The Bioactive Effect of Round Purple Turnip Roots against Carbon Tetrachloride-Induced Hepatotoxicity in Rats

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ABSTRACT

Many of the ordinarily used medications may cause liver injury as an adverse reaction that is unpredictable and produce a significant possibly serious acute and chronic liver injury. This study was carried out to investigate the bioactive effects of round purple turnip roots (RPTRs) against CCl_4-induced hepatotoxicity in rats. Thirty-five albino rats of Sprague-Dawley strain were randomly distributed into five groups, group 1 (negative control, 7 rats) was feeding on the basal diet. The rest groups (2, 3, 4 and 5) were intraperitoneal injected once a week for six weeks with CCl_4 to induce acute the liver toxicity. Group 2 was used as positive group and feed on the basal diet, while groups 3, 4 and 5 feed in the complementary diet with 5, 7.5 and 10% of RPTRs, respectively. The outcome commented that untreated hepatotoxicity-rats possess a significant ($P<0.05$) diminishing in FI, BWG, and BWG %, and serum TP and Alb levels, and activity of CAT, SOD, and GSH. While, the serum levels of UN, Cr, UA, TL, TG and TC, and activity of antioxidant and liver enzymes were increased, compared to the negative control group. Additionally, several degradation were marked in the hepatic lobules, congestion in both the blood sinusoids and central veins, ragmented nuclei hepatic cells and the presence of inflammatory cell infiltration and a dilated congested portal vein in positive group. In comparison to the positive group, the complemented diet with the three different levels of RPTRs caused a significant ameliorated in all of the biological and biochemical tested parameters, as well histological structure of liver, on dose dependent manner. Finally, this study concluded that RPTRs have a significant beneficial health effects that caused ameliorated on the functions of liver injury that may attribute to its bioactive compounds.

Keywords: Round Purple Turnip; Liver Toxicity; Lipid Peroxidation

1- INTRODUCTION

Liver is the largest and one of the most vital organs of the body which performs a major role in the metabolism of major nutrients (carbohydrates, proteins, and lipids). It also has a wide range of functions including detoxification, storage of glycogen, and the production of multiple coagulation factors and growth factors, hormones (**Abhilash *et al.,* 2014**). Liver disease accounts for about 2 million deaths per year globally (1 million due to complications of cirrhosis, and 1 million due to viral hepatitis and hepatocellular cancer). Cirrhosis is presently the 11th most common induce of death worldwide and liver cancer is the 16th leading cause of death; combined, they account for 3.5% of all deaths globally (**Asrani *et al*., 2018**).

Liver diseases are primarily caused by harmful chemicals, excess intake of alcohol, infections, and autoimmune disease. If the ability of the liver is not satisfactory to regenerate, or if damage to the liver is very serious, liver diseases may progress to liver failure and death (**Bischoff *et al.,* 2018**). Hepatotoxicity in most cases is owed to free radicals that are produced from a large number of biochemical processes, which constitute an essential component of aerobic and metabolism processes (**Abd El-Ghani and Nanees, 2010**).

In the experimental animals to induce liver injury, carbon tetrachloride (CCl_4) is a cooperative primary compound utilized for this purpose (**Kim *et al*., 2010**). Although the liver is not the only intended organ of CCl_4, it also affects other organs such as the kidneys, heart, and testicles (**Ozturk *et al*., 2003**). On the other hand, dietary antioxidants and anti-inflammatory components play an essential role in suppressive CCl_4 intoxication by scavenging active oxygen and free radicals, safely neutralizing lipid peroxides, and strengthening the natural cellular antioxidant action (**Unsal *et al*., 2021**).

Nowadays, the application of drugs in liver disorder treatment is at times costly, unsuitable, and can have important detrimental effects. Due to these important problems, it is required to investigate the natural local

substances that have been exploited before in popular medicine. Vegetables in the *Brassicaceae* family are considered part of the human diet, and consumed by nearly the whole world population (**Baenas *et al*., 2012**). The turnip one of the oldest vegetables and is widely consumed in most Asian countries. Round purple turnip roots (*Brassica rapa* subsp. *Rapa*.) are a yearly important plant belonging to the Brassicaceae family and native to the Near East, Central Asia, Europe, and Russia (**Sun, 2015**). Round purple turnip roots are rich source of flavonoids, phenolic compounds, sulfur compounds, glucosinolates, organic acids, and various volatile compounds that were discovered to have various beneficial health effects (**Paul *et al*., 2019**). In addition, turnips have several biological activities, as antimicrobial, antioxidant, anticancer, and antidiabetic as well as, the prevention of cerebral hypoxia and pulmonary edema (**Yuliya *et al*., 2023**). Therefore, the present study was conducted to investigate the bioactive effect of round, purple turnip roots on some biochemical parameters and liver histology assay in carbon tetrachloride-induced hepatotoxicity rats.

2- MATERIALS AND METHODS

2.1- Materials

2.1.1. **Plant Materials:** Fresh round purple turnip roots (RPTRs) were obtained in winter 2023 from agricultural fields in The Qalyubia Governorate, Egypt and was identified in National Center for Agricultural Research, Cairo, Egypt.

2.1.2. Rats: Thirty-five adult male rates (Sprague Dawley Strain), weighing about 200±5 g were purchased from Helwan farm of experimental animals, Ministry of Health and Population, Helwan, Cairo, Egypt.

2.1.3. Basal Diet Constituents: All the ingredient nutrients needed for the preparation of the basal diet (AIN 93-M) to the meet nutritional requirements of rats were purchased from the El-Gomhorya Company for

Trading Drugs and Chemicals, Cairo, Egypt. Sucrose, soybean oil and starch were purchased from the local market.

2.1.4. Chemicals: Kits for biochemical analyses were purchased from the Gamma Trade Co., for Pharmaceutical and Chemical, Dokki, Egypt. Carbon tetrachloride (CCL_4), diethyl ether and other chemicals used in this study were acquired from the El-Gomhoriya Company for Trading Drugs and Chemicals, Cairo, Egypt.

2.2. Methods

2.2.1. Preparation of Round Purple Turnip Roots (RPTRs): Fresh turnip roots were cleaned from dust and removed all invalid parts, green leaves and cut into slices about 1/2 cm thick. Then, sliced turnip roots will be dried using an oven under vacuum at 50-55 °C. A grinder mill and sieves were used to obtain a powder particle size of less than 0.4mm. The final powder was packaged and stored until further use.

2.2.2. Preparation of the Basal Diet: Diet (AIN-93M) was ready as depicted by **Reeves *et al.*, (1993)** to meet the reasonable suggested supplements levels of nutrients for keeping up with wellbeing rats as shown in **Table 1**.

Table 1: Components of Basal diet (AIN-93M)

Components	**Amount (g/kg)**
Casein (>85% protein)	140.00
Corn starch	465.692
Dextrinized cornstarch (90–94% tetra-saccharides)	155
Sucrose	100.00
Soybean oil	40.000
Fibers	50.000
Mineral mix.	35.000
Vitamin mix.	10.000
L-Cystine	1·800
Choline bitartrate	2.500
Tert-Butylhydroquinone	0.008

2.2.3. Induction of Hepatotoxicity: In this study, hepatic toxicity in all rats, except normal rats (7 rats) was induced by the intraperitoneal injection (IP) once a week for six weeks with 1 ml/kg b. wt of CCl_4 dissolved in paraffin oil in a 1:1 portion (v:v) as documented by **Karthikeyan and Deepa, (2010).**

2.2.4. Experimental Design: Firstly, all rats were housed in well-aerated cages for one week for adaptation in the animal house, Faculty of Home Economics, Helwan University under controlled environmental conditions of the light/dark cycle (12/12 hr), temperature (22±4°C) and relative humidity (45% to 50%). The supply of food and water was uninterrupted during the experimental period. Subsequently, rats were randomized into five groups, each with seven rats as follows.

