

**Full Length Research Paper****Role of Ethanolic Extract of Parsley (*Petroselinum Sativum*) and Rutin against CCl<sub>4</sub>-induced Nephrotoxicity on the Subcellular Fractions in Rat Kidney**<sup>1</sup>Nermien Z. Ahmed and <sup>2</sup>Teleb Z.A.<sup>1 and 2</sup> National Organization for Drug control and Research (NODCAR).<sup>1 and 2</sup> Department of Molecular Drug Evaluation, 6, Abou-Hazem st., Pyramids, Cairo, Egypt.**\*Corresponding Author: Teleb Z.A.****Abstract**

This study was designed to determine effect of *Petroselinum sativum* (Parsley) extract against CCl<sub>4</sub>-induced nephrotoxicity in rat kidney. Kidney injury was performed by given CCl<sub>4</sub> (1.5 ml/kg) orally twice per week. Ethanolic plant extract by two concentrations (100 and 50 mg.kg<sup>-1</sup>b.w.) were administered orally for one week, also rutin was administrated orally by single dose "100 mg.kg<sup>-1</sup>b.w." for two groups both induced and non-induced nephrototoxicity'. The results showed that CCl<sub>4</sub>group exerted a significant increase the MDA concentration and GST enzyme activity in the cellular fractions "mitochondrial, microsomal, and cytosolic fractions", also, the total protein was inhibited in these fractions. The lysosomal enzymatic activities (Acid phosphatase (ACP), β-galactosidase (GAL) and β-N-acetyl glucosaminidase (NAG)) in CCl<sub>4</sub>-induced toxicity were increased with variable percentage values. Administration of Parsley extract ameliorated the MDA concentration as compared to control, also, Rutin decrease the MDA concentrations. Total protein was increased in both protective and curative groups, GST activity in the subcellular fractions was inhibited as compared to control after administration of Parsley and Rutin. The activity of the lysosomal enzymes was ameliorated in rat kidney due to the inhibition and the stabilizing effect on the membrane permeability under the effect of Parsley extract and Rutin.

**Key words:** Parsley, Rutin, Subcellular fractions, lysosomal enzymes, Antioxidant parameters.**Introduction**

It is thought that antioxidants play a significant role in protecting living organism from the toxic effects of various chemicals by preventing free radical formation (Shewita et al., 2001). The free radical-mediated hepatotoxicity can be effectively managed upon administration of such agents possessing antioxidants, free radical scavengers and anti-lipid peroxidation activities (Lim et al., 2000). CCl<sub>4</sub> is an extensively used to induce lipid peroxidation and toxicity (Jeon et al., 2003). It is well established that CCl<sub>4</sub> is metabolized in the liver to highly reactive trichloromethyl radical which initiate free radical-mediated lipid peroxidation of the cytoplasmic membrane phospholipids and causes functional and morphological changes in the cell membrane leading to accumulation of lipid-derived oxidants causing kidney injury (Jeon et al., 2003).

Several studies have previously demonstrated that antioxidant prevent CCl<sub>4</sub> toxicity particularly hepatotoxicity, by inhibiting lipid peroxidation and increasing antioxidant enzyme activities (Teselkin et al., 2000 & Nevin and Vijayammal, 2005). Rutin, a natural flavone derivative, quercetin-3-rhamnosyl glucoside, is known for its anti-inflammatory and vasoactive properties, this flavonoid is an important anti-lipoperoxidant agent, and has also been found to be a strong scavenger of hydroxyl and peroxide radicals (Lindahl and Tagesson, 1997). Parsley has contains large amounts of flavonoids (Apigenin, kaempferol, quercetin, Hesperetin, Luteolin) polyphenols and tannins classified as flavones and flavonol class of flavonoids. It is considered as a source of natural antioxidant, anti-inflammatory and may be important in protecting cells against free radicals and chronic diseases (Lugasi and Hovari, 2000).

GST is considered as the main detoxifying enzyme for drugs xenobiotic compounds along with other supporting enzymes in liver and kidney. Hence its activity level was taken as toxicity marker in the target organs (Hassan et al., 2010). The aim of the present study was to investigate the ability of oral Parsley ethanolic extract administration to ameliorate the adverse effects of CCl<sub>4</sub>-induced nephrotoxicity in rat kidney. Different subcellular levels (mitochondrial, microsomal, and cytosolic fractions) were isolated from rat kidney, and the lysosomal enzymatic activities "ACP, β-GAL, and β-NAG" in kidney homogenate were determined.

