Original Article

Hepatoprotective Effect Of Rhubarb Roots Against Carbon Tetrachloride-Induced Hepatotoxicity In Rats

Aml F. Elgazar, Amr A. Rezq and Ahmed. M. Elsaied

Nutrition and Food Sciences Department, Faculty of Home Economics, Helwan University

Corresponding author: Aml Fawzy Elgazar
Email: *dr_aml_fawzy@yahoo.com*
DOI: 10.47750/pnr.2023.14.S01.31

Abstract

Rhubarb (*Rheum rhabarbarum* L.) is a perennial herbaceous plant widely used for public health, medicinal benefits and antioxidant properties. Hepatotoxicity refers to the dysfunction or damage in the liver associated with an overload of drugs or other foreign matters of the body. The present study distended to study the hepatoprotective effect of Rhubarb Roots (RRs) against carbon tetrachloride (CCL4) -induced hepatotoxicity in rats. Forty rats were divided into five groups, of eight rats each. Group 1, normal rats (negative control group) fed on the basal diet alone. Groups 2, 3, 4 and 5 were injected intraperitoneally with 1 ml/kg B. Wt. of CCl4 in paraffin oil (1:1) once weekly to induce liver toxicity. Hepatotoxic rats in the group 2 (positive control group) were fed only on the basal diet, while groups 3, 4 and 5 (treatment groups) were nourished on supplemented basal diet with 5, 10 and 15% of RRs, respectively. Hepatotoxic rats who received an enriched diet with the three different levels of RRs have a significant decrease ($p<0.05$) in the serum activity of AST, ALT and alkaline phosphatase enzymes. Additionally, serum concentrations of total bilirubin, direct and indirect, total protein, albumin, urea, creatinine, uric acid and malondialdehyde (MDA) decreased significantly ($P<0.05$). While the serum activities of CAT, GSH, GPx and SOD enzymes were significantly increased, compared to hepatotoxic rats that feed solely on the basal diet. Histopathological examination also showed an evident improvement in the liver and renal tissues of hepatotoxic rats fed on the enriched basal diet with different levels of RR. Finally, the existing study illustrated that RR could improve the liver and kidney functions and activity of antioxidant enzymes by eliminating the deleterious toxic effect of CCl_4, especially with the higher levels of RRs.

Keywords: Rhubarb Roots; Carbon tetrachloride; Hepatoprotective; Antioxidant; Liver functions; Kidney functions

INTRODUCTION

The Rhubarb (*Rheum rhabarbarum* L.; Polygonaceae) is a herbaceous plant. Its leaves are spherical heart-shaped inflorescences and the fruits are trigonated nuts with wings of white-milk or cream-colored (**Taheri and Assadi, 2013**). There are several species and/or cultivars of Rhubarb included in the genus *Rheum* L., and composed of about sixty species disseminated in the mountainous and desert regions in Asia and Europe, with the several species occurring in China (**Ruirui *et al.*, 2010**). Though many cultivars are considerably popular with a usual designate Rhubarb, mainly three categories have been scientifically evaluated for trading proposes, namely Indian (*R. emodi* and *R. webbianum*), Chinese (*R. officinal, R. tangticum* and *R. palmatium*) and Rhapontic Rhubarb (*R. rhapnticum* L) (**Agarwal *et al.*, 2001**).

Rhubarb plant is one of the traditional medicines and used widely for its nutritional value (**Lai *et al.*, 2015**). The petioles and/or stalks are the edible parts and are pink to red in colour with long leaves, as well as it has a fleshy leathery and are considered to be much sweeter than green (**Kalisz *et al.*, 2020**). **Singh and Rawat (2016)** reference that Rhubarb contains several biological effect components as flavonoids, anthocyanin's, anthraquinones, anthrones, acylglucosides and stilbenes, as well as organic acids and vitamins.

Chinese folk medicine motivates the use of Rhubarb roots (RRs) in the treatment of constipation, abdominal pain, appendicitis, fever, kidney failure, liver cancer, high blood pressure (**Wojcikowski *et al.*, 2004**) and weight loss

(**Cao *et al.,* 2017**). Traditional Indian medicine has also used Rhubarb roots as a laxative, anti-microbial and, in wound healing, skin ulcers, the treatment of boils and abdominal disorders (**Agarwa *et al.,* 2001**). Furthermore, experimental and clinical studies have shown that rhubarb roots have a diverse biologically beneficial effect as hypolipidemic, antibacterial, anti-inflammatory, antioxidant (**Fei *et al.,* 2017**), antidiabetic, anticancer, nephropotector and heart protector (**Ghorbani *et al.,* 2019**).

The liver is the most important organ and gland included in the nutrient metabolism and dominated by the levels of cholesterol, glucose, iron and some hormones, as well as playing an important role in removing blood toxins and blood clotting (**Blachier *et al.,* 2013**). The liver is permanently widely exposed to several foreign matters such as environmental toxins, drugs, chemicals and alcohol, which lead to various liver disorders or injuries, and can, in the latest, lead to hepatic failure and death (**Cooke *et al.,* 2010**). Also, inherits, virus infection and several different diseases including obesity, diabetes or an attack from own immune system are other factors that can permanently damage the liver and the bile duct or manger life (**Sivakrishnan and Pharm, 2019**). Hepatotoxicity defined as the damage in the liver cells or functions due to the long period of exposure to drugs or non-drug chemical substances (alcohol, CCL_4 and thioacetamide, etc.) (**Dhingra *et al.,* 2011**). Carbon tetrachloride (CCl_4) is a common fundamental compound used to cause liver injury (**Kim *et al.,* 2010**). Moreover, the liver is not the only target organ of CCl_4, but it also has effects on many organs of the body such as the kidneys, heart, testicles and brain. The mechanism by which CCL_4 induced hepatotoxicity, in particular liver necrosis and steatosis, may be the bioactivation of CCl_4 into trichloromethyl free radicals by the cytochrome P450 system in liver microsoms and therefore causes lipid peroxidation of the liver membranes leading to liver lesions (**Ozturk *et al.,* 2003**). However, Dietary antioxidants and anti-inflammatory agents play a critical role in combating CCl4 poisoning by scavenging active oxygen and free radicals, neutralizing lipid peroxides and reinforcing the natural cellular antioxidant action (**Unsal *et al.,* 2021**).

Today, the uses of the drug in the liver disease treat are sometimes expensive, inappropriate and can have serious adverse effects. Due to these important problems, it is necessary to research the prevalence of natural alternative sources locally and have been used before in popular medicine. Therefore, the present study distended to examine the hepatoprotective effect of Rhubarb roots against CCl4-induced hepatotoxicity in rats.

