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#### PROTECTIVE EFFECT OF RED RADISH PLANT ON RAT ACUTE LIVER INJURY

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#### **ABSTRACT**

The main target of this study was to evaluate the effect of red radish plant and its seeds on hepatic liver. Rats were injected with carbon tetrachloride (CCl<sub>4</sub>) to produced acute hepatic disease. After induced hepatitis, roots, leaves, whole plants and seeds of red radish were used to feed the rats. Chemical composition of roots, leaves, whole plants and seeds of red radish were determined. Body weight changes, total feed intake, feed efficiency (FE) and internal organs weight ratios were evaluated. Biochemical assay [liver functions, antioxidant enzyme activities superoxide dismutase (SOD) and glutathione-S-transferases (GST), lipid peroxidation level malondialdehyde, MDA and serum lipid profile] were measured at zero time and the end of experimental period (4 weeks). Liver of tested rat groups were histopathological examined. Significant (p < 0.05) differences were observed in weight gain, feed conversion efficiency (FCE), relative ratio weight of liver, kidney and brain of all tested rat groups compared to the positive control. Biochemical assay illustrated a significant (p < 0.05) decrease in alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) activities of rat groups fed on roots, leaves, whole plants and seeds of red radish. Total protein and A/G ratio had a significant decrease in negative control rats group. There was an increase of superoxide dismutase (SOD) activity and the red radish roots and leaves had the highest effect on SOD activity compared to positive control, whole plants and seeds. Significant (p < 0.05) differences between the tested rat groups in GST activity. No significant (p < p0.05) change of serum MDA in all tested treatments at the end of experimental period compared to zero time expect negative control rat groups Serum lipid profile show a significant different between the tested rats groups. Feeding on red radish leaves caused a high significant reduction in total cholesterol followed by seeds, roots and whole plants compared with negative and positive control. Histopathological examinations illustrate normal histological structure of rats fed on tested samples. These results indicate that continuous red radish supply effectively inhibits CCl<sub>4</sub> -induced acute liver injury and may suggest the possibility that radish plant would be useful in curing some liver diseases.

#### INTRODUCTION

Radish (Raphanus Sativur L.) belongs to the family cultivated in south east Asia. Korean varieties are rich in vitamin B and C and have important medicinal properties (Lee, 1987). The quality characteristics of radish fulfilling consumer quality requirements such as root color, glucorinolates, monosaccharides and pectin substances dependently on the seasonal climate conditions must be taken into consideration (Schreiner et al. 2002). Moreover, Popovic et al (1993) mentioned that the oral administration of radish juice had some beneficial effects although there was no significant hepato-protective activity. Lu et al (2000) monitored the hepato-protective activity by estimating the serum trans-aminases concentrations and histopathological changes in the liver of the experimental rats. They found that methyl extract significantly decreased the acute elevation of serum trans-aminases. The cruel extract ameliorates the central necrosis fatty changes or proliferation of bile duct epithelium focal necrosis caused by alphanaphthyl isothiocyanate or carbon tetra chloride hepatitis rats. Kocsis et al (2002) reported that the in-vitro studies, the juice from the tic black radish root hydrogen donating and d-field element chelating abilities. The juice exhibited strong reducing power property and radical scavenging effect of H<sub>2</sub>O<sub>2</sub>/OH Luminal system. The in-vivo experiment resulted in decreasing the in furious effect of liquid rich diet. Shishu et al (2003) reported that the sulpheraphene isolated from radish is a portent inhibitor of the S9-mediated mutagenicity of all tested heterocyclic amines (60-70% inhibition at dose of 500 n mole/ plate).

Liver is an organ that is actively concerned with nutrient metabolism. CCl<sub>4</sub> is an extensively used xenobiotic 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 liver injury (Poly et al., 1987 and Recknagel et al., 1989). It also induces hydropic degeneration, centrilobular necrosis, fatty changes, cirrhosis and hepatoma. CCl<sub>4</sub>induced damage also produces alteration in the antioxidant status of the tissues, which is manifested by abnormal histopathological changes (Rajesh and Latha, 2004). Several studies demonstrated that antioxidants prevent CCl<sub>4</sub> toxicity particularly hepatotoxicty, by inhibiting lipid peroxidation and increasing antioxidant enzyme activities (Kumaravelu et al., 1995, Basu, 2003 and Dwivedi et al., 2006). The present study carried out to study the effect of red radish plant on carbon tetrachloride induced acute liver of rats.

#### MATERIALS AND METHODS

#### Materials:

Red radish plants were purchased from the local market at Dokki, Giza, Egypt. Red radish seeds were obtained from the Horticulture Research Institute, Agriculture Research Center, Giza, Egypt. Red radish plants were cleaned and washed, divided into 2 groups (one group contains whole red radish plants and the second group comprises red radish roots and leaves separately) and dried at 50°C for 72h. The dried plants, roots, leaves and seeds were subjected to the chemical analysis, i.e. moisture, crude protein, total lipid, ash and crude fiber contents according to the method outlined in the A.O.A.C (2000). Total carbohydrates were calculated by difference.