**Materials and Methods****Chemicals**

Thiobarbituric acid (TBA) and carbon tetrachloride (CCl<sub>4</sub>) were obtained from Merck Company. All other chemicals such as rutin and the enzyme substrates were supported in analytical and purified grade from Sigma Co., St. Louse, MO.

### *Preparation of plant extract*

Leaves of Parsley were extracted three times successively using 70% ethanol at the rate of  $3 \times 10 \text{ ml.g}^{-1}$  of plant material. The supernatant was decanted centrifuged at  $5000 \text{ xg.10min}^{-1}$  and filtered (Lee et al., 2002). The collected supernatants were lyophilized under vacuum and freeze dried at  $-50^\circ\text{C}$  on the lypholizer

### *Animals*

100 Male albino rats weighed (200-250g) were supplied from the Animal House of "NODCAR". Animals were housed in a good condition and given free access to standard pellet diet and water, all rats were kept under controlled light/dark cycle (10h/14h); temperature conditions ( $24 \pm 1$ ) and relative humidity ( $65 \pm 10\%$ ) Naik et al. (2011). The experimental protocols were approved by the Ethics committee of Ministry of Health, Egypt and were conducted according to the National Organization for Drug control and Research (NODCAR).

Animal were distributed into the following groups and sub-groups, each box group containing 10 rats, the total animals about 90-100 rats.

**Group 1:** Normal control, rats received daily water only; **Group 2:** Negative control, rats received daily tween 20 (1%) only as a vehicle; **Group 3:** Positive control, rats received twice per week  $\text{CCl}_4$  (1.5 ml/kg) orally according to Janbaz et al. (2002).

**Group 4: For prophylactic experiment:** These animals were divided to subgroups as following, these group were taken plant extract at the first day up to 21 days,  $\text{CCl}_4$  (1.5 ml/kg) was given orally twice / week from the second week to the end of experiment:

- **Sub group a:** rats received parsley extract by high dose "100 mg/kg b.w." daily for three weeks.
- **Sub group b:** rats received parsley extract by low dose "50 mg/kg b.w." daily for three weeks (Havsteen, 2002).
- **Sub group c:** rats received rutin prepared freshly in dose "100 mg/kg b.w." daily for three weeks (Le Casa et al., 2000).

As well as, three subgroups for non-induced toxicity without  $\text{CCl}_4$  were received only two concentrations of Parsley and Rutin by single dose were performed for three weeks.

**Group 5: For curative experiment:** These animals were divided to subgroups, and firstly administrated orally with  $\text{CCl}_4$  (1.5 ml/kg) to induced toxicity, then treated with the plant extract by two concentrations and Rutin for 21 days.

After the last dose of plant extract with 24 hrs, rats were decapitated, kidney was removed and homogenized in 0.1 M Tris-HCl buffer, pH 7.4 (1.0 gm / 5ml), then centrifuged at 5000 r.p.m/10 min. The supernatant was used for estimation of (TBARS) for lipid peroxidation.

### *Biochemical parameters*

The activity of glutathione-S-transferase (GST) was measured according to the method of (Habig et al., 1974) using CDNB as a substrate, the absorbance was measured at 310nm using UV- Double beam spectrophotometer. Malondialdehyde (MDA) toxicity and oxidative stress levels of MDA were assayed by the method of Ohkawa et al. (1979) and Aboul-Enein et al. (2012) Lipid peroxidation (LPO) in tissue homogenate was estimated as the concentration of (MDA), which is the end product of LPO, and reacts with Thiobarbituric acid (TBA) and Trichloroacetic acid (TCA) giving pink color, which has an absorption on 535 nm. Total protein was determined in kidney homogenate according to the method of Lowry et al. (1951).

### *Isolation of tissue homogenate and sub-cellular fractions*

Rat kidney was promptly excised after decapitation, weighed and chilled in ice-cold 0.9% NaCl. Kidney was perfused with ice-cold 0.9% NaCl via the portal vein before homogenization. After washing, tissue homogenates were prepared in a ratio of 1 g of wet tissue to 9.0 ml of 1.15% KCl by using a glass or Teflon Potter-Elvehjem homogenizer. Mitochondria and microsomes of rat kidney were prepared according to the method of Hogeboom (1955). These fractions were finally suspended in 1.15% KCl, to contain approximately 1 mg protein in 0.1 ml suspension.