- **Group (1):** rats were kept as healthy negative control group (-ve group) and fed on the basal diet.
- **Group (2):** rats were IP injected with 1 ml/kg b. wt. of CCl_4 once a week for six weeks, maintained as a positive control group, and fed on the normal basal diet.
- **Group (3):** rats were IP injected with 1 ml/kg b. wt. of CCl_4 once a week for six weeks, and fed on the supplemented diet with 5% of dried round purple turnip roots (RPTRs).
- **Group (4):** rats were IP injected with 1 ml/kg b. wt. of CCl_4 once a week for six weeks, and fed on the supplemented diet with 7.5% of RPTRs.
- **Group (5):** rats were IP injected with 1 ml/kg b. wt. of CCl_4 once a week for six weeks, and fed on the supplemented diet with 10% of RPTRs.

2.2.5. Biological Evaluation: Fedd intake (FI) was calculated every day during the experimental period (6 weeks). The changes in body weight were determined by weighing rats at the initial of experiment (IBW) and at the end of the experimental period (FBW). Then, the body weight gain (BWG) and the relative body weight gain (BGW %) were calculated **Kratochvílova *et al.,* (2002).**

BWG = FBW - IBW

Change of body weight gain % = BWG/IBW X 100

2.2.6. Collection of Blood Samples: At the end of the experimental period (6 weeks), all rats were fasted for 12 hours, anesthetized with diethyl ether. Portal vein blood samples were collected in clean, dry centrifuge tubes and left to coagulate at room temperature. The clotted blood was centrifuged at 3000 rpm for 15 minutes to obtain serum. Then, clear serum samples were taken into the Eppendorf's tubes (1.5 mL) and stored at -20°C until they were used in biochemical analysis.

2.2.7. Biochemical Assay

4.2.7.1. Determination of Liver Functions: The serum activities of aspirate amino transaminase (AST) and alanine amino transaminase (ALT) were measured calorimetrically measure at 505 nm according to the method described by **(Reitman and Frankel, 1957)**. Enzymatic colorimetric determination of alkaline phosphatase (ALP) was carried out according to (**Bergmeyer and Brent, 1974)** using a spectrophotometer (Hum star 200, automatic biochemistry analyzer, Germany) adjusted at 510 nm.

Serum concentrations of total protein (TP) and albumin (Alb were measured colorimetrically using a spectrophotometer (Hum star 200, automatic biochemistry analyzer, Germany) as described by **Tietz (1994) and Young (2000**), respectively. The device was regulated at 545 and 628nm, respectively for measuring the color intensity that reflect the serum concentration of the tested parameters.

2.2.7.2. Determination of Kidney Functions: Quantitative-based colorimetric (ELISA Kits) assay was used for the measurements of serum levels of urea nitrogen (UN), creatinine (Cr), and uric acid (UA). The absorbance of the colored solutions was recorded by using a spectrophotometer (Hum star 200, automatic biochemistry analyzer, Germany) adjusted at 540, 530 and 750 nm, respectively as described by

Patton and Crouch, (1977), Friedman and Young, (1997) and Fossati *et al.*, (1980).

2.2.7.3. Determination of Serum TL, TG and TC Levels: Serum levels of total lipid (TL), triglycerides (TG) and total cholesterol (TC) were estimated using commercial reagent kits (Biomed diagnosis, Egypt) as described by **Hostmark *et al.*, (1991), Fassati and Prenciple (1982)** respectively.

2.2.7.4. Determination of Serum Oxidative Stress Marker Malondialdehyde (MDA) level was assayed quantitatively in serum as an oxidative stress marker by the MDA assay kit (ABCAM, UK). In this procedure, the MDA in the sample reacts with thiobarbituric acid (TBA) to generate an MDA-TBA adduct. The MDA-TBA adduct is quantified colorimetrically (OD = 532 nm) **(Ohkawa et al., 1979).**

2.2.7.5. Determination of Serum Activities of Antioxidant Enzymes: The serum activity of catalase (CAT), superoxide dismutase (SOD), and glutathione (GSH) enzymes was determined using commercial assaying kits (Cayman Practice ELISA Kits). The process that is used for the assessment of CAT activity was depended on the reaction of the enzyme with methanol in the presence of an optimum concentration of H2O2. The formaldehyde produced is measured spectrophotometrically at 540 nm as described by (**Wheeler *et al*., 1990**). To assay the activity of SOD, the principle technique used was enzyme-linked immunosorbent assay double-antibody. The color change was measured spectrophotometrically at 450 nm as described by (**Wheeler *et al*., 1990**). The serum activity of GSH was assayed according to the kit's instructions as described by **Ceballos-Picot *et al*., (1992)** using spectrophotometrically at 340nm.

2.2.8. Histopathological Examination: The liver and kidney were carefully removed from each rat following dissection, washed with normal saline for blood removal, and immersed in neutral formaldehyde (10%). Then, the submerged samples in the formaldehyde were removed,

cleaned, washed, and dehydrated in ascending-grade alcohols. Afterward, specimens were cleared in Xylol, fixed and deeply in paraffin mass, sectioned to 4-6 microns in thickness, and stained with the Hematoxylins and Eosin stain for examination as described by (**Bancroft and Gamble, 2002**).

2.2.9. Statistical Analysis: Data was evaluated statistically using computerized SPSS package program (SPSS 22.00 software for Windows) by one-way analysis of variance (ANOVA). To compare the means of the two bread samples (control and fortified bread) a t test was used. The obtained data was expressed as Mean ± SD and the significant difference among means was estimated at $p<0.05$ (**Snedecor and Cochran 1980)**.

3- RESULTS

3.1. The Effect of Supplemented Diet with Round Purple Turnips Roots on FI, IBW, FBW, BWG and BWG % in Rats with Hepatotoxicity: The effect of feeding hepatotoxicity-rats on the supplemented-diet with round purple turnip roots (RPTRs) on feed intake (FI), initial body weight (IBW), final body weight (FBW), body weight gain (BWG), and change in body weight gain (BWG %) is recorded in **Table 2**. Results showed that the positive control group (untreated hepatotoxicity-rats) had a significant ($P<0.05$) reduction in the amount of FI, IBW, FBW, BWG, and BWG %, compared to the negative control group (normal rats). Feeding hepatotoxicity-rats on a supplemented-diet with RPTRs at levels of 5% and 7.5% of RPTRs caused non-significant change in FI, while the supplemented-diet with 10% of RPTRs caused significant increase, compared to the positive control group.

Whereas, hepatotoxicity-rats fed on the supplemented diet with RPTRs at the three different levels (5, 7.5, and 10%) combined with IP injection by CCl_4 have a significant ($P<0.05$) increase in FBW, BWG, and BWG

(%) in comparison to injecting rats by CCl_4 alone. The superior results in FI, FBW, BWG, and BWG (%) were established in treated groups with 7.5 and 10% of RPTRs.

