CCl<sub>4</sub> toxicity significantly increase the TBARS concentration and decreased GSH and CAT, SOD concentrations due to increased oxidative stress, while on other hand, OLE administration significantly reduced the elevation in TBARS concentration and increased GSH, CAT and SOD, indicating a protective effect. Similar observations were noticed with black tea extract in rats after exposure to CCl<sub>4</sub> (Fadhel and Amran, 2002). In the present study, administration of CCl<sub>4</sub> to rats resulted in a decrease in serum uric acid levels. It is well documented that uric acid in blood is the most important antioxidant (Ames et al., 1981) and impairment in kidney function can result from oxidative stress (Muhammad et al., 2009).

The morphological changes in the kidney may be due to administration of CCl<sub>4</sub> that mediated lipid peroxidation of lipid components of the kidney. Similar findings were reported by Muhammad et al. (2009).

It can be concluded that OLE may be a therapeutic and nephroprotective agent against kidney failure induced by chemicals such as CCl<sub>4</sub>. However, further studies are needed with olive leaves from

different geographical locations and executing extraction by different method.

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