### *Preparation and isolation of lysosomal enzymes*

The whole kidneys were perfused in situ with 50 ml of 0.25M ice-cold sucrose buffer of pH 7.4. The kidney homogenate was centrifuged at 2500 r.p.m / 15 min, then the whole lysosomal fraction was prepared by centrifugation at 14.000 r.p.m / 15 min according to Tanaka and Iizuka (1968) and El-Deib and Nermien (2011).

### *Determination of lysosomal enzymatic activities*

The activity of the lysosomal enzymes has been measured according to the method described by Younan and Rosleff (1974) and El-Deib and Nermien (2011).

### *Statistical analysis*

The results are expressed as mean  $\pm$  S.E.M. for eight animals in each group. Differences between groups were assessed by one analysis of variance (ANOVA) using the SPSS/10 software package for windows. Post hoc testing was performed for inter-group comparisons using the least significance difference (LSD) test. Significance at P values  $< 0.05$  has been respective symbols in tables.

## Results

The present study showed the effect of Parsley ethanolic extract and rutin as standard antioxidant on sub-cellular particles (mitochondria, microsomes, and cytosolic fractions) on both induced and non-induced in rat kidney.

### **Effect of parsley ethanolic extract with two concentrations and Rutin with single dose on MDA concentrations in different subcellular fractions:**

The obtained results revealed that, parsley with the two concentrations have a significant inhibitory effect on MDA concentration as compared to the positive control in the different sub-cellular fractions.

In mitochondrial fractions, the inhibitory effect on lipid peroxidation (Table 1) either in protective or curative groups and non-induced toxicity exerted highly percentage values than in other fractions (microsomal and cytosolic fractions). The lowest inhibitory effect of the extract appeared obviously in cytosolic fractions.

The low concentration of Parsley in either toxicated or non-toxicated animals revealed high percentage of inhibition, while the high concentration of the plant extract has reversible effect as compared to the positive control. Rutin showed moderate inhibitory effect on lipid peroxidation in all subcellular fractions.

### **Effect of Parsley ethanolic extract with two concentrations and Rutin with single dose on the total protein:**

The data in Table 2 revealed that, in toxicated rats the total protein was significantly reduced either in mitochondrial, microsomal, and cytosolic fractions. While after the administration of plant extract and rutin with the two concentrations either intoxicated or non-toxicated treatments revealed an amelioration effect by enhancement the total protein in the three sub-cellular fractions.

As indicated from the results, that the highly protein content was appeared in the mitochondrial fraction then the microsomal and the cytosolic fraction. The highly percentage increase in the total protein was observed in the non-toxicated group than in CCl<sub>4</sub>-induced toxicity groups.

Also, the high concentration of parsley exerted significant increased values in non-induced group more than in toxicated curative treatment and intoxicated protective group in the three sub-cellular fractions.

### **Effect of Parsley ethanolic extract with two concentrations and Rutin with single dose on the enzyme activity GST in rat kidney:**

Table 3 revealed the effect of parsley extract by two concentrations and rutin on the enzyme activity of GST in the three subcellular fractions of rat kidney in either protective or curative group in rat induced hepatotoxicity. The enzyme activity of GST in CCl<sub>4</sub>-induced hepatotoxicity group exerted insignificant increase in all fractions of kidney.

After parsley administration with the two concentrations, the activity of GST was insignificantly increased as compared to positive control. Also, rutin with the single dose exerted insignificant effect on the enzyme activity for the three fractions "Mitochondria, Microsomal, and Cytosolic" in all treatments.

The effect of parsley and rutin on GST activity was highly in the cytosolic fraction much more than in microsomal and mitochondrial fractions, while the enzyme activity much higher in the mitochondrial fraction than microsomal and cytosolic fractions.

### **Effect of "Parsley" ethanolic extract with two concentrations and Rutin with single dose on the lysosomal enzymatic activities of "ACP, $\beta$ -GAL, and $\beta$ -NAG" in rat kidney:**

The data in Table (4) showed the effect of Parsley ethanolic extract with the two concentrations, as well as, rutin as antioxidant compound with single dose on the activity of three marker lysosomal enzymes isolated from rat kidney of CCl<sub>4</sub>-induced hepatotoxicity and non-induced group.