Table (2): The Effect of Supplemented-Diet with RPTRs on FI, IBW, FBW, BWG and BWG% in Rats with Hepatotoxicity

Parameters / Groups		Parameter as Mean ± SD				
		FI (g)	**IBW (g)**	**FBW (g)**	**BWG (g)**	**BWG (%)**
Negative group		15.14±0.90^{a}	202.42±1.14	300.20±2.12^{a}	97.78±1.12^{a}	48.31±1.09^{a}
Positive group		12.14±0.69^{c}	203.12±1.15	250.70±2.15^{d}	47.58±1.14^{d}	23.42±1.06^{d}
Hepatotoxicity rats fed on the supplemented diet with RPTRs at levels of:	5%	12.14±0.69^{c}	203.02±1.19	284.70±2.10^{c}	81.68±1.19^{c}	40.23±1.10^{c}
	7.5%	13.00±0.82^{bc}	203.02±1.20	294.20±3.15^{b}	91.18± 1.15^{b}	44.91±1.05^{b}
	10%	13.29±0.76^{b}	203.16±1.15	294.50±3.11^{b}	91.34±1.14^{b}	44.96±1.10^{b}

Values expressed as means ± SD; Means with different letters in each column are significantly differs at p< 0.05. **RPTRs:** Round Purple Turnip Roots; **FI**: Food Intake; **IBW**: Initial Body Weight; **FBW**: Final Body Weight; **BWG**: Body Weight Gain

3-2- The Effect of Supplemented Diet with Round Purple Turnips Roots on the Activity of AST, ALT and ALP Enzymes in Rats with Hepatotoxicity:

The recorded results in **Table 3**, explained that untreated hepatotoxic - rats (positive control group) have a significant (P<0.05) increase in the serum activity of aspartate aminotransferase (AST), Alanine aminotransferase (ALT) and alkaline phosphatase (ALP) enzymes, compared to normal rats. In comparison to the untreated hepatotoxic - rats fed on the basal diet, the results showed that the treated hepatotoxic - rats with feeding on the supplemented diet with 5, 7.5 and 10% of RPTRs have a significant (P<0.05) reductions in the serum activity of AST, ALT and ALP enzymes. The best enhanced results were reported in treated hepatotoxic - rats with increasing levels (10%) of RPTRs.

Table (3): The Effect of Supplemented Diet with RPTRs on the Activity of AST, ALT and ALP Enzymes in Rats with Hepatotoxicity

Parameters / Groups		Parameter as Mean ± SD		
		AST (µ/L)	ALT (µ/L)	ALP (µ/L)
Negative group		19.57±1.81^{e}	19.14±0.69^{e}	582.57±0.53^{d}
Positive group		57.57±1.72^{a}	39.86±1.57^{a}	733.29±1.11^{a}
Hepatotoxicity rats fed on the supplemented diet with RPTRs at levels of:	5%	51.14±0.69^{b}	32.86±1.57^{b}	678.14±1.21^{b}
	7.5%	46.00±1.15^{c}	24.43±1.72^{c}	638.85±1.68^{c}
	10%	23.86±1.35^{d}	21.86±0.90^{d}	580.86±0.89^{e}

Values expressed as means ± SD; Means with different letters in each column are significantly differs at p< 0.05. **RPTRs**: Round Purple Turnip Roots**; AST**: Aspartate transaminase; **ALT**: Alanine transaminase; **ALP**: Alkaline phosphatase.

3-3- The Effect of Supplemented Diet with Round Purple Turnips Roots on the Serum Concentrations of TP and Alb in Rats with Hepatotoxicity:

As shown in **Table 4**, the serum concentration of total Protein (TP) and Albumen (Alb) were reduced significantly (P<0.05) in hepatotoxic-rats fed on a basal diet alone, compared with normal rats fed on the basal diet. On the other hand, feeding hepatotoxic - rats on a supplemented diet with RPTRs at the three different levels (5, 7.5 and 10%) ameliorates serum levels of TP and Alb, as compared with that untreated hepatotoxic - rats fed on the basal diet alone. In addition, the results showed that the supplemented diet with 10% of RPTRs increases the improvement rate of serum concentration of the above parameters.

3-4- The Effect of Supplemented Diet with Round Purple Turnips Roots on the Serum Concentrations of UN, Cr and UA in Rats with Hepatotoxicity:

Results in **Table 5** outline the effect of the supplemented diet with RPTRs on the serum concentrations of urea nitrogen (UN), creatinine (Cr) and uric acid (UA) in hepatotoxicity rats. The recorded results proved a significant (p<0.05) rise in serum UN, Cr and UA

concentrations in rats treated with CCl_4 and fed basal diet alone (positive control group), compared to normal rats. Even so, the results performed a significant decrease in serum UN, Cr and UA concentrations of the CCl4-treated rats fed on the supplemented diet with the different levels of RPTRs by combining, compared to the treated rats with CCl4 fed on the basal diet alone. The most change for the better in serum levels of UN, Cr and UA was shown in the treated groups with increasing supplementation diet levels with RPTRs.

Table (4): The Effect of Supplemented Diet with RPTRs on the Serum Concentrations of TP and Alb in Rats with Hepatotoxicity

Parameters / Groups		Parameter as Mean ± SD	
		TP (gm/dl)	Alb (gm/dl)
Negative group		9.64± 0.11^{a}	4.01±0.09^{a}
Positive group		6.27± 0.15^{e}	3.23±0.30^{c}
Hepatotoxicity rats fed on the supplemented diet with RPTRs at levels of:	5%	6.84±0.10^{d}	3.53±0.27^{b}
	7.5%	7.19±0.24^{c}	3.84±0.13^{a}
	10%	8.67 ±0.25^{b}	3.91±0.09^{a}

Values expressed as means ± SD; Means with different letters in each column are significantly differs at p< 0.05. **RPTRs**: Round Purple Turnip Roots**; TP:** Total protein; **Alb:** Albumin

Table (5): The Effect of Supplemented Diet with RPTRs on the Serum Concentrations of UN, Cr and UA in Rats with Hepatotoxicity:

Parameters / Groups		Parameter as Mean ± SD		
		UN (mg/dl)	Cr (mg/dl)	UA (mg/dl)
Negative group		24.43±1.13^{d}	0.56± 0.05^{c}	3.03± 0.05^{d}
Positive group		36.29±1.25^{a}	0.96± 0.05^{a}	4.21± 0.15^{a}
Hepatotoxicity rats fed on the supplemented diet with RPTRs at levels of:	5%	28.71±1.25^{b}	0.79± 0.07^{b}	3.70± 0.13^{b}
	7.5%	27.29±1.80^{c}	0.64± 0.05^{c}	3.26±0.16 ^{c}
	10%	25.71± 0.76^{d}	0.56± 0.05^{d}	3.19± 0.12^{c}

Values expressed as means ± SD; Means with different letters in each column are significantly differs at p< 0.05. **RPTRs**: Round Purple Turnip Roots**; UN**: Urea Nitrogen; **Cr**: Creatinine; **UA**: Uric Acid

3-5- The Effect of Supplemented Diet with RPTRs on the Serum levels of TL, TG and TC in Rats with Hepatotoxicity:

Results in **Table 6**, make clear the effect of the supplemented diet with RPTRs on the serum levels of total lipids (TL), triglycerides (TG) and total cholesterol (TC) in hepatotoxic rats. In comparison to the negative control group, IP injection of CCl4 caused a significant ($P<0.05$) rise in serum concentrations of TL, TG and TC. Nevertheless, in comparison to the positive control group, feeding hepatotoxic-rats on the supplemented diet with the different levels (5, 7.5 and 10%) of RPTRs resulted in significantly diminished in serum levels of TL, TG and TC.
A superior enhancement in serum levels of TL, TG and TC was found out in treated hepatotoxic-rats with increasing the levels of RPTRs**.**

Table (6): The Effect of Supplemented Diet with RPTRs on the Serum levels of TL, TG and TC in Rats with Hepatotoxicity

Parameters / Groups		Parameter as Mean ± SD		
		TL (mg/dl)	TG (mg/dl)	TC (mg/dl)
Negative group		482.00±0.82^{e}	89.14±1.07^{e}	115.00±1.29^{e}
Positive group		714.71±0.76^{a}	236.29±1.11^{a}	253.14±1.35^{a}
Hepatotoxicity rats fed on the supplemented diet with RPTRs at levels of:	5%	668.71± 1.98^{b}	117.14±0.69^{b}	161.57±1.51^{b}
	7.5%	544.57± 1.51^{c}	103.29±1.80^{c}	144.00±0.82^{c}
	10%	486.71±0.76^{d}	93.14±1.35^{d}	126.57±1.27^{d}

Values expressed as means ± SD; Means with different letters in each column are significantly differs at $p< 0.05$. **RPTRs**: Round Purple Turnip Roots**; TL**= Total Lipid; **TG** =Triglyceride; **TC**= Total cholesterol.