Materials and Methods

Rhubarb Roots

Dried Rhubarb roots (RRs) as shown in photo (1) were obtained from the Dep., of Medic. and Aromatic Plants, Agricultural Research Centre, Dokki, Egypt. Dried RRs were sorted, removing all invalid parts and cleansing from dust. Afterwards, it was ground and sifted into 40 mm mesh powder and packed until it was used (photo 2).

Photo 1: Whole rhubarb roots **Photo 2**: Powder of rhubarb roots

Preparation of Purified Basal Diet

All the nutrient ingredients needed for the preparation of the basal diet (AIN 93-M) according to the 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. Afterwards, the diet was formulated based on the approbated quantities of **Reeves *et al.,* (1993)** to meet the nutritional requirements for rats during the experimental period.

Rats

Forty male Sprague Dawley rats weighing (200±5 g) were obtained from the experimental animal house of the Faculty of Veterinary Medicine, Cairo University. ♀ Thereafter, the rats were transferred and housed in wire cages at the animal house of the 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.

Chemicals and Biochemical Kits

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. Kits for biochemical assay were purchased from the Gamma Trade Co., for Pharmaceutical and Chemical, Dokki, Egypt.

Inducement of Hepatotoxicity in Rats

In this study, hepatic toxicity in all rats with the exception of normal rats was induced by intraperitoneal injection (IP) once a week with 1 ml/kg bwt of CCl_4 dissolved in paraffin oil in a 1:1 portion (v:v).as documented by **Karthikeyan and Deepa, (2010).**

Experimental Design and Grouping of Rats

Prior to the trial study, rats were kept for a week to acclimatize. Subsequently, rats were randomized into five groups, each with eight rats. In the first group (negative control group), rats were IP injected with an equivalent volume (0.1 mL) of paraffin oil once per week for six weeks and fed a normal basal diet. In Group 2, untreated hepatotoxic rats were maintained as a positive control group and fed only with the basal diet. Hepatoxic rats in groups 3, 4 and 5 were kept as treated groups by fed on the supplemented basal diet with Rhubarb roots (RRs) at the levels of 5, 10 and 15% of the diet, respectively.

Determining Feed Intake and Body Weight

The amount of feed intake (FI) for each rat was evaluated based on calculating the amount consumed daily for each group. To determine the change in body weight, rats were weighed before the experiment (IBW) and at the end of the experimental period (FBW). Next, we calculated the body weight gain (BWG) and the relative body weight gain (RGW%) as described by **Kratochvílova et al., (2002).**

Collecting Blood Samples.

At the end of the experimental period (6 weeks), rats in all groups were fasted for 12 hours, anesthetized with diethyl ether and scarified. 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.

Biochemical Analysis

Assessment of Liver Functions:

The serum activity of AST, ALT and ALP enzymes was measured colorimetric by utilizing (Diamond Co, Hanover, Germany) Kits according to instruction of **Young (2000)** and **Young (1997)**, respectively. The biometrics were measured using a spectrophotometer (Hum star 200, automatic biochemistry analyzer, Germany) adjusted at 505 and 510 nm, respectively.

Serum concentrations of total protein (TP), albumin (Alb), total bilirubin (TBL) and direct bilirubin (DBL) were measured colorimetrically using a spectrophotometer (Hum star 200, automatic biochemistry analyzer, Germany) as described by **Tietz (1994), Young (2000), Henry (1991)** and **Burtis and Ashwood (1999)**, respectively. The device was regulated at 545, 628, 520 and 548 nm, respectively for measuring the color intensity that reflect the serum concentration of the tested parameters. While indirect bilirubin (IDBL) was estimated by calculating the difference between total and direct bilirubin using the present formula (**Indirect bilirubin (mg/dl) = Total bilirubin- Direct bilirubin).**

Estimation of Kidney Functions

Quantitative ELISA-based colorimetric Kits assay were used for the measurements of serum levels of urea nitrogen (UN), creatinine (Cr) and uric acid (UA) based on colorimetric assay. 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, according to Kits procedures describe by **Friedman and Young (1997)**.

Estimation of Malondialdehyde and Activity of Antioxidant enzymes:

The serum concentration of MDA and activity of catalase (CAT), superoxide dismutase (SOD), glutathione (GSH) and glutathione peroxides (GPx) enzymes were determined using commercial assaying kits (Cayman Practice ELISA Kits). The principal method for the determination of oxidative stress depends on colorimetric by quantifying thiobarbituric acid (TBA) reactivity as malondialdehyde (MDA) in a spectrophotometer adjusted at 532 nm according to the described

method by **de- Zwart *et al.*, (1999).** The procedure that is used for the evaluation of CAT activity depends on the reaction of the enzyme with methanol in the presence of an optimal concentration of H2O2. The formaldehyde produced is measured spectrophotometrically at 540 nm as described by **Wheeler *et al.*, (1990).**
The standard technique to assay the activity of SOD is that the kits used use an enzyme linked immunosorbent assay-double antibody principle. The color change is measured spectrophotometrically at 450 nm as described by **Wheeler et al., (1990)**. The serum activity of GSH and GPx was assayed according to the kit's instructions as described by **Ceballos-Picot et al., (1992)** using spectrophotometrically at 340nm.

Histopathological Examination of the Liver and Kidney
Following rat dissection, the liver and kidney were carefully removed from each rat, washed with normal saline for blood removal and immersed in neutral formaldehyde (10%). The submerged samples were then cleaned, washed and dehydrated in ascending grade alcohols. Afterword, specimens were cleared in Xylol, fixed and deeply in paraffin mass, sectioned to 4-6 microns in thickness and stained with the Heamatoxylin and Eosin stain for examination as described by **Bancroft and Gamble (2002)**.

Statistical Analysis
Statistical analyses were performed using software system (SPSS version 22). The statistical tool used for the evaluation of significant differences ($P<0.05$) was the ANOVA test. The results obtained have been expressed in Mean ± SD.

Results

Recorded results in table (1) interpreted the effect of feeding supplemented diet with RRs on FI, FBW, BWG and RWG (%) in rats with hepatotoxicity. It showed that the injected rats with CCl_4 alone (positive control group) had a significant ($P<0.05$) reduction in FI, FBW, BWG and RWG (%), compared to normal rats (negative control group). Whereas, feeding rats on a supplemented diet with RRs at the three different levels (5, 10 and 15%) combined with IP injection by CCl_4 has a significant ($P<0.05$) increase in FI, FBW, BWG and RWG (%) in comparison to injected rats by CCL4 alone. The superior results in FI, FBW, BWG and RWG (%) were established in treated groups by 10 and 15% of RRs.