As revealed from the results, that in hepatotoxicity group, the three enzymes "ACP,  $\beta$ -GAL, and  $\beta$ -NAG" activities exerted highly significant release by 2438% for ACP ; 274% for  $\beta$ -GAL, and 206% for  $\beta$ -NAG as compared to negative control.

**Table 1.** Effect of *Parsley* ethanolic extract with two concentrations (100, 50 mg.kg<sup>-1</sup>b.w.) and *Rutin* with single dose (100 mg.kg<sup>-1</sup>b.w.) on Malondaldehyde concentrations (MDA) in kidney of rats induced and non- induced hepatotoxicity treatments.

Treatments Fractions	Normal control	Negative Control	Positive Control	Rat induced hepatotoxicity						Non- induced hepatotoxicity		
				(Protective)			(Curative)			Par(H)	Par(L)	Rutin
				Par(H)	Par(L)	Rutin	Par(H)	Par(L)	Rutin			
<b>Mitochondrial Fractions</b> nmole/ml % Change	13.0 ± 0.43 ↓31.2%	11.83 ± 0.14 ↓37.4	18.90 ± 0.28 --(a)--	2.03 ± 0.011 *** ↓89.3%	2.78 ± 0.041 *** ↓85.3%	2.48 ± 0.049 *** ↓86.9%	6.78 ± 0.006 *** ↓64.1%	2.27 ± 0.002 *** ↓88.0%	1.51 ± 0.001 *** ↓92.0%	3.66 ± 0.003 *** ↓80.6%	1.733 ± 0.033 *** ↓90.8%	1.6 ± 0.40 *** ↓91.5%
<b>Microsomal Fractions</b> nmole/ml % Change	10.28 ± 0.011 ↓36.5%	8.86 ± 0.018 ↓45.3%	16.19 ± 0.036 --(a)--	5.54 ± 0.027 ** ↓65.8%	6.38 ± 0.012 ** ↓60.6%	5.52 ± 0.024 ** ↓65.9%	11.58 ± 0.093 ** ↓28.5%	10.18 ± 0.048 ** ↓37.1%	5.26 ± 0.018 ** ↓67.5%	9.46 ± 0.042 ** ↓41.6%	3.26 ± 0.027 *** ↓79.9%	5.1 ± 9.7x10 <sup>-3</sup> ** ↓68.5%
<b>Cytosolic Fractions</b> nmole/ml % Change	11.88 ± 0.062 ↓30.2%	9.33 ± 0.021 ↓45.2%	17.02 ± 0.11 --(a)--	10.35 ± 0.18 ** ↓39.2%	11.92 ± 0.15 *** ↓88.7%	6.97 ± 0.042 ** ↓59.0%	13.79 ± 6.5x10 <sup>-3</sup> * ↓19.0%	8.49 ± 0.015 ** ↓50.2%	7.84 ± 0.011 ** ↓53.9%	9.84 ± 0.049 ** ↓42.2%	5.46 ± 0.068 ** ↓67.9%	8.01 ± 0.030 ** ↓52.9%

The values are the mean (n=8) ± S.E. of experiments performed in triplicate.

(a): Significant vs. control treated (Positive control)

Induced: CCl<sub>4</sub>-induced groups

\*\*\*: Very highly significant =P<0.0005

Non-induced: Groups without CCl<sub>4</sub>

\*\* Highly significant = P<0.0025

\*: Significant = P<0.0125

**Table 2.** Effect of *Parsley* ethanolic extract with two concentrations (100, 50 mg.kg<sup>-1</sup>b.w.) and *Rutin* with single dose (100 mg.kg<sup>-1</sup>b.w.) with protein content (TP) in kidney of rats induced and non- induced hepatotoxicity treatments.