#### Biological experiment:

Adult male *albino* rats weighing  $200 \pm 5$  g, Sprague Dailey strain (36 rats) obtained from the Food Technology Research Institute, A.R.C., Giza were housed in plastics cages in healthy conditions and fed on basal diet for one week. Water and diet were provided *ad libitum*. After this period, the animals were divided into two main groups. The first group (6 rats) was fed on basal diet and considered as a positive control. The second group (30 rats) was carried to induce acute hepatotoxicity in rats by three doses of CCL<sub>4</sub> ( $25\mu$ L/Kg body

weight, IP) according to the method mentioned by Gyamfi et al (1999). The inject period was 3 days (one dose of each) and was divided into the following subgroups according to the feeding materials:

Subgroup (1) fed on basal diet and considered as negative control.

Subgroup (2) fed on basal diet contained red radish leaves.

Subgroup (3) fed on basal diet contained red radish roots.

Subgroup (4) fed on basal diet contained red radish seeds.

Subgroup (5) fed on basal diet contained whole plants of red radish.

Experimental diets were designed to substitute 4 % of cellulose content of basal diet with the tested samples as a source of fiber and 10 % protein level from tested samples and casein as shown in Table (1) and the animals were fed on experimental diet for 4 weeks.

Table (1): Composition (%) of experimental diets.

Rat	Protein	(10 %)	Oil	Cellulose	Salt	Vitamin	
group	Radish gm	Casein gm	(10 %)	(4%)	(4%)*	(4%)*	Starch
Positive control		11.11	10	4	4	1	69.89
Negative control		11.11	10	4	4	1	69.89
Roots	3.96	7.15	10		4	1	73.89
Leaves	4.27	6.84	10		4	1	73.89
Whole plants	3.76	7.35	10		4	1	73.89
Seeds	3.37	7.74	10	<b></b>	4	1	73.89

<sup>\*</sup> Ref A.O.A.C. (2000) 960.48

#### Rat body weight changes:

Body weight changes, total feed intake and feed conversion efficiency (FCE) of rats fed on different diets were measured.

#### Internal organs weight ratios:

At the end of the experiment, rats were weighed and killed. The carcasses were dissected. Internal organs (liver, kidney, heart, spleen, lung and brain) were removed and kept in a saline solution (NaCl, 5 %), and weighed. The internal organ percentages were calculated by divided weight of organ / total body weight x 100.

#### Biochemical assay:

Blood samples were collected at zero time and at the end of the experimental period. Blood samples were obtained from the orbital venous plexus of each rat using heparinized capillary tube. Each sample was collected in a clean centrifuge tube, left to cool and the serum was separated after centrifugation for 10min. at3000 rpm and kept frozen at -20°C in clean dry plastic tubes for the determination of blood biochemical parameters.

#### **Biochemical parameters:**

Alanine aminotransferase (ALT: EC 2.6.1.2) and aspartate aminotransferase (AST: EC 2.6.1.1) activities were assayed by the method of Bergmeyer and Harder (1986). Alkaline phosphatase (AlP; EC 3.1.3.1) activity was measured at 405nm by the formation of paranitrophenol from para-nitrophenylphosphate as a substrate using the method of Varley et al., (1980). Also, total proteins and albumin were measured according to the methods of Lowry et al, 1951 and Doumas et al,1977, respectively. Globulin was calculated by difference between serum total proteins and serum albumin The activity of superoxide dismutase (SOD) and glutathione-S-transferases (GST) activity of rats blood serum were estimated according to Marklund and Marklund (1974) and Habig et al., (1974), respectively. Lipid peroxidation level (malondialdehyde, MDA) was determined according to the method showed by Meltzer et al (1997). Total lipids, trigycerides and total cholesterol, HDL-cholesterol and LDLcholesterol were determined by using the methods described by Knight et al (1972), Fossati and Prencipe (1982) and Wastson (1960), Assmann (1979) and Wieland and Seidel (1983), respectively.

#### Histopathological examination:

After the experimental period animals were decapitated, livers removed immediately, sliced and washed in saline. Liver pieces were preserved in 10% formalin for histopathological studies. The pieces of liver were processed and embedded in paraffin wax. Sections were taken and stained with hematoxylin and eosin and photographed. Tissue specimens from liver of rats were fixed in 10% neutral buffered formalin solution. The fixed specimens were trimmed, washed and dehydrated in ascending grades of alcohol, cleaned in xylene, embedded in paraffin then sectioned (4-6 micron) and stained with hematoxyline and eosin according to Bancroft *et al* (1996). The

degree of hepatic injury was estimated using an ordinal scale modified from Plaa and Charbonneau (1994).

#### Statistical analysis:

All values are mean  $\pm$  S.E. obtained from six animals. For statistical analysis, one-way ANOVA with Duncan's variance (SPSS 11.1) was used to compare between the groups. In all the cases a difference was considered significant when p was  $\leq$  0.05.

#### RESULTS AND DISCUSSION

#### Chemical composition of red radish:

Table (2) shows that red radish roots characterized with the highest protein content (26.56%) followed by whole plants (22.75%), seeds (19.81%) and leaves (18.38%). It could be noticed that the ether extract contents of the tested samples were below 1%. In contrast, the crude fiber levels of tested samples were high; it ranged from 26.80% in roots to 17.23% in leaves of red radish. Moreover, the highest value of ash was 5.80% in the leaves and the lowest value of ash was 2.01% in the seeds of red radish. Total carbohydrate contents varied according to plant part, it was ranged from 43.12%, in roots, to 57.94% in the leaves. The data were in the harmony with the previously results reported by Giusti et al (1998) and Schreiner et al (2002).