Table (1): Effect of feeding on supplemented diet with RRs on FI, FBW, BWG and RWG (%) in rats with hepatotoxicity**.**

Parameters Groups		Parameter as Mean ± SD				
		FI (g)	IBW (g)	FBW (g)	BWG (g)	RWG (%)
Negative control group		16.41±0.20 [a]	200.43±0.14	305.00±0.12[a]	104.57±0.02[a]	52.17±0.07[a]
Positive control group		13.51±0.30 [e]	201.10±0.15	255.50±0.15[d]	54.40± 0.04[d]	27.05± 0.04[d]
Treated groups with RRs at levels of:	5%	13.79±0.19 [d]	201.00±0.19	289.50±0.10[c]	88.50±0.09[c]	44.30± 0.08[c]
	10%	15.07±0.21[c]	201.00±0.20	301.10±1.15[b]	100.10± 0.05[b]	49.800± 0.03[b]
	15%	15.71±0.16 [b]	201.14±0.15	301.30±1.11[b]	100.16±0.04[b]	49.80± 0.08[b]

Means with different letters in each row are significantly differs at $p< 0.05$

The tabulated results in table (2) explained that untreated rats with hepatotoxicity have a significant increase in the serum activity of AST, ALT and ALP enzymes, compared to normal rates. Whilst the treatment of hepatotoxicity rats by feeding on a supplemented diet with 5, 10 and 15% of RRs caused significant ($P<0.05$) reductions in the serum activity of AST, ALT and ALP enzymes, compared to untreated hepatotoxicity rats. The best improved results were reported in rats treated with high levels (15%) of RRs.

Table (2): Effect of feeding on supplemented diet with RRs on serum 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 control group		48.14±4.22 ^{e}	13.01±2.65 ^{e}	91.01±2.31 ^{d}
Positive control group		88.14±3.98 ^{a}	55.43±2.23 ^{a}	165.71±1.60 ^{a}
Treated groups with RRs at levels of:	5%	77.29±1.80 ^{b}	36.29±1.11 ^{b}	123.14±6.20 ^{b}
	10%	70.57± 2.30 ^{c}	33.86±1.46 ^{c}	120.71±2.29 ^{b}
	15%	55.29±3.20 ^{e}	15.57±1.27 ^{d}	97.29±1.70 ^{c}

Means with different letters in each row are significantly differs at $p< 0.05$

As shown in table (3), the serum concentration of TP, Alb, TBL, DBL and IDBL were reduced significantly ($P<0.05$) in injected rats with CCL4 and fed on a basal diet alone, compared with normal rats. On the other hand, feeding rats on a supplemented diet with RRs at the three different levels (5, 10 and 15%) incorporated injected with CCl_4 significantly ($P<0.05$) ameliorates serum levels of TP, Alb, TBL, DBL and IDBL as compared with that treated by CCL4 and fed on the basal diet alone. In addition, the results showed that the supplemented diet with 15% of RRs increases the improvement rate of serum concentration of the above parameters.

Table (3): Effect of feeding on supplemented diet with RRs on serum concentration of TP, Alb, TBL, DBL. And IDBL in rats with hepatotoxicity.

Parameters Groups		Parameter as Mean ± SD				
		TP (gm/dl)	Alb (gm/dl)	TBL (gm/dl)	DBL (gm/dl)	IDBL (gm/dl)
Negative control group		6.35±0.20^{d}	2.69±0.12^{d}	0.55±0.02^{d}	0.19±0.01^{c}	0.36±0.02^{c}
Positive control group		9.60±0.19^{a}	3.63±0.25^{a}	0.85±0.02^{a}	0.35±0.06^{a}	0. 50±0.06^{a}
Treated groups with RRs at levels of:	5%	8.58±0.23^{b}	3.50±0.08^{ab}	0.79±0.01^{b}	0.37±0.01^{a}	0.42±0.01^{b}
	10%	7.54±0.25^{c}	3.34±0.03^{b}	0.65±0.01^{c}	0.30±0.01^{b}	0.35±0.02^{c}
	15%	6.47±0.26 ^{d}	3.04±0.37^{c}	0.57±0.01^{d}	0.21±0.01^{c}	0.36±0.02^{c}

Means with different letters in each row are significantly differs at $p< 0.05$

Results in table 4 describe the effect of a fortified diet by RRs on serum concentration of UN, Cr and UA in hepatotoxicity rats. The results exhibited a significant ($p<0.05$) increase in serum UN, Cr and UA concentrations in rats treated with CCL4 and fed on basal diet alone (positive control group), compared to normal rats. However, the results showed that there was a significant decrease in serum UN, Cr and UA concentrations of the CCl_4-treated groups given the different levels of RRs in combination, compared to the treated rats with CCL4 and fed on basal diet alone. The best amelioration in serum level of UN, Cr and UA is shown in the treated groups by RRs at a level of 15% compared to the other treated levels.

Table 5 represents lipid peroxidation as indicated by serum MDA level and activity of CAT, SOD, GSH and GPx in normal rats, injected rats with CCL4alone and that treated along with supplemented diet by 5, 10 and 15% of RRS. In comparison to normal rats, administration of CCl4 encourages a significant ($P<0.05$) increase in serum MDA level and decrease in the activity of CAT, SOD, GSH and GPx enzymes. Feeding rats on the fortified diet at the different levels of RRs along with injection with CCL4 caused significant amelioration in serum MDA levels and activities of CAT, SOD, GSH and GPx enzymes when compared to the positive control group fed on a normal basal diet alone.
The superior result in serum concentration of MDA and activity of antioxidant enzymes was shown in the treated group by the upper levels (15%) of RRs.

Table (4): Effect of feeding on supplemented diet with RRs on serum concentration 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 control group		41.86±0.54^{e}	41.86±0.54^{e}	41.86±0.54^{e}
Positive control group		67.24±1.97^{a}	67.24±1.97^{a}	67.24±1.97^{a}
Treated groups with RRs at levels of:	5%	54.16±1.07^{b}	54.16±1.07^{b}	54.16±1.07^{b}
	10%	48.89±0.63^{c}	48.89±0.63^{c}	48.89±0.63^{c}
	15%	43.41±1.84^{d}	43.41±1.84^{d}	43.41±1.84^{d}

Means with different letters in each row are significantly differs at $p< 0.05$

Table (5): Effect of feeding on supplemented diet with RRs on serum concentration of MDA and activity of CAT, SOD, GSH and GPx enzymes in rats with hepatotoxicity.