Treatments Fractions	Normal control	Negative Control	Positive Control	Rat induced hepatotoxicity						Non- induced hepatotoxicity		
				(Protective)			(Curative)					
				Par(H)	Par(L)	Rutin	Par(H)	Par(L)	Rutin	Par(H)	Par(L)	Rutin
<b>Mitochondrial Fractions</b>	134.1	137.0	124.6	146.3	177.6	135.7	165.4	144.7	153.2	192.8	156.9	175.5
±	±	±	±	±	±	±	±	±	±	±	±	±
<b>Mg/ml</b>	8.2x10 <sup>-3</sup>	0.013	0.015	0.013	0.024	0.020	0.00043	0.014	0.016	0.047	0.039	0.029
<b>% Change</b>				**	**	*	**	**	**	**	**	**
	↑7.1%	↑9.1%	-----	↑17.4%	↑42.5%	↑8.9%	↑32.7%	↑16.1%	↑23.0%	↑54.7%	↑25.9%	↑40.9%
<b>Microsomal Fractions</b>	74.4	64.8	45.7	70.7	79.4	51.2	74.4	62.1	55.1	94.9	62.1	65.3
±	±	±	±	±	±	±	±	±	±	±	±	±
<b>Mg/ml</b>	5.5x10 <sup>-3</sup>	0.036	0.019	0.012	0.022	0.011	0.012	0.00093	0.018	0.027	0.031	0.012
<b>% Change</b>				**	***	**	**	**	**	***	**	**
	↑62.8%	↑41.8%	-----	↑54.7%	↑73.7%	↑12.0%	↑62.8%	↑35.9%	↑20.6%	↑107.7%	↑35.9%	↑42.9%
<b>Cytosolic Fractions</b>	25.8	22.4	20.4	24.5	28.4	25.1	27.4	23.3	22.0	36.5	29.2	27.7
±	±	±	±	±	±	±	±	±	±	±	±	±
<b>Mg/ml</b>	0.024	0.038	0.020	0.00059	0.021	0.029	0.011	0.012	0.026	0.033	0.042	0.011
<b>% Change</b>				**	**	**	**	**	*	***	**	**
	↑26.5%	↑9.8%	-----	↑20.1%	↑39.2%	↑23.0%	↑34.3%	↑14.2%	↑7.8%	↑78.9%	↑43.1%	↑35.8%

The values are the mean (n=8) ± S.E. of experiments performed in triplicate.

(a): Significant vs. control treated (Positive control)

Induced: CCl<sub>4</sub>-induced groups

\*\*\*: Very highly significant =P<0.0005

Non-induced: Groups without CCl<sub>4</sub>

\*\* Highly significant = P<0.0025

\*: Significant = P<0.0125

**Table 3.** Effect of *Parsley* ethanolic extract with two concentrations (100, 50 mg.kg<sup>-1</sup>b.w.) and *Rutin* with single dose (100 mg.kg<sup>-1</sup>b.w.) on Glutathione-S-Transferase activity (GST) in kidney of rats induced and non- induced hepatotoxicity treatments.

Treatments Fractions	Normal control	Negative Control	Positive Control	Rat induced hepatotoxicity						Non- induced hepatotoxicity		
				(Protective)			(Curative)			Par(H)	Par(L)	Rutin
				Par(H)	Par(L)	Rutin	Par(H)	Par(L)	Rutin			
<b>Mitochondrial Fractions</b> M/min % Change	179.79 ± 3.8x10 <sup>-3</sup> ↓34.0%	177.29 ± 5.7x10 <sup>-3</sup> ↓34.9%	272.50 ± 0.010 -----	273.54 ± 0.00049 ↓0.4%†	276.46 ± 0.00042 ↓1.5%†	276.46 ± 0.00091 ↓0.5%†	279.17 ± 0.00019 ↓2.4%†	284.93 ± 0.00058 ↓4.6%†	276.15 ± 0.00023 ↓1.3%†	280.52 ± 0.00012 ↓2.9%†	275.73 ± 0.012 ↓1.2%†	277.60 ± 0.00056 ↓1.9%†
<b>Microsomal Fractions</b> M/min % Change	162.40 ± 0.013 ↓27.5%	145.83 ± 8.3x10 <sup>-3</sup> ↓34.9%	223.96 ± 0.053 -----	246.35 ± 0.019 ↓10%*	251.25 ± 0.021 ↓12.3%*	216.67 ± 0.039 ↓3.1%†	248.23 ± 0.017 ↓10.8%*	232.5 ± 0.00042 ↓3.8%†	213.75 ± 0.00091 ↓4.6%†	277.71 ± 0.00012 ↓24%*	228.65 ± 0.067 ↓2.1%†	224.90 ± 0.028 ↓0.4%†
<b>Cytosolic Fractions</b> M/min % Change	48.75 ± 0.014 ↓63.6%	50.21 ± 0.016 ↓62.5%	134.0 ± 0.011 -----	42.08 ± 0.011 ↓6.0%*	156.25 ± 0.015 ↓16.6%*	135.63 ± 0.018 ↓1.2%†	142.5 ± 0.00048 ↓6.3%*	135.0 ± 0.013 ↓0.7%†	133.13 ± 0.017 ↓0.7%†	166.67 ± 0.00077 ↓24.4%*	134.17 ± 0.018 ↓0.1%†	132.29 ± 0.00073 ↓1.3%†

The values are the mean (n=8) ± S.E. of experiments performed in triplicate.