Table (2): Chemical composition (%) of roots, leaves, whole plants and seeds of red radish (on dry weight basis).

Red radish part	Crude protein	Ether extract	Crude fibers	Ash	Total carbohydrates	
Roots	26.56	0.94	26.80	2.58	43.12	
Leaves	18.38	0.65	17.23	5.80	57.94	
Whole plants	22.75	0.72	24.18	4.68	47.67	
Seeds	19.81	0.51	23.51	2.01	54.16	

## Effect of feeding red radish on weight gain, feed intake and feed conversion efficiency (FCE) of experimental rats:

Data in Table (3) show that the initial weights of the experimental animals were 200  $\pm$  5 g and ranged from 273.14  $\pm$  26.27 g to 297  $\pm$  12.21 g at the end of the experimental period. The data reveal that the most effective material was the leaves which exhibited a body weight gain of 96.30  $\pm$  8.80 g followed by roots (88.30  $\pm$  4.21 g) and positive control (85.82  $\pm$  4.54 g). On the other hand, negative control and whole plant resulted significant ( $p \le 0.05$ ) decreases compared with other treatments.

Table (3): Weight gain, feed intake and feed conversion efficiency (FCE) of rats fed on red radish roots, leaves, whole plants and seeds.

Rat group	Initial weight (g)	Final Weight (g)	Weight Gain (g)	Total feed intake (g)	Feed conversion efficiency (FCE)
G1 (positive control)	199.76±6.83ª	285.58±11.37 <sup>ab</sup>	85,82±4.54 <sup>ab</sup>	330.08±22.98ª	0.26±0.01 <sup>ab</sup>
G2 (Negative control)	198.34± 6.49ª	268.12±11.59 <sup>b</sup>	69.78±9.74 <sup>b</sup>	317.18±10.75ª	0.22±0.02
G3 Roots	201.45±3.90 <sup>a</sup>	289.75±4.87 <sup>ab</sup>	88.30±4.21ab	339.61±11.42ª	0.26±0.02ab
G4 Leaves	200.91±6.55ª	297.21±12.21ª	96.30±8.80ª	356.66±25.29ª	0.27±0.02ª
G5 Whole plant	199.53±6.54ª	273.14±26.27 <sup>ab</sup>	73.61±24.71 <sup>b</sup>	308.59±15.80 <sup>a</sup>	0.24±0.06 <sup>b</sup>
G6 Seeds	201.45±3.10 <sup>a</sup>	281.19±6.61 <sup>ab</sup>	79.74±4.34 <sup>ab</sup>	332.25±14.04 <sup>a</sup>	0.24±0.01 <sup>b</sup>
L.S.D.	10.254	24.851	21.051	30.28	0.051

Each value represents the mean  $\pm$  SE.

The mean values with different superscript alphabets indicate significant differences (P≤0.05) using LSD test.

Total feed intake showed no significant ( $p \le 0.05$ ) differences and ranged between  $308.59 \pm 15.80$  and  $356.66 \pm 25.29$ g. Feed conversion

efficiency (FCE) data show no significant ( $p \le 0.05$ ) differences between treatments except that of negative control which resulted in significant ( $p \le 0.05$ ) decrease compared with the tested rat groups. Meanwhile, whole plant treatment had the highest ratio of FE. In this situation, Hashimoto *et al* (1999) and Seppanen and Saari Csallany (2002) found that the body weight of rats after administered carbon tetrachloride was significantly less than that of normal rats. Therefore, the red radish induces curing effect compared with the negative effect of carbon tetrachloride.

### Effect of feeding red radish on the relative ratios of rat organs to body weight:

Table (4) shows the relative ratios of organs to body weight of the experimental animals as affected by the feeding on red radish. The data reveal no significant ( $p \le 0.05$ ) difference between the relative ratios of liver fed on red radish roots, leaves, whole plant and positive control which resulted significant ( $p \le 0.05$ ) increase compared with negative control and that fed on red radish seeds. Kidney resulted significant ( $p \le 0.05$ ) increase when fed on red radish roots than all other treatments. Meanwhile brain showed a significant increase in its relative ratio due to positive control, negative, rats fed on roots and seeds than that of other feeding treatments. On the other hand, heart, spleen and lung showed no ( $p \le 0.05$ ) significant differences due to feeding materials.

From the above mentioned data, it could be concluded that feeding on red radish plant affect the relative ratio of liver, kidney and brain while no effect was found regarding heart, spleen and lung. Martin et al. (1981) demonstrated that the increase or decrease of liver weight, is relative to the control under the influence of different diets and that might be due to the accumulation or reduction of either fats or glycogen contents. Moreover, Ohyama et al (1990) found that kidney weight of experimental rats was effected by administration CCL<sub>4</sub> and there was kidney damage.

Table (4): Relative ratios of organ to body of rats fed on red radish roots, leaves,

whole plants and seeds.