Parameters Groups		Parameter as Mean ± SD				
		MDA (u/ml)	CAT (u/ml)	SOD (u/ml)	GSH (u/ml)	GPx (u/ml)
Negative control group		1.58±0.12^{c}	31.41±3.32^{a}	46.10±1.06^{a}	6.78±0.30^{a}	19.83±0.50^{a}
Positive control group		3.41±0.56^{a}	17.12±0.67^{c}	24.51±0.33^{e}	3.37±0.08^{e}	8.78±0.19^{e}
Treated groups with RRs at levels of:	5%	2.38±0.06^{b}	18.73±2.98^{c}	28.15±1.44^{d}	4.22±0.10^{d}	14.30±0.22^{d}
	10%	2.13±0.03^{b}	25.98±0.78^{b}	35.50±0.80^{c}	6.15±0.03^{c}	16.31±0.18^{c}
	15%	1.55±0.19^{c}	27.16±1.15^{b}	43.60±1.28^{b}	6.42±0.19^{b}	18.88±0.32^{b}

Means with different letters in each row are significantly differs at $p< 0.05$

Histopathological Examination

Microscopically, the liver of rats from group 1 (negative rats) revealed the normal histological architecture of lobules as shown in **Photo 3**. In contrast, as shown in **Photo 4**, the liver of rats from group 2 (positive rats) have marked hepatocellular steatosis, fibroblasts proliferation encircles the hepatocytes and hepatocellular apoptosis, as well as marked fibroplasia in the portal triad, newly formed bile ductules and congested blood vessel (**Photo 5**). Meanwhile, the liver of rats from group 3 treated with CCL4 + 5% of RRs exhibited Kupffer cell activation, hepatocellular steatosis and a few strands of fibroblast proliferation shown in **Photo 6**. Liver sections of rats from group 4 treated with CCL4 + 10% of RRs showed Kupffer cell activation and a few fine strands of fibroblasts encircle the hepatocytes (**Photo 7**). However, liver sections of rats from group 5 treated with CCL_4+15% of RRs revealed moderate fibroblasts proliferation encircles the hepatocytes (**Photo 8**).

Light microscopic examination of kidneys of rats from group 1 (negative rats) showed normal histological architecture of renal parenchyma as shown in **Photo 9**. In contrast, kidney sections of rats from group 2 (positive rats) showed histopathological damage characterized by congestion of renal blood vessels and proteinaceous materials in the lumen of renal tubules (**Photo 10**) as well as Pyknotic nuclei of renal tubular epithelium and congestion of glomerular tuft as shown in **Photo 11**. Meanwhile, kidneys from treated rats with CCL_4 + 5% of RRs (group 3) showed proteinaceous materials in the lumen of some renal tubules (**Photo 12**), periglomerular and perivascular inflammatory cell infiltration (**Photo 13**). On the other hand, kidneys from treated rats with CCL_4 + 10% of RRs (group 4) revealed congestion of the renal blood vessels and glomerular tuft (**Photo 1**4). Otherwise, kidneys from treated rats with CCL_4 + 15% of RRs (group 5) exhibited no histopathological alterations except congestion of glomerular tuft in some examined sections as shown in **Photo 15**.

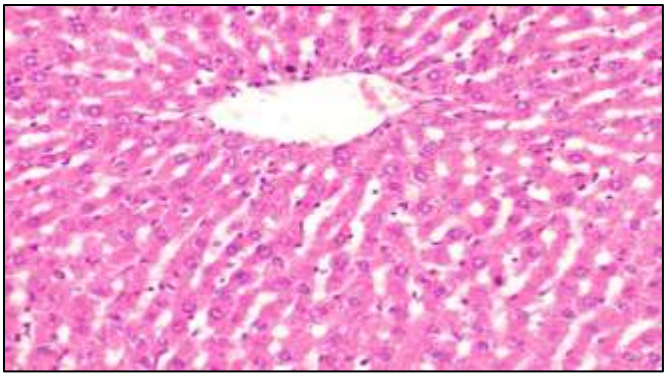

Photo 3: Photomicrograph of liver of rat from group 1 (negative rats) showing the normal histological architecture of hepatic lobule (H & E X 400).

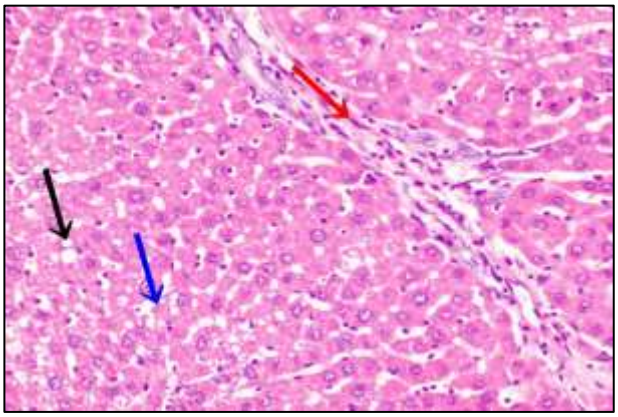

Photo 4: Photomicrograph of liver of positive rats showing hepatocellular steatosis (black arrow), hepatocellular apoptosis (blue arrow) and fibroblasts proliferation encircle the hepatocytes (red arrow) (H & E X 400).

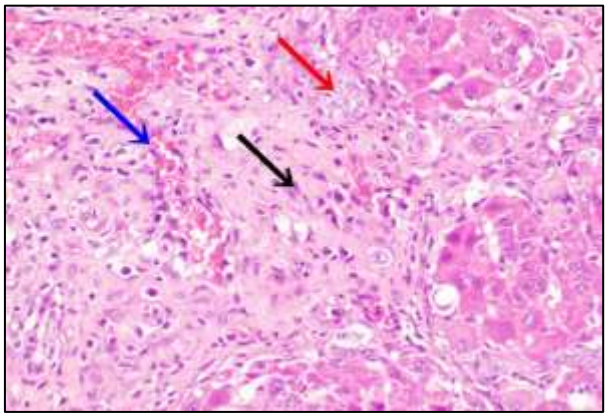

Photo 5: Photomicrograph of liver of positive rats showing marked fibroplasia in the portal triad (black arrow), newly formed bile ductules (red arrow) and congested blood vessel (blue arrow) (H & E X 400).

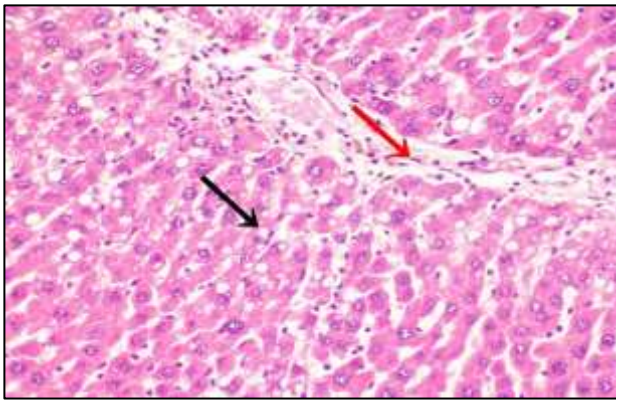

Photo 6: Photomicrograph of liver from treated rats with CCL_4+ 5% of RRs showing Kupffer cells activation (blue arrow) hepatocellular steatosis (black arrow) and few strands of fibroblasts proliferation (red arrow) (H & E X 400).