(a): Significant vs. control treated (Positive control)

Induced: CCl<sub>4</sub>-induced groups

\* Significant = P<0.025

Non-induced: Groups without CCl<sub>4</sub>

†: Insignificant = P< 0.25

**Table 4.** Effect of *Parsley* ethanolic extract by two concentrations (100, 50 mg.kg<sup>-1</sup>b.w.) and *Rutin* as standard by (100 mg.kg<sup>-1</sup>b.w.) a single dose on the activity of the three lysosomal enzymes isolated from rat kidney induced and non- induced hepatotoxicity treatments.

Treatments	Lysosomal enzymatic activity nmole/ml/hr					
	ACP		β-GAL		β-NAG	
Normal control	722.35 ± 0.32		698.58 ± 0.0073		585.02 ± 0.023	
Negative control	639.48 ± 0.039		530.34 ± 0.058		527.04 ± 0.068	
Positive control	16231.65 ± 0.041		1984.73 ± 0.0074		1611.73 ± 0.058	
<i>Petroselinum HD</i>	a↑2438%    b----		a↑274%    b----		a↓206%    b----	
	10570.96 ± 0.021		1107.36 ± 0.020		554.4 ± 0.014	
	a↑1553%    **	b↓34.9%	a↑109%    **	b↓44.2%	a↑5.0%    **	b↓65.6%
<i>Petroselinum LD</i>	11993.48 ± 0.042		1288.01 ± 0.015		752.40 ± 0.008	
	a↑1776%    **	b↓26.1%	a↑143%    **	b↓35.1%	a↑43%    **	b↓53.5%
	8130.51 ± 0.039		1264.12 ± 0.017		519.87 ± 0.016	
<i>Rutin</i>	a↑1171%    **		a↑138.4%    **		a↑2.0%    **	
	b↓49.9%	b↓36.3%	b↓67.7%			
	10009.79 ± 0.028		1121.80 ± 0.073		447.56 ± 0.032	
<i>Petroselinum HD</i>	a↑1465%    **		a↑112%    **		a↑15%    ***	
	b↓38.3%	b↓43.5%	b↓72.2%			
	12332.79 ± 0.026		925.95 ± 0.059		613.36 ± 0.053	
<i>Petroselinum LD</i>	a↑1828%    **		a↑75.0%    **		a↑16%    **	
	b↓24.0%	b↓53.3%	b↓61.9%			
	7373.57 ± 0.058		908.72 ± 0.047		418.89 ± 0.029	
<i>Rutin</i>	a↑1053%    **		a↑71.0%    **		a↑21%    ***	
	b↓54.6%	b↓54.2%	b↓74.0%			
	7092.99 ± 0.049		899.59 ± 0.0035		595.23 ± 0.041	
<i>Petroselinum HD</i>	a↑1009%    **		a↑70.0%    **		a↑13%    **	
	b↓44.0%	b↓54.7%	b↓63.1%			
	8587.28 ± 0.113		925.60 ± 0.020		761.30 ± 0.032	
<i>Petroselinum LD</i>	a↑1243%    **		a↑75.0%    **		a↑44%    **	
	b↓47.1%	b↓53.4%	b↓52.8%			
	8404.57 ± 0.023		742.84 ± 0.025		652.90 ± 0.051	
<i>Rutin</i>	a↑1214%    **		a↑40.0%    **		a↑24%    **	
	b↓48.2%	b↓62.6%	b↓59.5%			

The values are the mean (n=8) ± S.E. of experiments performed in triplicate.