3-6- The Effect of Supplemented Diet with RPTRs the Serum Concentrations of MDA and the Activity of antioxidant enzymes in Rats with Hepatotoxicity:

Table 7 represents lipid peroxidation as stated by serum monoaldehyde **(**MDA) level and activity of catalase (CAT), superoxide dismutase (SOD), and glutathione (GSH) in normal rats, untreated hepatotoxic-rats and treated hepatotoxic-rats with 5, 7.5 and 10% of RPTRs. In comparison to the normal rats, administration of CCl_4 promotes a

significant (P<0.05) raise in serum MDA level and decline in the activity of tested antioxidant enzymes. In contrast, feeding hepatotoxic-rats on the accompanied diet with the different levels of RPTRs resulting significant enhancement in serum MDA levels and activities of CAT, SOD and GSH enzymes when compared to the positive control group fed on a normal basal diet alone. The best result in lower serum concentration of MDA and increasing activity of antioxidant enzymes was displayed in the treated hepatotoxic group with the augment the levels of RPTRs.

Table (7): The Effect of Supplemented Diet with RPTRs on the Serum Concentrations of MDA and the Activity of CAT, SOD and GSH Enzymes in Rats with Hepatotoxicity:

Parameters / Groups		Parameter as Mean ± SD			
		MDA (u/ml)	CAT (u/ml)	SOD (u/ml)	GSH (u/ml)
Negative group		84.00± 1.83^{e}	91.29±0 .76^{a}	991.29±1.98^{a}	4.87± 0.36^{a}
Positive group		461.71±1.50^{a}	37.86±1.57^{e}	466.00±2.31^{e}	1.84± 0.21^{e}
Hepatotoxicity rats fed on the supplemented diet with RPTRs at levels of:	5%	188.00±1.91^{b}	55.57±1.90^{d}	891.71±2.69^{d}	2.76± 0.25^{d}
	7.5%	144.86±1.86^{c}	74.43±0.79^{c}	927.00±2.94^{c}	3.20± 0.10^{c}
	10%	109.0± 1.91^{d}	83.71±1.38^{b}	984.71±2.56^{b}	4.58± 0.11^{b}

Values expressed as means ± SD; Means with different letters in each column are significantly differs at p< 0.05. **RPTRs**: Round Purple Turnip Roots**; MDA:** Malondialdehyde; **CAT:** Catalase; **SOD**: Superoxide Dismutase. **GSH:** Reduced Glutathione.

4-6- Histopathological Examinations:

4-6-1- Histopathological Examination of Kidneys: Light microscopic examination of kidneys of rats from group 1 (negative rats) showed normal histological architecture of renal parenchyma as shown in **Photo 1**. In contrast, kidney sections of rats from group 2 (positive rats) showed destruction and tubule injuries. Most of the tubules were dilated with the presence of cellular debris in their lumens (**Photo 2**), as well as the tubular cells were vacuolated cytoplasm and dark pyknotic nuclei (**Photo 3**). In addition, dilated and congested peritubular space (**Photo 4**), and mononuclear inflammatory cells (**Photo 5**). Moderate rate of small

glomeruli with wide capsular space, and large glomeruli with obliterated capsular space and degenerated membrane around Glomeruli were detected (**Photo 6**). On the other hand, kidneys from treated rats with CCl_4 + 5% of RPTRs (group 3) revealed restoration of normal renal architecture. The renal corpuscles and the renal tubules appeared nearly normal, there was a moderate level of inflammatory cell infiltration (**Photo 7**). The glomeruli, the proximal convoluted tubules, and the distal convoluted ones appeared nearly normal. Luminal casts inside the renal tubules were evident (triangle). Dilated and congested peritubular space (**Photo 8**). Meanwhile, kidneys from treated rats with CCl_4 + 7.5% of RPTRs (group 4) showed a histological profile of the renal cortex comparable to the negative control group. However, there were congestion, degenerated epithelial cells that lined renal tubules and minor mononuclear cell infiltration as shown in **Photo 9**. In addition, most glomeruli were in typical size and shape within the bowman's capsule with normal capsular space and the proximal convoluted tubules and the distal convoluted ones appeared almost normal (**Photo 10**). Otherwise, kidneys from treated rats with CCl_4 + 10% of RPTRs (group 5) exhibited no histopathological alterations except a mild level of congestion and deteriorated epithelial cells that lined the renal tubules (**Photo 11**). Also, the invasion of mononuclear cells was quite slow. Most glomeruli in the bowman's capsule was the usual size and shape, normal capsular space, and nearly normal-looking proximal and distal convoluted tubules (**photo 12**).

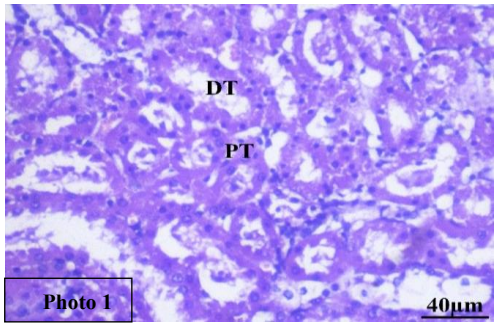
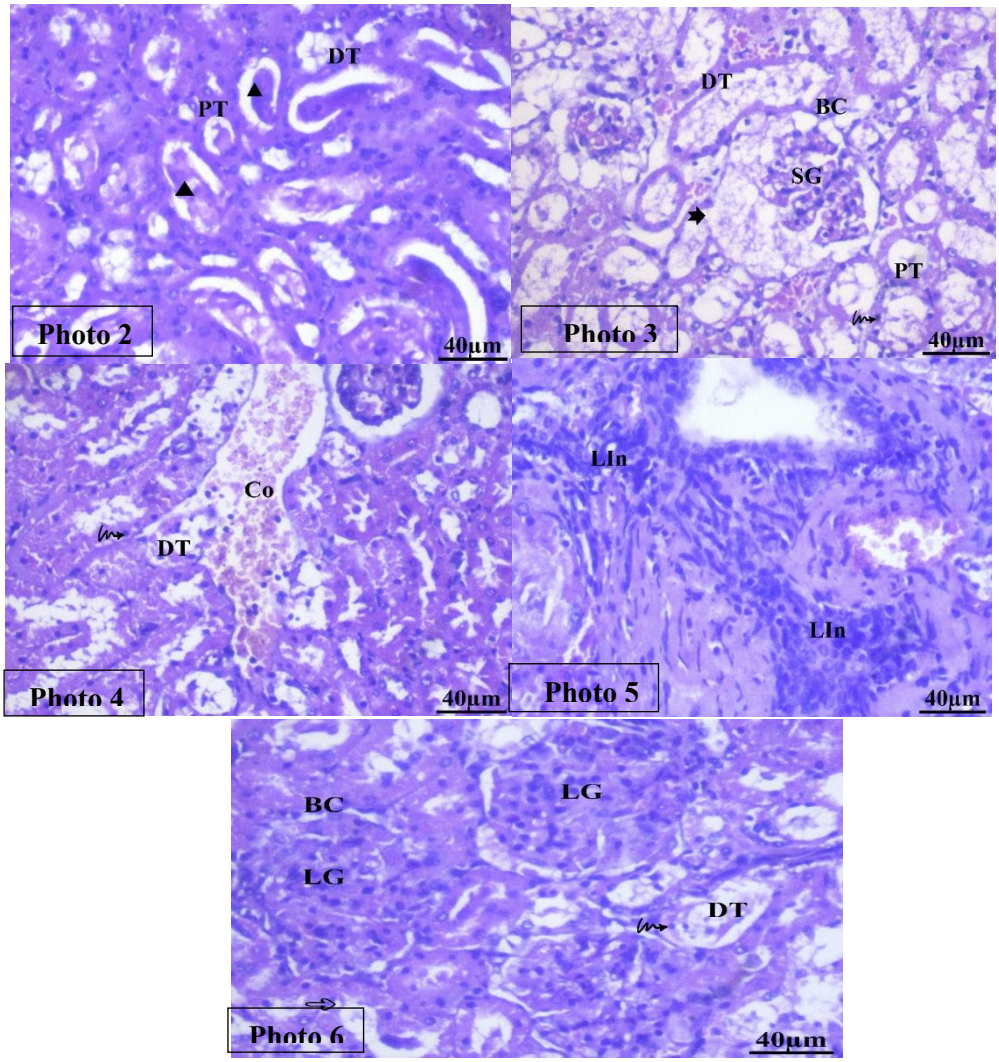
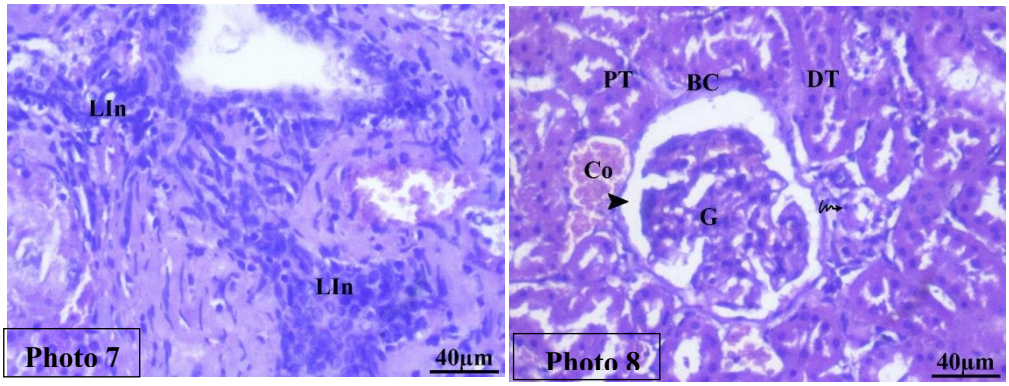