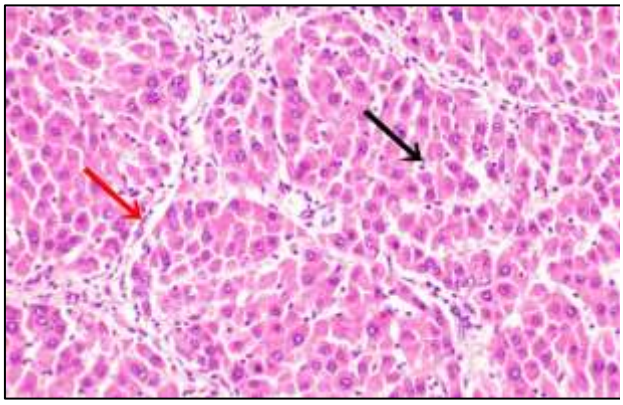

Photo 7: Photomicrograph of liver from treated rats with CCL_4+ 10% of RRs showing Kupffer cells activation (black arrow) and few fine strands of fibroblasts encircles the hepatocytes (red arrow) (H & E X 400).

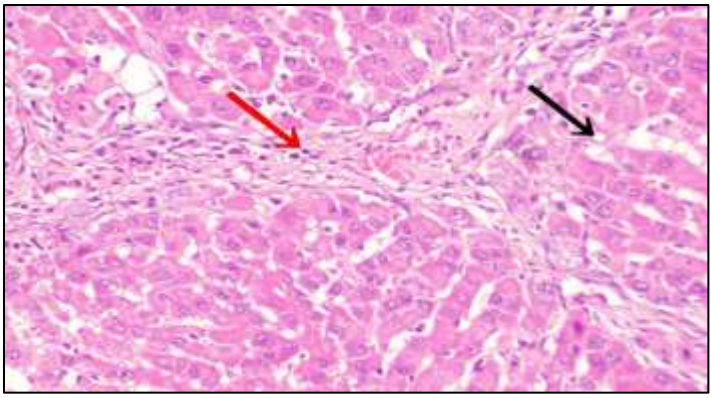

Photo 8: Photomicrograph of liver from treated rats with CCL_4+ 15% of RRs showing steatosis of sporadic hepatocytes (black arrow) and moderate fibroblasts proliferation encircles the hepatocytes (red arrow) (H & E X 400).

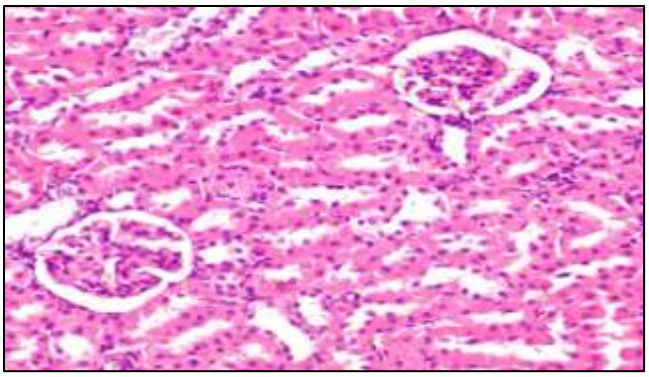

Photo 9: Photomicrograph of kidney sections from negative rats showing the normal histological architecture of renal parenchyma (H & E X 400).

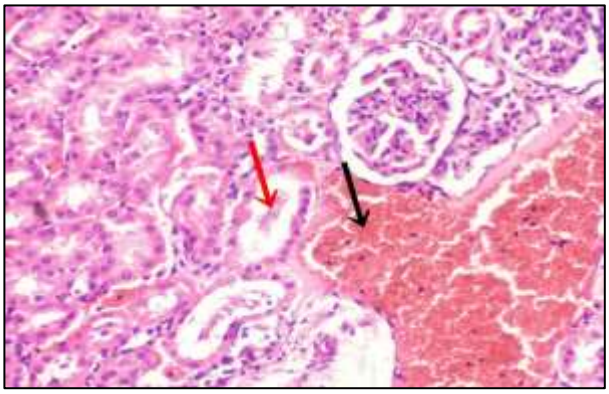

Photo 10: Photomicrograph of kidney sections from positive rats showing congestion of renal blood vessel (black arrow) and proteinaceous materials in the lumen of renal tubules (red arrow) (H & E X 400).

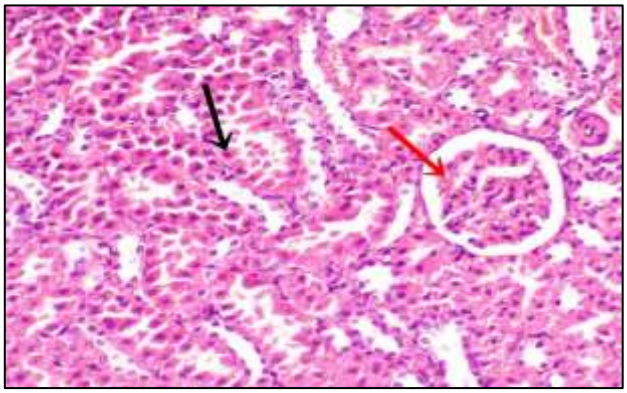

Photo 11: Photomicrograph of kidney sections from positive rats pyknosis of some nuclei of renal tubular epithelium (black arrow) and congestion of glomerular tuft (red arrow) (H & E X 400).

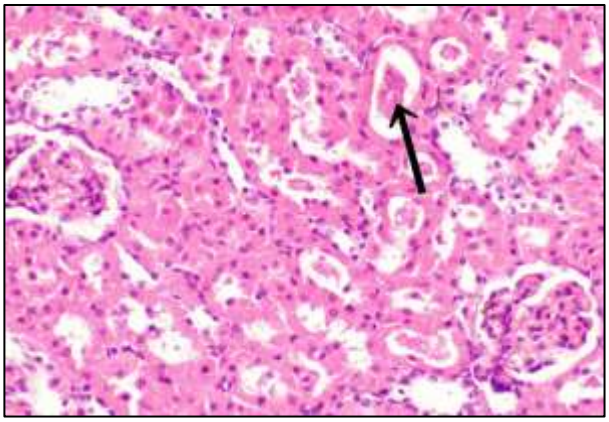

Photo 12: Photomicrograph of kidney sections from treated rats with CCL_4+ 5% of RRs showing proteinaceous materials in the lumen of some renal tubules (arrow) (H & E X 400).