Induced: CCl<sub>4</sub>-induced groups

Non-induced: Groups without CCl<sub>4</sub>

a: % change between treatments from Negative control    \*\*\*: Very highly significant =P<0.0005

b: % change between treatments from positive control    \*\* Highly significant = P<0.0025

## Discussion

Effect of parsley ethanolic extract with two concentrations and Rutin with single dose on MDA concentrations in different subcellular fractions indicated that under the effect of  $\text{CCl}_4$ , the tissue injury was seen and lipid peroxidation was increased, this finding in agreement to Nelson and Pearson (1990). They investigated that the damage to biomacromolecules by covalent binding of electrophils or by proton obstruction by radicals can lead to loss of the biological activity of the macromolecules, then the cell lost the activity and may lead to cell death. An increased cytosolic calcium concentration resulted from damage to the plasma membrane due to lipid peroxidation.

MDA a degradation product from lipid peroxide, provides an index of the peroxidation of lipids in biological tissue. It was observed by Kuhad et al. (2007) that an increased production of MDA measured as TBARS in the kidney under the effect of nephrotoxicity. Also, it was obviously that the antioxidant activity of the antioxidant compound mainly depend on the phenolic OH-group, although a small fraction may be due to the  $>\text{CH}_2$  site Kuhad et al. (2007). Ippoushi et al. (2003) found that MDA lipid peroxidation reacts with DNA bases and induces mutagenic lesions, the activated oxygen species can induce cellular events such as enzyme inactivation, DNA cleavage and membrane lipid peroxidation.

Badami et al. (2003) found that lipid peroxidative degradation of the biomembrane is one of the principle causes of toxicity of  $\text{CCl}_4$ . This evidence appeared by the elevation of TBARS and decreased in the activity of radical scavenging enzymes such as SOD and CAT in the  $\text{CCl}_4$  treated animals.

It was found that, reactive oxygen species (ROS) such as hydroxyl radical ( $\text{OH}\cdot$ ); superoxide radical ( $\text{O}_2^-$ ); peroxy radical and hydrogen peroxide can be detoxified by the effect of SOD; CAT;  $\text{GP}_x$  or non-enzymatic system by the scavenging action of GSH, while organic peroxides can be detoxified by GST (Schlorff et al., 1999). Modulation of these enzymes and concentration of GSH is important in the balance of the redox status through the reduction of ROS and peroxides produced in the organism, as well as, in the detoxification of xenobiotics (Ramiro-Puig et al., 2007 and Ramos, 2008).

The formation of reactive oxygen species (ROS), which can result in lipid peroxidation (LPO) is estimated by greater concentrations of thiobarbituric acid reactive substance (TBARS), which is assessed by its end product malondialdehyde (MDA) (Aboul-Soud et al., 2001). The observation of oxidative damage to the liver in rats exposed to a subchronic dose of the drug, as reflected by the significant increase in MDA production and the substantial inhibition at the present of parsley and rutin (Aboul-Soud et al., 2001).

Rutin has been found to be an important antioxidant agent and also has well-established properties against lipid peroxidation (Le Casa et al., 2000), although the anti-inflammatory properties of Rutin have been observed (Lindahl and Tagesson, 1997). It was found that rutin as a flavonols most effective on chronic and subchronic process inflammation by  $\text{CCl}_4$  induced nephrotoxicity (Rotelli et al., 2003 and Galati et al., 2005).

It was known that under conditions of severe oxidative stress, free radical generation as performed by carbon tetrachloride using in liver injury, this leads to protein modification. Proteins may be damaged directly by specific interactions of oxidants or free radicals with particularly susceptible amino acids: they are modified indirectly, with reactive carbonyl compounds formed by the auto-oxidation of carbohydrates and lipids with eventual formation of advanced glycation / lipoxidation and products (Gumieniczek, 2005). The levels of plasma total proteins were found to be decreased in this study. In addition, Beal (2005) investigated that the free radicals react with lipids, protein, and DNA often causing irreparable damage that can lead to cell death. One of the deleterious consequences of oxidative stress is lipid peroxidation, which involves hydrogen abstraction from fatty acids by free radicals such as OH and once initiated is a self-propagating process (Wang et al., 2006).

As indicated from the results, parsley extract and rutin have protective and curative effects, this effect was varied according to the subcellular fraction, also it may be due to the phenolic and hydroxyl groups (Derasena et al., 2002 and Justesen and Knuthsen, 2001). Parsley containing high levels of the flavone apigenin which possesses an antioxidant activity on the GST enzyme activity (Nielsen et al., 1999). Also, parsley contains a main compound "Myristicin" as antioxidant active agent (Wei and Shibamoto, 2007). The degenerative changes in rat liver tissue were significantly reduced or absent in the hepatocytes treated with parsley (BolKent et al., 2005). As well as, rutin as a flavonoids fraction exerts a protective and curative effect against  $\text{CCl}_4$ -induced degenerative process in rat liver (Galati et al., 2005).