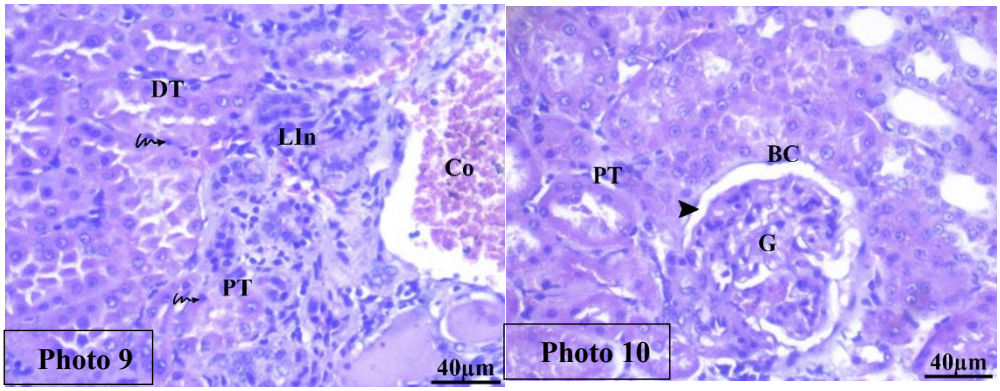
Photo (1): Photomicrograph of kidney sections from negative rats showing the normal histological architecture of renal parenchyma (H & E X 400).



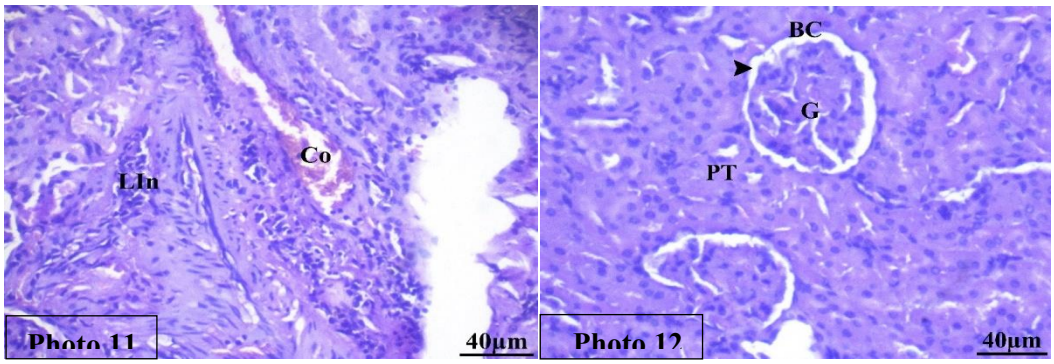
Photos (2- 6): Photomicrograph of kidney sections from positive rats showing destruction and exhibited tubule injuries. Most of the tubules were dilated with the presence of cellular debris in their lumens (triangle). Most of the tubular cells had vacuolated cytoplasm (bold arrow) and dark pyknotic nuclei (wavy arrow). Dilated and congested peritubular space (Co), and mononuclear inflammatory cells (LI) were detected. Moderate rate of small glomeruli (SG) with wide capsular space, and large glomeruli (LG) with obliterated capsular space. Degenerated membrane (notch arrow) around Glomeruli. (H & E X 400).



Photos (7 and 8): Photomicrograph of kidney sections from treated rats with CCl4 + 5% of RPTRs showing restoration of normal renal architecture. The renal corpuscles and the tubules appeared nearly normal, and moderate level of inflammatory cell infiltration (LI). The glomeruli (G), the proximal convoluted tubules (PT), and the distal convoluted ones (DT) appeared nearly normal. Luminal casts inside the renal tubules were evident (triangle). Dilated and congested peritubular space (Co) (H & E X 400).



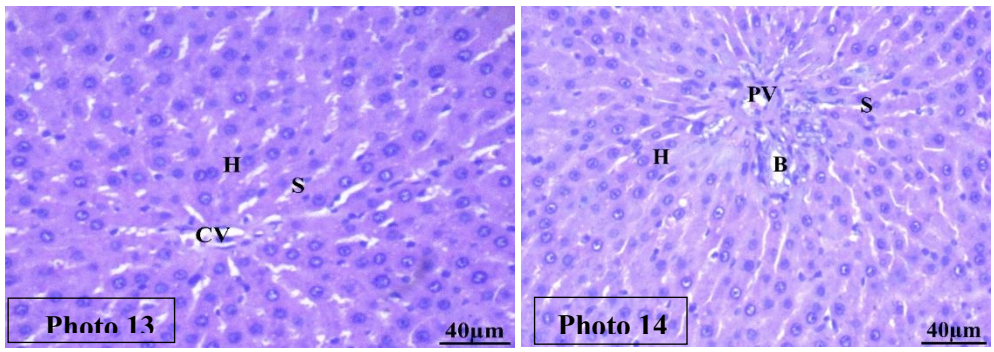
Photos (9 and 10): Photomicrograph of kidney sections from treated rats with CCl4 + 7.5% of RPTRs showing a histological profile of the renal cortex comparable to the negative control group. However, there were congestion (Co), degenerated epithelial cells that lined renal tubules (hollow arrow) and minor mononuclear cell infiltration (LI). Most of glomeruli (G) were in typical size and shape within the bowman's capsule (BC) with normal capsular space (arrowhead) and the proximal convoluted tubules (PT) and the distal convoluted ones (DT) appeared almost normal (H & E X 400).