Whole pands and seeds.											
Rat group	Internal organ (%) / body weight										
	Liver	Kidney	Heart	Spleen	Lung	Brain					
G1 (positive control)	3.86±0.07a	0.66±0.03bc	0.34±0.05a	0.30±0.04a	0.81±0.02a	1.04±0.02ab					
G2 (Negative control)	2.44±0.14c	0.59±0.04c	0.34±0.035a	0.28±0.03a	0.76±0.03a	1.15±0.09a					
G3 Roots	3.62±0.13a	0.76±0.09a	0.33±0.05a	00.36±0.02a	0.78±0.05a	1.10±0.04a					
G4 Leaves	3.48±0.36a	0.74±0.03ab	0.357±0.04a	0.36±0.08a	0.78±0.08a	0.96±0.12bc					
G5 Whole plants	3.62±0.064a	0.64±0.05bc	0.347±0.05a	0.35±0.07a	0.73±0.06a	0.86±0.02c					
G6 Seeds	3.08±0.27b	0.69±0.04ab	0.383±0.01a	0.35±0.02a	0.77±0.06a	1.07±0.10ab					
L.S.D.	0.3701	0.0992	0.0742	0.0898	0.0947	0.1428					

Each value represents the mean  $\pm$  SE. The mean values with different superscript alphabets indicate significant differences ( $P \le 0.05$ ) using LSD test.

#### Biochemical assay: Liver functions:

Table (5) shows the effect of red radish fed to experimental animals suffering from acute hepatic disease as a result of CCl<sub>4</sub> infection. ALT, AST, ALP, total proteins, albumin, globulin, and A/G ratio were determined in order to show the effect of red radish on these hepatic parameters. ALT shows a highly significant  $(p \le 0.05)$ increase regarding negative control which was about two times as that of zero time. Meanwhile a significant  $(p \le 0.05)$  decrease was found as a result of feeding on red radish at the end of experiment compared with that of zero time. The decrease in ALT was about 14%, 12%, 21.5%, and 14.8% when animals were fed on red radish roots, leaves, whole herbs and seeds, respectively. In contrast, a slight increase was found regarding AST as a result of all tested treatments. Alkaline phosphate (ALP) activity showed that a significant ( $p \le 0.05$ ) decrease due to feeding on different red radish fractions. In this connection, Lu et al. (2000), Nevin and Vijayammal (2005) and Wen et al (2006) reported that acute CCl<sub>4</sub> administration significantly increased the level of liver injury marker enzymes like AST, ALT and ALP. Moreover, Ozardal et al. (2004) showed that reactive metabolites are produced during biotransformation of CCl<sub>4</sub> and these metabolites may cause cellular death in the liver. AST, ALT and ALP may be released into blood plasma and serum levels of these enzymes may increase as due to the cellular damage in the liver. Thus, the levels of ALP in blood plasma may also increase in the early periods of liver damage.

On the other hand, total proteins, albumin, globulin and A/G ratio are performed as a routine test to evaluate the toxicological nature of various chemicals. The data reveal that positive control, leaves, whole plant and seeds of red radish resulted fairly decrease in serum total proteins compared to values at zero time.

Negative control had the lowest total protein value at the end of the experimental period (5.47 u/ml). Albumin level slightly increased in all tested rat groups and vice versa concerning globulin. The decreased total proteins and A/G ratio in negative control rats group may be due the damage caused in the liver tissues by CCl<sub>4</sub>. The ability of red radish to maintain the total proteins and A/G ratio may be due to the non-toxic antioxidant constituents associated with the extract. Wen et al (2006) found that the acute dose of CCl<sub>4</sub> possessed negative effects on total proteins and A/G ratio.

Table (5) Effect of feeding red radish on rat liver functions.