It was demonstrated that at one-time administration of anticancer induced significant oxidative stress and decreased hepatic levels of SOD, CAT, GSH-Px, GSH and GST enzymes and increased LPO in liver mice (Prenkumar et al., 2001 and Bhattacharya et al.,



2003). GST is considered as the main detoxifying enzyme for drugs and xenobiotics compounds along with other supporting enzymes in liver and kidney. Hence its activity level was taken as toxicity marker in the target organs (Hassan et al., 2010).

Oxidative stress, implicated in the pathogenesis of a wide variety of clinical disorders, refers to the cytological consequence of a mismatch between the production of free radicals and the ability of the cell to defend against them. Oxidative stress can thus occur when the generation of free radicals increases or the capacity to scavenge free radicals and repair of oxidatively modified macromolecules decreases or both (Sies, 1997). This imbalance leads to the accumulation of oxidatively modified molecules, predominantly end products of superoxide ( $O_2^{\cdot-}$ ) and hydroxyl ( $OH^{\cdot}$ ) action. Hydrogen peroxide ( $H_2O_2$ ) and peroxynitrate ( $ONOO^{\cdot}$ ), although not free radicals themselves, contribute to the cellular redox state. Collectively, these molecules, referred to as reactive oxygen species (ROS), produce significant functional alterations in lipids, proteins, and DNA molecules. Oxidative lipid damage, referred to as lipid peroxidation, produces a gradual loss of cell membrane fluidity, reduces membrane potential and increases permeability to ions like  $Ca^{2+}$ . Oxidative stress has been proposed to be involved in the pathophysiology of many chronic diseases like atherosclerosis and is known to accelerate the aging process (Bhattacharya et al., 2003 and De la Fuente and Victor, 2000).

On the other hand, all groups "curative, protective" exerted a highly percentage release for ACP with different variability.  $\beta$ -NAG activity appeared to be the lowest percentage release for the three groups. The  $\beta$ -GAL activity revealed a moderate percentage release for ACP activity. These results exerted that the enzyme release of the lysosomal activities was type of enzyme – dependent and type of treatment group-dependent. This may be due to the behavior of each enzyme and also to the treatment. Many biochemical informations indicated that some of the lysosomal enzymes are involved in the constituents of the membrane and that portion of the soluble enzymes is bound to the membrane and many form a protective lining (Abdel Gawad et al., 2004). It has been demonstrated that the phenolic ring of polyphenolic compounds and flavonoids which are found in the plants with antioxidant activity, these groups have a main action on the membranes. It was found that the reactive oxygen species (ROS) which generated from the toxicity of compounds may be responsible for the release of lysosomal enzymes through the lysosomal membrane, also may be due to the decrease in membrane integrity and the enzyme leakage from the enclosed sacs. This effect may lead to the intracellular dysfunction, disruption of potential substrates and organelles as mitochondria and sarcolemma (El-Deib et al., 2009). It is assumed that such changes in marker enzymes activities could be attributed to the variability in lysosomal membrane labialization, which affects the outward leakage of these enzymes (Teleb et al., 1990). It was found that the process of lipid peroxidation activates phospholipase and remove the oxidized lipid from the membranes, the membrane that surround cell organelles such as mitochondria, lysosomes and peroxisomes contain large amounts of poly unsaturated fatty acids so they are a target for lipid peroxidation by free radicals, the oxidation of unsaturated fatty acids in biological membranes by free radicals leads to a decrease in membrane fluidity and disruption of membrane structure and function (Haragushi et al., 1997). Also, receptors present in the membranes are released or inactivated, furthermore, activation of lysosomal proteases; nucleosides and other lipases cause degradation of biological molecules and subsequently cell death (Teleb et al., 2006).

It has been demonstrated that, the protective effect of some medicinal plants was attributed to its antioxidant properties by inhibiting free radical generation (Manikandan et al., 2004). It caused a decrease in the degree of degradation of the existing collagen matrix and collagen synthesis, these effects were attributed to free radical scavenging properties and inhibition of lysosomal enzymes release (Maheshwari et al., 2006).

## Conclusion

Based on the results obtained, it was concluded that, the parsley ethanolic extract have the curative and prophylactic effects.

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