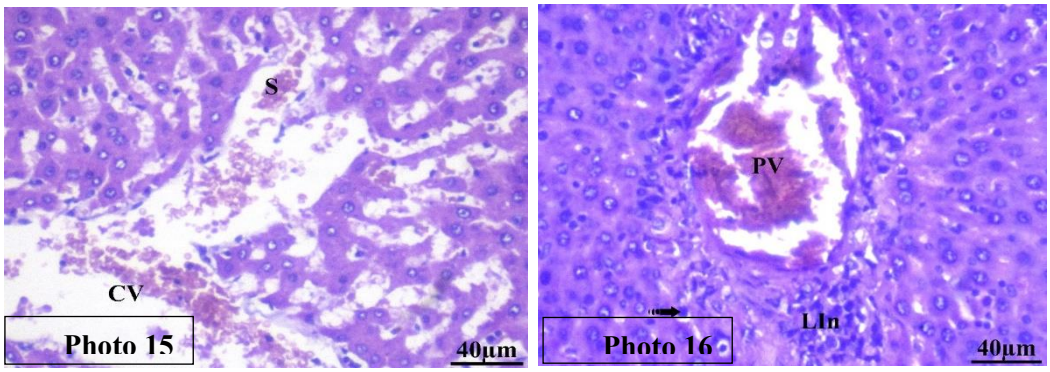
Photos (11 and 12): Photomicrograph of kidney sections from treated rats with CCl_4 10% of RPTRs showing no histopathological alterations except a mild level of congestion (Co) and deteriorated epithelial cells that lined the renal tubules. Also, the invasion of mononuclear cells was quite slow. Most glomeruli (G) in the bowman's capsule (BC) was the usual size and shape, normal capsular space, and nearly normal-looking proximal and distal convoluted tubules (H & E X 400).

4-6-2- Histopathological Examination of Liver: Microscopically, the liver of rats from group 1 (negative rats) revealed the hepatic tissue displayed the characteristic lobular architecture, consisting of hepatocytes arranged in cord-like structures that appeared to be branching and anastomosing. As well as, the hepatocyte cords were extending from the central vein located at the center, towards the portal tract areas at the periphery and the hepatocytes were a polygonal morphology, with acidophilic cytoplasm and centrally located spherical, vesicular nuclei as shown in **Photo 13**. In addition, the hepatic sinusoids in the interstitial spaces between the cords of hepatocytes, with the Kupffer cell, the bile duct and portal vein branches were observed to be situated within the portal tract sites (**Photo 14**). In contrast, as shown in **Photo 15**, the liver of rats from group 2 (positive rats) have marked the hepatic lobules exhibited dilation and congestion of both the blood sinusoids and central veins, which were shown to have a detached endothelial wall. In addition, certain hepatic cells have a ghost-like appearance characterized by fragmented nuclei, while others display dark pyknotic nuclei. Furthermore, the presence of inflammatory cell infiltration and the

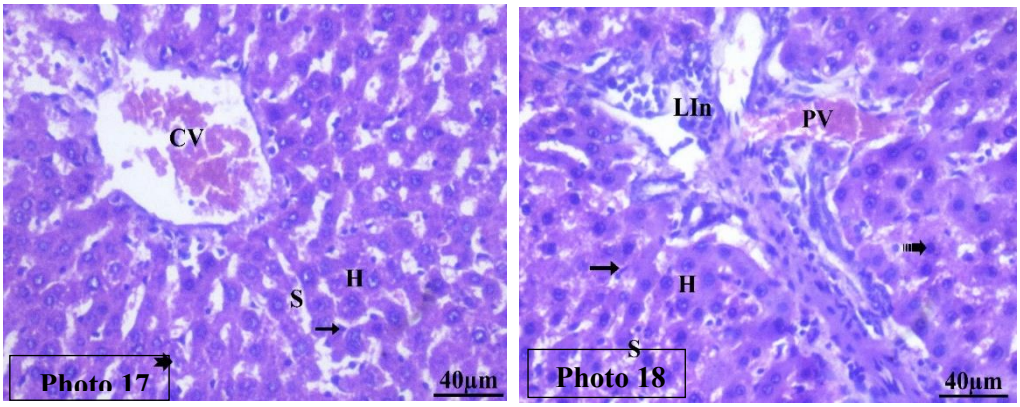
observation of a dilated congested portal vein were noted as shown in **Photo 16**. As shown in **Photo 17**, the liver sections of rats from treated group with CCl_4 + 5% of RPTRs (group 3), the hepatic lobules exhibited a morphology characterized by the presence of branching and anastomosing cords of hepatocytes emanating from the central vein. These cords were interspersed with blood sinusoids and Kupffer cells. In addition, the hepatocytes exhibited acidophilic cytoplasm and singular central spherical vesicular nuclei, resembling those observed in the negative control rats. As well, scattered in the portal areas, there was a moderate quantity of mononuclear cellular infiltrations. However, there was evidence of dilation and congestion in certain central veins, portal veins, and sinusoids. Among the liver sections, the presence of pyknotic hepatic cells and disintegrating hepatic cells was observed to a mild extent as shown in **Photo 18**. As shown in **Photos 19 and 20**, liver sections of rats from group 4 treated with CCl_4 + 7.5% of RPTRs showed that the most hepatocytes exhibited vesicular nuclei and acidophilic cytoplasm, with a small subset still displaying nuclei that were darkly stained. As well, the observed structures, the central veins, sinusoids, portal vein, and bile duct, exhibited normal characteristics, except for limited certain areas where congestion was still evident in the central veins and portal vein, accompanied by a detached endothelial wall lining. Furthermore, the absence of periportal mononuclear cellular infiltration was noted. Discrete areas of hepatic tissue exhibited hepatocytes displaying signs of degeneration. Liver sections of rats from group 5 treated with CCl_4 + 10% of RPTRs shown that the hepatic cords were suitably arranged in proximity to the sinusoids and a mild level of congestion was observed in both the central vein and portal vein. As well, there was evidence of a slight degree of mononuclear cell infiltration (LIn) and pyknotic nuclei and disintegrating nuclei were seen (**Photo 21**).



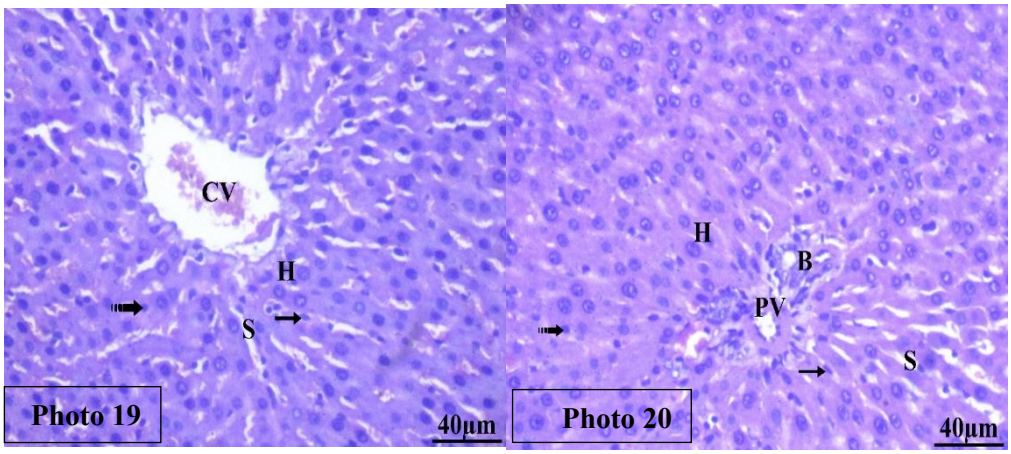
Photos (13 and 14): Photomicrographs of liver sections from negative rats showing the hepatic tissue displayed the characteristic lobular architecture, consisting of hepatocytes (**H**) arranged in cord-like structures that appeared to be branching and anastomosing. As well as, the hepatocyte cords were extending from the central vein (**CV**) located at the center, towards the portal tract areas at the periphery and the hepatocytes were a polygonal morphology, with acidophilic cytoplasm and centrally located spherical, vesicular nuclei. In addition, the hepatic sinusoids (**S**) in the interstitial spaces between the cords of hepatocytes, with the Kupffer cell, the bile duct (**B**) and portal vein (**PV**) branches were observed to be situated within the portal tract sites (H & E X 400).