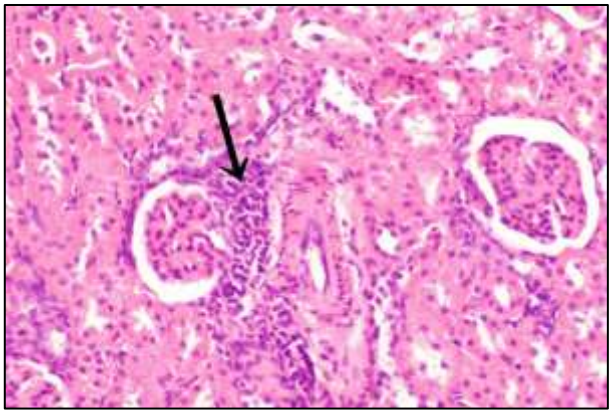

Photo 13: Photomicrograph of kidney sections from treated rats with CCL_4+ 5% of RRs showing periglomerular and perivascular inflammatory cells infiltration (arrow) (H & E X 400).

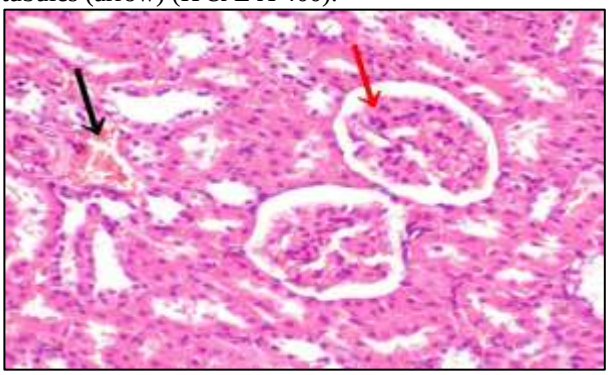

Photo 14: Photomicrograph of kidney sections from treated rats with CCL_4+ 10% of RRs showing congestion of renal blood vessel (black arrow) and glomerular tuft (red arrow) (H & E X 400).

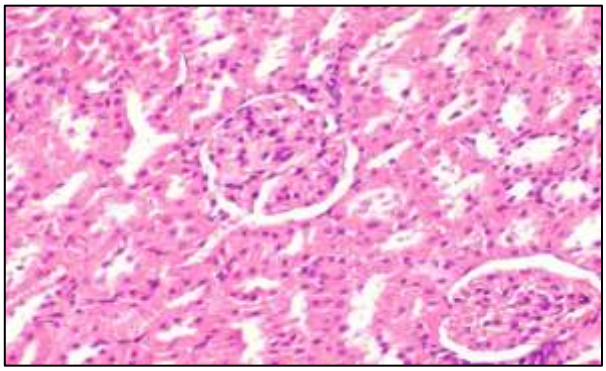

Photo 15: Photomicrograph of kidney sections from treated rats with CCL_4+ 15% of RRs showing no histopathological changes (H & E X 400).

Discussion

The hepatoprotective effect of Rhubarb roots (RRs) against carbon tetrachloride (CCL_4) - induced hepatotoxicity in rats was examined. That method has been verified by exploring its effect on some biological parameters such as changes in body weight, liver and kidney functions, lipid peroxidation levels and the activities of antioxidant enzymes. In addition to histopathological examination of liver and kidney tissues.

The existing results documented that intraperitoneal injection (IP) with CCl_4 gives rise to a significant decrease in food consumed, body weight and activity of antioxidant enzymes tested (CAT, SOD, GSH and GPx). In addition, there is a significant increase in serum activities of liver enzymes (AST, ALT and ALP), serum concentrations of total proteins (TP), albumin (Alb) and total, direct and indirect bilirubins, as well as urea nitrogen (UN), creatinin (Cr) uric acid(UA) and MDA. The histological examination pronounced the presence of hepatocellular steatosis, fibroblast proliferation encircles the hepatocytes and apoptosis, as well as fibroplasia in the portal triad, newly formed bile ductuoles and congested blood vessels. Furthermore, kidney sections showed congestion of renal blood vessels and proteinaceous materials in the lumen of renal tubules, as well as pyknosis of nuclei of renal tubular epithelium and and congestion of glomerular tuft.

The acquired results were in conformity with **Weber *et al.*, (2003)** who stated that CCL_4 has a hepatic toxic effect and causes critical liver injury such as necrosis and steatohepatitis. This consequence of strength is due to the liberation of free radicals, which are composed from trichloromethyl (CCL_3) and proxy trichloromethyl (OOCCL3) radicals. These free radicals can produce lipid peroxide which damage cell membranes, alter enzyme activities and lead to liver damage and death of most cells. In addition, **Naik and Panda (2007)** exhibit that CCl4-treated animals have a significant increase in serum activity of liver enzymes as an indication of liver deterioration. Further, **Mahmoed and Rezq (2013)**

and **Adewale *et al.*, (2014)** mentioned that CCl4 caused a significant increase in serum activity of AST, ALT and ALP enzymes, and the levels of TP, Alb and total, direct and indirect bilirubins, UN, Cr and UA. These elevations may be related to hepatic cholestasis and hepatocellular damage. Additionally, **Ruidong *et al.*, (2011)** proved that the CCl4-treated rats have a significant increase in serum levels of MDA and depletion in the activities of SOD, CAT, GSH and GPx enzymes.

With regard to the accomplishment of RRs on hepatotoxicity rats, the present findings state that feeding hepatotoxicity rats on a diet contains different levels (5, 10 and 15%) of RRs results in a significant increase in FI, BWG and RWG %, comparable to the positive control group fed on the basal diet alone. In addition, adding the three different levels of RRs to the diet successfully recovers liver and kidney functions and preserves their tissues from deterioration, as well as recovers the activities of the antioxidant enzymes and decreases the levels of lipid peroxidation. The highest results were established in treated heptotoxicty rats with moderate (10%) and higher levels (15%) of RRs respectively. The results of the biological examination concurred with the consequence of the histological investigation, which consequences incremental advancement with rising levels of RRs.

The current study was in agreement with the results of **Bahnasy, (2020)** who said that feed concumed, body weight gaind and feed efficiency ratio were improved in the rhubarb –feeding rats. As well, **Bu *et al*., (2018)** illustrated that RRs obviously diminished the serum activities of ALT, AST and ALP, as well as ameliorated the injury rate of liver fibrosis. Additionally, **Srinivasarao (2015)** concluded that RRs extract decreased serum levels of ALT, AST, ALP, TP and bilirubin. The hepatoprotective effect of RRs reduced liver alteration evidently directing antioxidant free radical scavenging properties. **Lai *et a*l., (2015)** mentioned that Anthraquinone as an active compound in Rhubarb remarkably reduces the serum concentrations of ALT, AST and MDA, and increase the activity SOD enzyme. **Hosseini *et al.*, (2017)** revealed that the extract of RRs enhanced the serum biological indexes related to the liver and kidney functions of diabetic rats.