Rat group	ALT (U/L)		AST (U/L)		ALP(U/L)		Total proteins (mg/dl)		Albumin (mg/dl)		Globulin (mg/dl)		A/G ratio	
	Zero time	end	Zero time	end	Zero time	End	Zero time	end	Zero time	end	Zero time	end	Zero time	end
Positive	22.37	24.08	48.61	52.89	60.36	49.32	6.56	6.27	3.62	3.66	2.94	2.21	1.23	1.65
control(G1)	± 0.725°	1.39 <sup>ab</sup>	± 1.69 <sup>a</sup>	0.52 <sup>b</sup>	± 2.29 <sup>a</sup>	± 1.07 <sup>a</sup>	0.31 <sup>a</sup>	± 0.65°	± 0.17°	0.22 a	0.38 a	0.81 a	± 0.28 <sup>a</sup>	0.24 <sup>ab</sup>
Negative	23.1	47.97	49.82	62.11	55.63	64.0	6.77	5.47	3.62	3.77	3.18	3.98	1.13	0.94
control(G2)	± 1.51 <sup>a</sup>	± 2.6	± 2.21 <sup>a</sup>	± 4.47	2.06 <sup>b</sup>	± 4.15	± 0.37°	± 0.92	0.17 <sup>a</sup>	± 0.11 a	± 0.52 <sup>ab</sup>	± 0.26 <sup>a</sup>	± 0.18 <sup>a</sup>	± 0.18
Roots	22.67	19.55	49.30	51.06	57.97	47.18	6.48	6.75	3.81	4.27	3.16	2.54	1.21	1.37
(G3)	±	±	±	± .	± .	±	±	±	±	± .	±	, ±	±	
(03)	2.48 <sup>a</sup>	1.03°	1.06ª	0.89 <sup>ab</sup>	1.51 <sup>ab</sup>	2.2 <sup>ab</sup>	0.22 a	0.44 a	0.16*	0.35 <sup>b</sup>	0.44 a	0.52 ª	0.26 <sup>a</sup>	0.12°
Leaves	23.57	20.76	51.79	52.17	56.61	50.76	6. 97	6.26	3.65	3.72	3.22	2.40	1.13	1.55
(G4)	±	±	±	± .	±	±	±	±	±	±	± .	±	±	±
(0.)	2.67°	2.05°	1.6 <sup>ab</sup>	1.36 <sup>b</sup>	3.06 <sup>b</sup>	1.5°	0.33*	0.53 a	0.13 a	0.24 <sup>ab</sup>	0.45 a	0.49 a	0.18 <sup>a</sup>	0.27 <sup>ac</sup>
Whole plants	23.88	18.73	50.48	54.66	55.36	42.44	6.82	6.51	3.77	4.11	3.28	2.71	1.15	1.52
(G5)	±	±	±	±	±	±	± 0.41a	±	±	±	±	±	±	±
(03)	2.85a	2.46 <sup>c</sup>	2.30 <sup>a</sup>	2.45 <sup>b</sup>	3.7 <sup>b</sup>	4.38	0.41	0.40 a	0.22 a	0.18 <sup>b</sup>	0.38 a	0.51 <sup>a</sup>	0.27ª	0.33ac
Seeds	24.71	21.06	50.08	52.02	58.97	45.16	6.91	6.19	3.82	4.11±	3.46	2.08	1.10	1.97
(G6)	± ±	±	± 1	±	±	±	±	±	±	a ach	±	±	± 0.158	±
	2.09 <sup>ab</sup>	2.9°	2.21	0.96 <sup>b</sup>	3.216 <sup>a</sup>	0.93 <sup>a</sup>	0.81 a	0.98 a	0.19ª		0.44 <sup>ab</sup>		0.15 <sup>a</sup>	0.42 <sup>b</sup>
L.S.D	3.4		3.	291	6.842 1.044		0.421		0.351		0.4	0.415		

Each value represents the mean  $\pm$  SE. The mean values with different superscript alphabets indicate significant differences (P $\leq$ 0.05) using LSD test.

#### Antioxidant enzymes:

Aerobic organisms employ a battery of defense mechanisms such as antioxidant enzymes to prevent oxidative tissue damage (Halliwell and Gutteridge, 1989). Among biological tissue antoxidants mostly studied, is superoxide dismutase (SOD) that constitutes the first line of defenses against superoxide anion mediated injury. Superoxide dismutase (SOD) enzyme activity was estimated based on the generation of superoxide radicals produced by xanthine and xanthine oxidase, which react with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride (INT) to form a red formazan dye (Sarban et al., 2005). Glutathione-S-transferases (GST) are a major contributor to the eukaryotic cell's defences against chemical and oxidative stress (Wallig et al., 1998 and Kazi and Ellis, 2002). Tissue-specific expression of the glutathione S- transferase subunits may occur at or prior to the level of RNA processing and pretranslationally (Tu et al, 1983 and Lai et al, 1984).

Data in Table (6) clearly show superoxide dismutase (SOD) and glutathione-S-transferases (GST) activities of rats fed on red radish. It could be observed that significant ( $p \le 0.05$ ) differences between the tested rat groups in SOD and GST activities. SOD activity ranged from  $73.11\pm2.76$  to  $77.76\pm2.24$  (U/ml) at zero time. At the end of the experimental period, the positive control resulted an increase in SOD activity by about 39.3 % fold as that of zero time while the negative control showed a decrease in SOD activity by about 17.4% than that of zero time. Rat groups fed on tested red radish roots, leaves, and whole plant seeds resulted in an increase in SOD activity by about 50.35, 40.01, 16.01 and 22.00 % fold as that of the corresponding area at zero time, respectively.

It could be noticed that the red radish roots and leaves had the highest effect on SOD activity compared to positive control, whole plants and seeds. Additionally, gultathion-s-transferase showed an activity ranged from  $2048\pm99.2$  to  $2156.09\pm101.53$  (U/L) at zero time. At the end of the experimental period, the data revealed an increase in its activity by about 12.04, 12.41, 11.71, 12.47 and 12.89% fold as that of zero time for the positive control and rat groups fed on red radish roots, leaves, whole plant and seeds, respectively.

Table (6): Effect of feeding red radish on antioxidant enzymes.

Pat group	SOD	(U/ml)	Glutathion-s-transferase (U/L)			
Rat group	Zero End		Zero	End		
Positive control (G1)	77.76±2.24 <sup>a</sup>	108.31±2.72 <sup>a</sup>	2156.09±81.5 <sup>a</sup>	2596.06±136.3 <sup>ab</sup>		
Negative control (G2)	76.50±2.14 <sup>a</sup>	63.17±4.52	2066.06±103.7 <sup>b</sup>	2027.03±173.0		
Roots (G3)	73.11±2.76 <sup>b</sup> 109.92±6.47		2048.03±99.2 <sup>b</sup>	2541.04±152.0		
Leaves (G4)	$77.21\pm1.98^{a}$	$108.16\pm4.07^{a}$	2134.70±103.2 <sup>a</sup>	2499.9±149.6 <sup>ab</sup>		
Whole plants (G5)	$75.81\pm2.03^{a}$	87.95±5.27 <sup>b</sup>	2105.92±111.7 <sup>ab</sup>	2626.05±117.8 <sup>b</sup>		
Seeds (G6)	74.92±2.13 <sup>b</sup>	91.41±3.64 <sup>b</sup>	$2111.87\pm110.9^{a}$	2722.52±123.6 <sup>b</sup>		
L.S.D	17.	.513	231.532			

Each value represents the mean  $\pm$  SE. The mean values with different superscript alphabets indicate significant differences (P $\leq$ 0.05) using LSD test.