Photos (15 and 16): Photomicrographs of liver sections from positive rats showing the hepatic lobules dilation and congestion of both the blood sinusoids (**S**) and central veins (**CV**), which were shown to have a detached endothelial wall. In addition, certain hepatic cells have a ghost-like appearance characterized by fragmented nuclei, while others display dark pyknotic nuclei (**dotted arrow**). Furthermore, the presence of inflammatory cell infiltration (**LIn**) and the observation of a dilated congested portal vein (**PV**) were noted (H & E X 400).



Photos (17 and 18): Photomicrographs of liver sections from treated group with CCl_4 + 5% of RPTRs shown the hepatic lobules exhibited a morphology characterized by the presence of branching and anastomosing cords of hepatocytes (**H)** emanating from the central vein (**CV**). These cords were interspersed with blood sinusoids (**S**) and Kupffer cells (**arrow**). In addition, the hepatocytes exhibited acidophilic cytoplasm and singular central spherical vesicular nuclei, resembling those observed in the negative control rats. As well, scattered in the portal areas, there was a moderate quantity of mononuclear cellular infiltrations (**LIn**). However, there was evidence of dilation and congestion in certain central veins (**CV**), portal veins (**PV**), and sinusoids (**S**) and pyknotic hepatic cells (**dotted arrow**) and disintegrating hepatic cells (**notch arrow**) to a mild extent (H & E X 400).



Photos (19 and 20): Photomicrographs of liver sections from treated group with CCl_4 + 7.5% of RPTRs shown that the most hepatocytes exhibited vesicular nuclei and acidophilic cytoplasm, with a small subset still displaying nuclei that were darkly stained (**dotted arrow**). As well, the observed

structures, the central veins (**CV**), sinusoids (**S**), portal vein (**PV**), and bile duct (**B**), exhibited normal characteristics, except for limited certain areas where congestion was still evident in the **CV** and **PV**, accompanied by a detached endothelial wall lining. Furthermore, the absence of periportal mononuclear cellular infiltration was noted. Discrete areas of hepatic tissue exhibited hepatocytes displaying signs of degeneration (**notch arrow**) (H & E X 400).

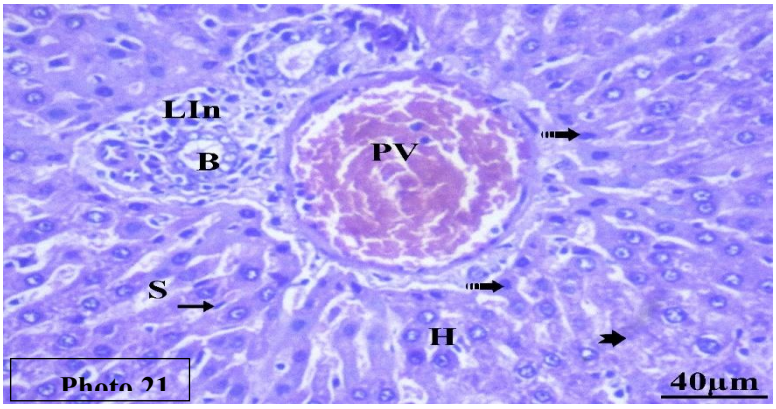


Photo (21): Photomicrographs of liver sections from Liver sections of rats from group 5 treated with CCl_4 + 10% of RPTRs shown that the hepatic cords (**H**) were suitably arranged in proximity to the sinusoids (**S**) and a mild level of congestion was observed in both the central vein (**CV**) and portal vein (**PV**). As well, there was evidence of a slight degree of mononuclear cell infiltration (**LIn**) and pyknotic nuclei (**dotted arrow**) and disintegrating nuclei (**notch arrow**) were seen.

4- DISCUSSION

The hepatoprotective performance of round, purple turnip roots (RPTRs) upon carbon tetrachloride (CCl_4)-induced hepatotoxicity in rats was discovered. These effects have been shown by considering its outcome on the alteration in body weight, and liver and kidney functions, as well as some biochemical variable such as lipid profile (TL, TG and TC), lipid peroxidation levels (MDA) and the activities of antioxidant enzymes (CAT, SOD and GSH). In addition to the histological investigation of liver and kidney tissues.

CCl_4 is a well-known poison, and it is the best-characterized example to provoke free radical-mediated inducing hepatotoxicity brought about from CCl_4 which is thought to be one of the mechanisms leading to hepatotoxicity in animals (**Al-Harbi *et al.*, 2014**). Therefore, hepatotoxicity induced by CCl_4 is the most widely used model to assess hepatoprotective activity of plant extracts/drugs in the laboratory, since the pathological lesions induced by CCl_4 in animals closely resemble the symptoms of human liver diseases. Where, CCl_4 is metabolized by hepatic cytochrome P450 into the highly reactive trichloromethyl free radicals (CCl_3) and trichloromethyl peroxyl (CCl_3O_2) radicals in the liver, which can encourage lipid peroxidation and therefore affect the transmitted functions and membrane receptiveness of hepatocytes, leading to the leakage of enzymes from the hepatic cells into blood. This outcome of strength is due to the liberation of free radicals, which are composed from trichloromethyl (CCL_3) and proxy trichloromethyl ($OOCCL_3$) radicals (**Meng *et al.*, 2018)**. As well, CCl_4 also raises the activation of immune systems through the infiltration of inflammatory cells to the location of damage (**Al-Harbi *et al.*, 2014**). Further **Dongare *et al.*, (2013)** stated that low dose of CCl_4 (1ml/kg) generated a significant increase in the activities of ALT, AST, ALP, and TBIL and other biochemical parameters as total, direct and indirect Bilirubin, thus disclose liver damage.

The current results are proven that intraperitoneal injection (IP) with CCl_4 to encourage liver toxicity consequence a significant ($P<0.05$) decline in consumed food, body weight, serum levels of TP and Alb, and activities of the tested antioxidant enzymes (CAT, SOD and GSH). As well as, there is a significant increase in serum levels of MDA, activities of liver enzymes (AST, ALT and ALP), concentrations of UN, Cr and UA, compared to the normal rats. Also, the histological investigation of kidney sections noticeable devastation, kidney tubule damage with distended, the existence of cellular debris in their lumens, vacuolated cytoplasm and dark pyknotic nuclei. In addition to, the appearance of the hepatic lobules expressed dilation and congestion of both the blood

sinusoids and central veins, which were shown to have a detached endothelial wall. In addition, particular hepatic cells have a ghost-like occurrence described by fragmented nuclei, while others display dark pyknotic nuclei. Additionally, the being of inflammatory cell infiltration and the observation of a dilated congested portal vein. The obtained results were in conformity with (**Ren *et al*., 2019**).

Also, the obtained results were in accordance with **Akinloye *et al*., (2012)** who mentioned that the administration of CCl_4 resulted in notable change in serum activities of hepatic (AST, ALT and ALP) and antioxidant enzymes, and level of MDA indicative of hepatic injury. In addition, **Kang, (2015)** reported that CCl_4 generated liver toxicity in treated animals was obvious by elevated serum activities of AST, ALT, ALP and GGT enzymes. As well, induction of oxidative stress in the liver tissue was shown evidence of a decline in the levels of NP-SH and TP, and an augmented level of MDA concentration and dwindling in the activities of SOD, CAT, GSH and GPx enzymes. An increase in MDA concentration in the liver suggested the enhancement of peroxidation level leading to the liver tissue injury and the failure of antioxidant-defense system. On the other hand, the rise in serum activities of AST, ALT and the level of total cholesterol have been attributable to the damaged of liver structural integrity, due to the fact that they are located in the cytoplasm and are released into the circulation after cellular damages. **El gazar *et al*., (2023)** reported that intraperitoneal injection of CCl_4 caused hepatotoxic-rats as indicated by the significant raise serum activity of AST, ALT and ALP enzymes, levels of TP, Alb, UN, Cr, UA and MDA.