Moreover, the obtained results were in agreement with **Lee *et al.*, (2012)** who discovered that RRs enhance the antioxidant status of GSH enzyme in CCl_4-induced hepatic injury, aiding with mitigating hepatocellular injury resulted by oxidation. Also, **Wang *et al.*, (2015)** found out that RRs have the capacity to scavenge free radicals, reduce liver MDA levels, improve total antioxidant capability, diminish lipid peroxidation and remediate cell membranes, and thus RRS has the benefit of protecting hepatocytes. **Hosseini *et al.*, (2018)** reported that RRS is a rich source of phenolic compounds with antioxidant properties. The administration of RRs extract prevents mercury-induced liver toxicity in rats as honoured by the lowering liver enzymes. This hepatoprotective effect was induced via suppression of oxidative stress. Recently, **Ghorbani *et al.*, (2021)** reporte that RRs extract decreased the blood levels of liver enzymes, total bilirubin, direct bilirubin, albumin, urea, creatinine, attenuated of oxidative stress and improved pathological changes in the liver.

The earlier studies identified the existence of multiple bioactive components of the RRs as enthrone, citreorosein, hydroxyanthraquinone, aloe-emodin, emodin, Rhein, chrysophanol, Byron, plumbagin, acylglucoside, chromenes, Steuben and resveratrol as well as glycoside of anthraquinone and chromone, and acids of protocatechuic and vanillic (**Shang *et al.*, 2019**). Furthermore, the bioactive antioxidant component includes polygons, Gallic acid, flavonoids, procyanidins, catechin, epicatechin, and tannin (**Rajkumar *et al.*, 2011**), marsupsin and maesopsin (**Krenn *et al.*, 2003**).

These results imply that rats feeding RRs possess a protective effect on the oxidative stress caused by CCl_4 injection. The mechanism by which RRs augment liver functions may be attributed to their antioxidant properties. **Ibrahim *et al.*, (2008)** reported that emodin components exhibited hepatoprotective activity in vitro and in vivo conditions in CCl_4- induced liver injury. Anthraquinone compounds showed to be principally accountable for the liver protective effects (**Wu *et al.*, 2014**). Additionally, **Lai *et al.*, (2015)** found out that the anthraquinone component in RRs hinders oxidative stress and hepaticoxidation by way of inhibiting lipid peroxidation, intracellular ROS and augmenting intracellular antioxidant action as well as scavenging oxygen-free radicals.

Conclusion

THE obtained results from the biochemical and histopathological investigation showed the Rhubarb roots have a potential positive hepatopotective, antioxidant and nephuroprotective effects contrary to the toxic emphasise resulted by CCl4. Nevertheless, in the eventual we need more researches specifically relevant to toxic effect of rhubarb in vivo. Moreover the scientific database needs to be created to understand the fundamental mechanisms of Rhubarb roots bioactivity.

References

1. **Adewale B., Adekeye O., Akintayo O., Onikanni A. and Saheed S. (2014)**: Carbon tetrachloride (CCl4)-induced hepatic damage in experimental Sprague Dawley rats: Antioxidant potential of *Xylopia aethiopica.* The J. of Phytopharmaco., 3(2): 118-123.
2. **Agarwal K., Singh S., Lakshmi V., Verma S. and Kumar S. (2001)**: Chemistry and pharmacology of rhubarb (Rheum species)—a review, J. Sci. Ind. Res., 60 (1): 1–9.
3. **Bahnasy R. (2020):** Rhubarb (Rheum emodi) improves some immunity markers and blood glucose in rats exposed to glucocorticoids. Al-Azahar J. of Home Econo., 36 (1): 111-128.
4. **Bancroft J. and Gamble M. (2002)**: Theory and practice of histological techniques,5th ed. John D. Bancroft, Marilyn Gamble, London, New York and Philadelphia.
5. **Blachier M., Leleu H ., Peck-Radosavljevic M., Valla C. and Roudot-Thoraval, F. (2013):** The burden of liver disease in Europe: a review of available epidemiological data. *Journal of hepatology*. 58(3): 593-608.
6. **Bu T., Wang C., Meng Q., Huo, X., Sun, H., Sun P. and Liu K. (2018)**: Hepatoprotective effect of rhein against methotrexate-induced liver toxicity. European Journal of Pharmacology. 834: 266-273.
7. **Burtis A. and Ashwood R. (1999)**: editors. Tietz Textbook of Clinical Chemistry, 3rd ed. Philadelphia, PA: WB Saunders.1169.
8. **Cao J., Pu J., Tang P., Shen J., Chen Y., Kang A., Zhou S. and Duan A. (2017):** Advances in bio-active constituents, pharmacology and clinical applications of rhubarb. Chin Med., 12 (36):1–12.
9. **Ceballos-Picot I., Trivier M., Nicole A., Sinet M. and Thevenin M. (1992):** Age correlated modifications of copper-zinc superoxide dismutase and glutathione-related enzyme activities in human erythrocytes. Clinical Chemistry. 38(1): 66-70.
10. **Cooke S., Main J.and Thursz, R. (2010).** Treatment for hepatitis B. Bmj., 340.
11. **de-Zwart L., Meerman H., Commandeur N. and Vermeulen P. (1999):** Biomarkers of Free Radical Damage Applications in Experimental Animals and Humans, Free Rad. Biol. Med. 26:202-226.
12. **Dhingra M., Nain P., Nain J. and Malik M. (2011):** Hepatotoxicity V/S hepatoprotective agents-a pharmacological review. Int Res J Pharm, 2, 31-37.
13. **Fei, Y., Wang, J., Peng B., Peng J., Hu H., Zeng P. and Yan L. (2017).** Phenolic constituents from Rheum nobile and their antioxidant activity. Natural product research, 31(24), 2842-2849.
14. **Friedman R.B. and D.S. (1997):** Young Effects of Disease on Clinical Laboratory Tests, 3rd Ed. Washington, DC; AACC Press.
15. **Ghorbani A., Amiri M. and Hosseini A.,(2019):** Ghorbani, A., Amiri, M. S., & Hosseini, A. (2019). Pharmacological properties of Rheum turkestanicum Janisch. Heliyon. 5(6): e01986.
16. **Ghorbani A., Hosseini A., Mirzavi F., Hooshmand S., Amiri, S., Jafarian, H. and Rajabian A. (2021):** Protective Effects of Rheum Turkestanicum Janischagainst Diethylnitrosamine-Induced Hepatocellular Carcinoma in Rats. a journal of Research Square, 2-23.
17. **Henry d. (1991):** Clinical Diagnosis and Management by Laboratory Methods, 18th Edition, W.B. Saunders, Philadelphia, PA.
18. **Hosseini A., Rajabian A., Fanoudi S., Farzadnia M. and Boroushaki M. (2018):** Protective effect of Rheum turkestanicum root against mercuric chloride-induced hepatorenal toxicity in rats. Avicenna Journal of Phytomedicine, 8(6), 488.
19. **Hosseini A., Mollazadeh H., Amiri S., Sadeghnia H. and Ghorbani A. (2017):** Effects of a standardized extract of Rheum turkestanicum Janischew root on diabetic changes in the kidney, liver and heart of streptozotocin-induced diabetic rats, *Biomed.Pharmacother*. 86–605–611.
20. **Ibrahim M., Khaja N., Aara A., Khan A., Habeeb A., Devi P. and Habibullah M. (2008):** Hepatoprotective activity of Sapindus mukorossi and Rheum emodi extracts: in vitro and in vivo studies. World Journal of Gastroenterology: WJG, 14(16), 2566.
21. **Kalisz S., Oszmiański J., Kolniak-Ostek J., Grobelna A., Kieliszek M. and Cendrowski A. (2020):** Effect of a variety of polyphenols compounds and antioxidant properties of rhubarb (Rheum rhabarbarum). Lwt, 118, 108775.
22. **Karthikeyan M. and Deepa M. (2010):** Hepatoprotective effect of Premna corymbosa (Burm f.) Rottl. and Willd leaves extract on CCL4 induced Hepatic Demage in wistar albino rats. Asian Pacific J. Trop. Med., 3: 17-20.
23. **Kim Y., Kim K., Choi H., Jung Y., Oh Y., Kim C. and Lee M. (2010).** Hepatoprotective effect of pinoresinol on carbon tetrachloride–induced hepatic damage in mice. Journal of pharmacological sciences, 112(1), 105-112.
24. **Kratochvílova M., Hyankova L., Knizetova H., Fiedler J. and Urban F. (2002):** Growth curve analysis in cattle from early maturity and mature body size viewpoints. *Czech J. Anim. Science* 47 4: 125–132.
25. **Krenn L., Presser A., Pradhan R., Bahr B. and Paper DH. (2003):** Sulfemodin 8-O-b-D-glucoside, a new sulfated anthraquinone glycoside, and antioxidant phenolic compounds from Rheum emodi. J Nat Prod 66:1107–1109.
26. **Lai F., Zhang Y., Xie DP., Mai ST. and Weng YN. (2015):** A systematic review of rhubarb (a traditional Chinese medicine) used for the treatment of experimental sepsis. Evid Based Complement Altern Med:1–12.
27. **Lee H., Huang Y., Duh D. and Wu C. (2012)**: Hepatoprotection of emodin and Polygonum multiflorum against CCl4-induced liver injury. Pharmaceutical biology, 50(3), 351-359.
28. **Mahmoed Y and Rezq A. (2013).** Hepatoprotective Effect of Avocado Fruits Against Carbon Tetrachloride-Induced Liver Damage in Male Rats. World Applied Science Journal 21 (10): 1445-1452.
29. **Naik R. and Panda S. (2007).** Antioxidant and hepatoprotective effects of Ginkgo biloba phytosomes in carbon tetrachloride - induced liver injury in rodents. Liver international, 27(3), 393-399.
30. **Ozturk F., Ucar M., Ozturk C., Vardi N. and Batcioglu K. (2003):** Carbon tetrachloride-induced nephrotoxicity and protective effect of betaine in Sprague-Dawley rats. Urology, 62(2), 353-3.
31. **Rajkumar V., Guha G. and Kumar RA (2011):** Antioxidant and anti-cancer potentials of Rheumemodi rhizome extracts. Evid BasedComplementary Altern Med 697986:1–9.
32. **Reeves P., Nielsen F. and Fahmy G. (1993):** AIN-93. Purified diets for laboratory rodents: Final reports of the American Institute of Nutrition adhoe 9 wriling committee of reformulation of the AIN-76 A Rodent Diet. J. Nutr., 123: 1939-1951.
33. **Ruidong G., Wenyuan F., Zhiren D. Guoshan and Zhengxin W. (2011):** Hepatoprotective action of radix paeoniae rubra aqueous extract against CCl4-induced hepatic damage. Molecules, 16: 8684-8693.
34. **Ruirui L., Wang A., Tian X., Wang D. and Liu J. (2010):** Uniformity of karyotypes in Rheum (Polygonaceae), a species-rich genus in the Qinghai-Tibetan Plateau and adjacent regions. Caryologia, 63(1), 82-90.
35. **Shang XF., Zhaoa ZM. and Li JC. (2019):** Insecticidal and antifungal activities of Rheum palmatum L. anthraquinones and structurally related compounds. Ind Crop Prod 137:508–520.