Negative control rat group had the lowest value of gultathions-transferase activity and it was decreased compared to the zero time. In this respect, various studies showed that the antioxidative activity or inhibition of free radicals is important in the protection against CCl<sub>4</sub>-induced liver lesions (Johnston and Kroening, 1998). Damage to liver cells causes leakage of cellular enzymes into serum. In the present study, treatment with red radish showed increased activity of antioxidant enzymes compared to CCl<sub>4</sub>- treated rats indicating the potentiality of red radish to act as an antioxidant by preventing the peroxidative damage caused by CCl<sub>4</sub> (Fraga *et al.*, 1987). Also, Wen *et al* (2006) found that the SOD activity was decreased form 82.2 u/ml at zero time to 58.4 U/ ml after 36 h of administered CCl<sub>4</sub>. Also, Lu *et al.* (2002) noticed that the gultathion-s-transferase activity was decreased in acute liver damage in rats given CCl<sub>4</sub>.

#### Lipid peroxidation (Malondialdehyde, MDA):

Lipid peroxidation is a crucial step in the pathogenesis of free radical-related diseases including inflammatory injury and hepatic dysfunctions (Datta *et al.*, 1998). MDA, the last product of lipid breakdown caused by oxidative stress, was evaluated by thiobarbituric acid reactive substances method (TBARS) (Draper and Hadley, 1990).

The level of serum MAD is shown in (Fig.1). It could noticed that no significant ( $p \le 0.05$ ) change of serum MAD in all tested treatments at the end of experimental period compared to zero time expect negative control rat group.

These results indicate that roots, leaves, whole plants and seeds of red radish maintained the level of serum MAD from the negative effect of CCl<sub>4</sub> and negative control rat group appeared significant ( $p \le 0.05$ ) increase in serum MDA. It has been shown that CCl<sub>4</sub> administration increased the lipid peroxidation in liver and microsomal (Nevin and Vijayammal, 2005 and Wen *et al*, 2006). Moreover, Aleynik *et al* (1997) showed that CCl<sub>4</sub> generates peroxidation of lipid membrane to the formation of free radicals. These radicals are capable of initiating a chain of lipid peroxidation reactions by abstracting hydrogen from polyunsaturated fatty acids (PUFA). Peroxidation of lipids, particularly the ones containing PUFA, can dramatically change properties of biological membranes, resulting in severe cell damage and could play a significant role in pathogenesis of diseases.

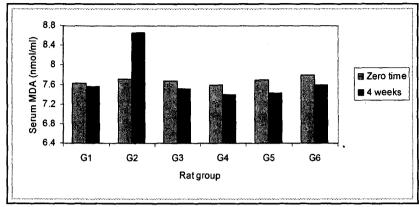


Fig (1): Levels of serum malondialdehyde (MDA) of rats fed on red radish

G2 = Negative control G5 = Whole plants

G3 = Roots G6 = Seeds

#### Serum lipid profile:

Table (7) shows the lipid profile, i.e. total lipids, triglycerides, total cholesterol, HDL-C and LDL-C of rats fed on red radish. It could be shown that no significant ( $p \le 0.05$ ) differences among the tested rat groups at zero time. The data reveal that total lipids ranged from  $0.363 \pm 0.036$  to  $0.497 \pm 0.028$  (mg/dl) at the end of the experimental period. Also, the rat group fed on red radish roots was the most effective treatment for the total lipids still constant. Meanwhile, all treatments were significantly ( $p \le 0.05$ ) increased total lipids.

From the above mentioned data, it could be concluded that red radish improved lipid profile especially the cholesterol components. The data are in parallel with those reported by Satoh *et al* (1993), Nishimura *et al* (2000), and Balasinka *et al* (2005). They indicated that radish lowered plasma cholesterol in mice fed on the cholesterol-enriched diet. It is conceivable that the cholesterol-lowering action of horseradish could be due to the interference with exogenous cholesterol absorption. Moreover, Wang *et al* (2002) showed that the effects of peroxidase on hyperlipidemia, mice fed on a diet high in cholesterol and fat by using different- purity peroxidase (radish juice, crude radish and horseradish peroxidase). They suggested that peroxidase may be a contributing factor in the prevention of hyperlipidemia.

Table (7) Effect of feeding red radish on the serum lipid profile of rats.