With regard to the effect of the compliment diet with the different levels of RPTRs (5, 7.5 and 10%) on hepatotoxicty -rats, the obtained findings state that supplemented diet with different RPTRs caused a significant increment in FI, BWG and RWG %, compared to the basal diet. Additionally, the consequences indicated that there is a get preferable in liver and kidney functions and marmalade their tissues from damage, as well as recovers the activities of the antioxidant enzymes and decline the

levels of TL, TG and TC in -rats fed on the supplemented diet with the three different levels of RPTRs. The highest results were established in treated hepatotoxicity-rats with increasing the level of RPTRs. The results of the biological examination agreed-upon with the consequence of the histological inquiry of kidney and liver, which consequences gradual improvement with on the rising levels of RPTRs. The current study was in agreement with the results of **Mohajeri *et al*., (2012)**, who reported that the body weight and feed intake, feed efficiency ratio, as well significant the activities of ALT, AST, LDH and concentrations of bilirubin, and serum levels of TP and Alb in were improved in hepatotoxicity-rats fed on the RPTRs than to normal control group. **Khader *et al*., (2021)** revealed that diabetic rats fed on 5 % turnip roots have a significant improvment in liver enzymes (AST, ALTand ALP) and serum Cr, UA, UN, TC and TG, compared to the positive group. Also, **Sobh and Shalan (2022)** reported that supplemented diet with 2.5% of turnip roots caused significant increase in FI and BWG%, and decrease in serum activites of AST and ALT enzymes, levels of TC, TG, uric acid, urea and creatinine in hypercholesterolemic-rat.

In other previous experimental research have shown that CCL_4 administration resulting in an increase in serum activities of GOT, GPT and ALP in mice. Although, treated mice with RPTRs juice significantly diminished the elevated serum liver enzymes and bilirubin (**Al-Shabanah *et al*., 2000**). Furthermore, it has also been shown that CCL_4-induced hepatotoxicty is significantly hindered by turnip fresh juice. The suppressant effect of it on CCL_4-induced hepatotoxicty suggest the capability of juice component to reverse the damage exerted by CCl_4, on the cytochrome P450 involved in metabolism of phenobarbitone (**Rafatullah *et al.,* 2006**). In another study, oral administration of turnip ethanolic extract after liver injury caused significant reduction in serum activities of AST, ALT, and LDH enzyme and improved the activities of CAT and SOD enzymes in liver microsomal cytosol (**Choi *et al*., 2006**). As well that the presented flavonoid in turnip roots was established to be

able to suppress the levels of plasma ALP, ALT and AST in mice with CCl4-induced liver injuries (**Igarashi *et al.*, 2008**).

As previous study indicated that Brassica vegetables are consumed for health betterment, which relates to their antioxidant activity (**Rafatullah *et al*., 2006**). Additionally, **Li et al., (2010)** revealed particular beneficial effects of turnip in treating liver related problems. Therefore, the positive effect of RPTRs on treating liver toxicity may relate to its content of the active biological components such as flavonoids including kaempferol, isorhamnetin, quercetin and glycosides; Insole alkaloids, phenylpropanoid derivatives and sterile glycosides, as well as vitamins as niacin, riboflavin ascorbic acid and vitamin A (**Zare *et al*., 2018**), anthocyanins, and sulfur compounds which exhibit hepatoprotective activity on CCl_4-induced hepatotoxicity in rats, by lowering the activities of serum GOT, GPT, ALP, and bilirubin (**Paul *et al*., 2019**). Additionally, lipid peroxidation is a detrimental process in liver injury due to administration of hepatotoxicants. However, Brassica rapa contain nutrient /health-promoting components, like diversified types of glucosinolates and phenolic compounds (**Baenas *et al*., 2017**). Therefore, the biological activity formulated by these compounds is mainly due to their antioxidant ability, which could lower the injurious consequences of excessively high levels of Reactive oxygen species (ROS) in cells, thus, decline oxidative stress (OS) by supplying cells with molecular tools to fight the imbalance between the production of ROS and the ability to modulate the redox balance. These characteristics have forthright effects on a number of cellular operation activated by ROS, which are related to inflammation and oxidative reactions on DNA, proteins, and cell lipids (**El-Dreny, 2019**).

5. Conclusion:

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التأثير الحيوي لجذور اللفت المستدير الأرجواني ضد السمية الكبدي التي يسببها رابع كلوريد الكربون في الفئران

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الملخص

قد تتسبب العديد من الأدوية الشائعة الاستخدام في إصابة حادة ومزمنة بالكبد، والتي قد تكون خطيرة كتأثير جانبي غير متوقع. أُجريت هذه الدراسة للتحقيق في التأثيرات الحيوية لجذور اللفت الأرجواني المستدير ضد التسمم الكبدي الناتج عن رابع كلوريد الكربون في الفئران. تم توزيع خمسة وثلاثين فأرًا ألبينو من سلالة سبراجيو داولي عشوائيًا إلى خمس مجموعات، حيث تغذت المجموعة ١ (مجموعة التحكم السلبي، ٧ فئران) على النظام الغذائي الأساسي. أما بقية المجموعات (٢، ٣، ٤، و٥) فتم حقنها داخل الصفاق مرة واحدة في الأسبوع لمدة ستة أسابيع برابع كلوريد الكربون لإحداث التسمم الكبدي الحاد. و استُخدمت المجموعة ٢ كمجموعة ضابطة إيجابية وتغذت على النظام الغذائي الأساسي، بينما تغذت المجموعات ٣، ٤، و٥ على النظام الغذائي المكمل بنسب ٥، ٧,٥ و١٠٪ من جذور اللفت الأرجواني المستدير الجاف على التوالي. أظهرت النتائج أن الفئران غير المعالجة التي تعاني من التسمم الكبدي كانت لديها انخفاض كبير في كلاً من كمية الطعام المتناولة، الوزن، ونسبة الزيادة في الوزن، ومستويات البروتين الكلي والألبومين في السيرم، وكذلك نشاط إنزيمات الكاتالاز، السوبر أكسيد ديسموتاز، والجلوتاثيون . بينما كانت هناك زيادة في مستويات اليوريا، الكرياتينين، حمض اليوريك، الدهون الكلية، الدهون الثلاثية والكوليسترول الكلي، وكذلك نشاط الانزيمات المضادة للأكسدة وإنزيمات الكبد مقارنةً بمجموعة التحكم السلبي. بالإضافة إلى ذلك، لوحظت عدة تغييرات تدهورية في الفصوص الكبدية،مثل احتقان في كل من الاوعية الدموية والأوردة المركزية، تحلل أنوية الخلايا الكبدية، ووجود التهابية وتوسّع واحتقان الوريد البابي في المجموعة الضابطة الإيجابية. كمقارنةً بالمجموعة الضابطة الإيجابية، أدى النظام الغذائي المكمل بالمستويات الثلاثة المختلفة من جذور اللفت الأرجواني المستدير إلى تحسن كبير في جميع المعايير البيولوجية والبيوكيمائية المحددة التي تم اختبارها، وكذلك في التركيب النسيجي للكبد، وذلك بشكل يعتمد على الجرعة. وأخيرا، لخصت هذه الدراسة إلى أن اللفت الأرجواني المستدير له تأثيرات صحية مفيدة كبيرة تسببت في تحسين وظائف الكبد المصابة والتي قد تعزى إلى مركباته النشطة بيولوجيا.

الكلمات المفتاحية :اللفت الأرجواني المستدير؛ التسمم الكبدي؛ بيروكسيد الدهون