36. Singh, P. and Rawat M. (2016): Phytochemistry and biological activity perspectives of Rheum species. The natural products journal, 6(2), 84-93.
37. Sivakrishnan S. and Pharm M., (2019): Liver disease overview. World Journal of Pharmacy and Pharmaceutical Sciences, 8(1), 1385-1395.
38. Srinivasarao M. (2015): Hepatoprotective Potential of Total Anthraquinones Fraction of Rheum emodi: In vitro and In vivo Studies. Journal of Medical and Pharmaceutical Innovation, 2(11):15-22.
39. Taheri G. and Assadi, M. (2013): A synopsis of the genus Rheum (Polygonaceae) in Iran with description of three new species. (1)14 93-85
40. Tietz W. (1994): Specimen Collection and Processing; Sources of Biological Variation in Textbook of Clinical Chemistry, 2nd Ed. Philadelphia; W.B. Saunders.
41. Unsal V., Cicek M. and Sabancilar I. (2021): Toxicity of carbon tetrachloride, free radicals and role of antioxidants. Rev Environ Health 2021; 36(2): 279–295.
42. Wang W., Guo M., Ma D. and Wang Q. (2015): Effects of Rhubarbs from different regions on blood lipid and autoxidation of hyperlipidemia rats. Zhongguo Ying Yong Sheng li xue za zhi= Zhongguo Yingyong Shenglixue Zazhi= Chinese Journal of Applied Physiology, 31(3), 278-281.
43. Weber L., Boll M. and Stampfl A. (2003): Hepatotoxicity and mechanism of action of haloalkanes: Carbon tetrachloride as a toxicological model. Crit. Rev. Toxicol., 33: 105-136.
44. Wheeler R., Salzman A., Elsayed M., Omaye T. and Korte W. (1990): Automated assays for superoxide dismutase, catalase, glutathione peroxidase, and glutathione reductase activity. Analytical biochemistry, 184(2), 193-199.
45. Wojcikowski K., Johnson W. and Gobe G. (2004): Medicinal herbal extracts - renal friend or foe:part two: herbal extracts with potential renal benefits. Nephrol 9:400–405.
46. Wu W., Hu N., Zhang Q., Li Y. and Li P. (2014): In vitroglucuronidation of five rhubarb anthraquinones by intestinal and liver microsoms from humans and rats. Chem Biol Interact 219:18–27.
47. Young S. (1997): Effects of Preanalytical Variables on Clinical Laboratory Tests, 2nd Edition, AACC Press, Washington, D.C.
48. Young S. (2000): Effects of Drugs on Clinical Laboratory Tests, fifth edition, AACC Press, Washington, D.C.