	Total lipids (g/dl)		Triglycerides (g/dl)		Total cholesterol(mg/dl)		HDL-C (mg/dl)		LDL-C (mg/dl)	
Rat group	Zero time	End of experiment	Zero time	End of experime nt	Zero time	End of experime nt	Zero time	End of experi ment	Zero Time	End of experime nt
Positive	0.363±	0.497±	87.37±	108.28±	217.0±	230.62±	62.83±	65.23±	154.16±	162.48±
control(G1)	$0.037^{a}$	0.028 <sup>b</sup>	7.79	2.97 <sup>b</sup>	15.68 <sup>a</sup>	8.15	$4.04^{a}$	4.47 <sup>a</sup>	12.54 <sup>a</sup>	4.4
Negative	0.353±	0.485±	96.99±	122.41±	218.54±	254.06±	62.92±	65.55±	155.61±	188.50±
control(G2)	$0.057^{a}$	0.091 <sup>b</sup>	1.29 <sup>a</sup>	3.93 <sup>d</sup>	19.05 <sup>a</sup>	7.10	$6.06^{a}$	6.04 <sup>a</sup>	23.3 <sup>a</sup>	9.4
Roots (G3)	0.361±	0.363±	97.56±	119.63±	217.91±	208.87±	61.70±	67.67±	156,20±	141.2±
Koots (G5)	$0.036^{a}$	0.035 <sup>a</sup>	5.97 <sup>a</sup>	8.30 <sup>cd</sup>	17.40 <sup>a</sup>	224 <sup>a</sup>	4.5 <sup>a</sup>	1.4 <sup>ab</sup>	12.8 <sup>a</sup>	3.6 <sup>b</sup>
Leaves (G4)	$0.373 \pm$	0.407±	98.16±	107.09±	219.16±	171.53±	65.06±	73.07±	154.09±	99.89±
Leaves (04)	0.012 <sup>a</sup>	$0.057^{a}$	6.47 <sup>a</sup>	5.25 <sup>b</sup>	17.97 <sup>a</sup>	14.13 <sup>b</sup>	1.6ª	2.07	18.9 <sup>a</sup>	16.5
Whole plants	0.35±	0.437±	98.20±	115.95±	217.69±	209.81±	63.2±	70.73±	154.48±	138.93±
(G5)	$0.063^{a}$	0.035 <sup>ac</sup>	3.19 <sup>a</sup>	6.99 <sup>e</sup>	14.26 <sup>a</sup>	2.10 <sup>a</sup>	3.7 <sup>a</sup>	5.1 <sup>b</sup>	18.01 <sup>a</sup>	6.3 <sup>b</sup>
Soods (CG)	0.367±	0.457±	89.66±	113.15±	216.90±	177.72±	63.95±	70.13±	152.94±	141.05±
Seeds (G6)	0.028 <sup>a</sup>	$0.032^{ac}$	2.41 <sup>a</sup>	2.31°	16.19 <sup>a</sup>	9.26 <sup>b</sup>	5.03 <sup>a</sup>	6.9 <sup>a</sup>	11.9 <sup>a</sup>	9.8 <sup>b</sup>
L.S.D	0.0614		8.9584		24.215		7.45		20.449	

Each value represents the mean  $\pm$  SE. The mean values with different superscript alphabets indicate significant differences (P $\leq$ 0.05) using LSD test.

#### Histopathological examination:

Histopathological examination of positive control liver revealed normal histological structure (Fig. 2). The hepatocytes appeared within normal histological structure characterized by central basophilic nucleus and eosinophilic cytoplasm. Meanwhile, liver of CCL<sub>4</sub> exposed group (negative control) showed necrobiotic changes of hepatocytes including vacuolar degeneration, nuclear pyknosis and necrosis. Some hepatocytes showed fatty degeneration characterized by intracellular fat globules occupied most of the cell with eccentric nucleus (Fig. 3). Liver of rats fed on roots of red radish showed ballooning degeneration of hepatocytes with narrowing of its hepatic sinusoids. Some hepatocytes showed fatty degeneration characterized by fat droplet and / or globules occupied most of the cells. Single cell necrosis was also noticed (Fig. 4). Liver of rat fed on leaves of red radish showed mild swelling of hepatocytes. Hyperplasis of kupffer cells with few numbers of mononuclear cells infiltration were seen (Fig. 5). Additionally, liver of rat fed on whole plants showed degenerative changes in the form of vacuolation and fatty degeneration of hepatocytes. Disorganization of hepatic cords with mononuclear cells infiltration was also noticed (Fig. 6). Liver of rat fed on seeds of red radish showed the portal triads and the surrounding hepatocytes showed normal histological structure in comparisons with rat control group (Fig. 7). The hepatic injury appeared as swelling of hepatocytes. It could be noticed that feeding on red radish plants, roots, leaves and seeds prevented CCl4 induced liver damage and slightly repair hepatocytes.

Generally, in the liver it has been shown that toxicity of CCl<sub>4</sub> is mediated by the CytP450 dependent mixed oxidase-mediated biotransformation product, trichloromethyl free radical (CCl<sub>3</sub>) and subsequent derivative Cl<sub>3</sub>COO. These free radical combine with the cellular lipids and proteins to produce lipid peroxidation, measured through its catabolite, malondyaldehyde (MDA), resulting in structural changes of metabolic activity leading to liver damage (Lim et al., 2000 and Kadiiska et al., 2000). Here the study clearly demonstrate that the red radish ameliorated the deleterious effects of CCl<sub>4</sub> on lipid peroxidation in whole liver and microsomal fraction by acting as an antioxidant.



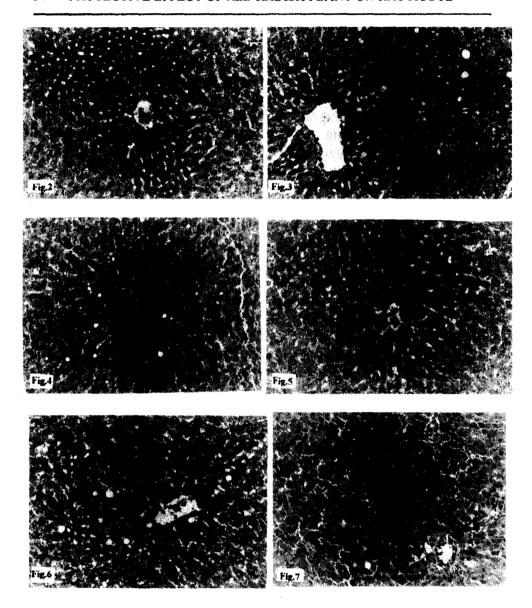


Fig. (2-7): Histopathological examination of liver rats fed different parts of red radish

Fig. 2 = Positive control, G1

Fig. 3 = Negative control, G2

Fig. 4 = Roots, G3

Fig. 5 = Leaves, G4

Fig. 6 =Whole plants, G5

Fig. 7 =Seeds, G6

On the other hand, (Lin et al, 1998 and Weng et al, 2006) reported that normal rats received to administer CCl<sub>4</sub> showed abnormal histological change started to appear at 3 h, and classic damages of liver cells were evident at 24 h, demonstrated by severe hepatocyte necrosis or apoptosis, inflammatory cells infiltration, fatty degeneration, hemorrhage, and hydropic degeneration, etc. It can be concluded that the significant hepatoprotective and antioxidant effect exhibited by red radish may be due to the presence of alkaloids and its inhibitory effect on microsomal Cyt P450 enzyme or on lipid peroxidation. Red radish may be interfering with the Cyt P450 and ultimately hindering the bio transformation of CCl<sub>4</sub>. Red radish may also possesses antioxidant activity which inhibited the deleterious effect of free radicals generated by CCl<sub>4</sub> influencing the membrane rigidity by preventing/inhibiting the membrane peroxidation. Further studies are undergoing on red radish and structurally identify the active component/components of medicinal importance.

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## التاثير الواقى لنبات الفجل الأحمر على كبد الفنران داليا محمد النحال — سحر أحمد عرفه — أنصاف محمد خليل مركز البحوث الزراعيه- معهد بحوث تكنولوجيا الاغذيه- قسم الاغذيه الخاصه والتغذيه

الهدف الرئيسي من هذه الدراسه هو تقييم أثر نبات الفجل الأحمر و بذوره على الكبد المصاب تم ذلك عن طريق حقن فئران التجارب برابع كلوريد الكربون الحدات أصابه حاده للكبد. بعد الأصبابه أستخدم جذور و أوراق و النبات الكامل و البذور للفجل الأحمر لتغذيه فنران التجارب كلا على حدى. تم دراسه الركيب الكيمائي لاجزاء النبات. :ما تم در اسه مدى تأثير المعاملات المختلفه على التغيير في و زن الجسم و مجموع الغذاء المتناول و نسب وزن الأعضاء الداخليه و التقييم البيوكيمائي [ وظائف الكبد - نشاط أنزيمات الأكسدة (السوبر أوكسيد ديسميتيز - جلوتاثيون-أس-ترانز فيراز) - معدلات أكسده الليبيدات malondialdehyde, MDA – الليبيدات الكليه ] في سيرم الدم و دلك في بدايه التجربه و في نهايه مده التجريه. أوضحت النتائج أختلافًا معنوياً في وزن الجسم - نسب وزن الأعضاء في مجاميع الفئران المختبرة بالمقارنه بالموجبه النضابطه الموجبه. التقييم alanine aminotransferase (ALT). البيوكيمائي أظهر أنخفاض معنوي في نشاط aspartate aminotransferase (AST) and alkaline phosphatase (ALP) للمجاميع المغذاه على جذور و أوراق و النبات الكامل و البذور للفجل الأحمر. جذور و أوراق الفجل الأحمر هما الأعلى في نشاط السوبر أوكسيد ديسميتين و وجود أختلافات معنويه بين المجاميع المختبره في نشاط جلوتاثيون-أس- ترانز فيراز و لا يوجد أختلافات معنويه في معدلات الاكسده للبيدات نتائج تقديرات الليبيدات الكليه والكولستيرول الكلي والجلسريدات الثلاثية والكولستيرول عالى ومنخفض الكثافة في السيرم أظهرت أختلافات بين المجاميع المختبره. أعلى انخفاض في الكولستيرول الكلي ظهر في مجاميع الفئران المغذاه على الأوراق وثم البذور ثم الجذور ثم النبات الكامل مقارنته بالمجموعة الضابطة الموجبه و السالبه. أظهرت الدراسه الهستوبولوجيه أن الأجزاء المختلفه من الفجل الأحمر كان لها تأثير متثبط للأصابه الحادثه برابع كلوريد الكربون و أن له تأثير ايجابي على خلايا