ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

Therapeutic effects of curcumin and royal jelly as natural antioxidants on some biochemical parameters in hepatotoxicity induced by carbon tetrachloride (CCl_4) in male albino rats

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Manuscript Info

Manuscript History:

Received: 14 September 2015
Final Accepted: 19 October 2015
Published Online: November 2015

Key words:

curcumin; royal jelly; carbon tetrachloride; oxidative stress; antioxidants biomarker, liver functions

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Abstract

The present study was designed to determine the possible therapeutic effects of curcumin, royal jelly and both against carbon tetrachloride (CCl_4) induced oxidative stress and biochemical changes in liver of albino rats. A patch of 135 male Wister albino rats averaged weights (190±10 g) at the beginning of the experiment were divided into 9 main groups according to the treatment and requirements of the experiment. The rats injected i.p. with CCl_4 at dose (2 mL/kg, 1:1 in olive oil) twice per week and received, via gavage, curcumin at dose (150 mg/kg b.wt), royal jelly at dose (500 mg/kg b.wt) and the combined supplementation of both curcumin (150 mg/kg b.wt) and royal jelly (500 mg/kg b.wt) along the experimental period. Each group contains 15 rats and five rats were sacrificed on the 2nd, and 4th and 6th week from each group.

The results refer to a significant elevation of some hepatic parameters (TBARS, ASAT, ALAT, and ALP) while a significant decrease in some other parameters (Total protein, albumin, CAT, GSH and SOD). on the 2nd ,4th and 6th week in rats injected intraperitoneal with CCl_4 as compared to the control groups.

The administration of the curcumin, royal jelly or both had beneficial and decrease side effects against the deleterious changes of CCl_4.

In conclusion, according to the results obtained the administration of the curcumin and royal jelly or both provides considerable hepatoprotective and hepatotherapeutic effects against intoxicated with CCl_4 in male Wister albino rats by preventing oxidative stress through ROS scavenger and improvement in the former biochemical parameters.

INTRODUCTION

The liver plays a crucial role in metabolisms of endogenous and exogenous substances, and the hepatic injury is associated with distortion of these functions. Liver function is generally impaired by xenobiotics or infections. Chronic or excessive exposure of xenobiotics is finally terminated to cirrhosis or malignant lesions in untreated cases. Nowadays, many people are suffering from hepatic damage induced by alcohol, chemicals and infections. Thus, acute and chronic liver diseases continue to be serious health problems in the world. Some natural compounds are demonstrated to have a protective role against liver diseases **(Pramyothin *et al*., 2007; Wan *et al*., 2009 and Shaker *et al*., 2010).** Hence, there has been considerable interest in role of naturally originated agents for the treatment of liver disease.

Carbon tetrachloride (CCl_4) is one of the oldest and most widely used toxins for experimental induction of liver fibrosis in laboratory animals (**Tsukamoto *et al.*, 1990).** This model has been used in various studies on examined the deposition of extracellular matrix in the fibrotic and cirrhotic liver **(Hernandez-Munoz *et al*.,1994; Muriel et al., 1998)**. CCl_4 is a selective hepatotoxic chemical agent. CCl_4- induced reactive free radicals initiate cell damage through two different mechanisms of covalent binding to the membrane proteins and cause lipid peroxidation **(Parola *et al.*, 1992).** Production of reactive oxygen species and lipid peroxidation induced by iron overload **(Bacon and Britton, 1990),** cholestatic injury **(Parola *et al.*, 1996)** and intoxication by ethanol **(Kamimura *et al.*, 1992)** and CCl_4 is associated with liver fibrosis and cirrhosis.

Sanmugapriya and Venkataraman (2006) and Shaker *et al.*, (2010) proved that natural products containing antioxidants protect liver against peroxidation of lipids and depreciation of the antioxidant status induced by CCl_4.

Curcumin has a hepatocyte-protective effect against carbon tetrachloride and D-galactosamine in-vitro **(Donatus *et al*., 1990 and Kiso *et al*., 1983).** Royal Jelly (RJ) is a secretion of the hypopharyngeal and mandibular glands of worker honeybees, and it plays an important role in queen honeybee feeding and development. RJ contains several substances including protein, sugar, lipids, vitamin, salt, free amino acids and water **(Tamura *et al.*, 2009).** RJ is widely used product for a variety of medicinal, antioxidant and nutritional purposes **(Viuda-Martos *et al*., 2008)**.

Materials and methods

Experimental design:

135 male Wister albino rats' averaged weights (190±10 g) were conducted in accordance with the criteria of the investigations and Ethics Committee of the Community Laws governing the use of experimental animals. The rats obtained from the Egyptian Holding Company for Biological Products and Vaccines were used as experimental animals. The rats were transferred to the animal house in Zoology Department, Faculty of Science, Al-Azhar University, and placed in regular designed cages and maintained in conditions of good ventilation, normal temperatures, and humidity range. Five rats were placed into each cage. Food and water were provided *adlibitum* to the animals.

The rats were classified in to main nine groups and 27 sub-groups as follow: **Group 1**: Normal control, **Group II**: Rats were treated orally with olive oil with (5 ml/kg body weight) **(Fang *et al*., 2008)**, **Group III** Rats were treated orally with curcumin in dose (150 mg/kg) suspended in olive oil daily, **Group IV** Rats treated orally with royal jelly in dose (500 mg/kg) suspended in distilled water daily, **Group V** Rats treated orally with curcumin and royal jelly in doses (150 mg/kg and 500 mg/kg) daily, **Group VI** Rats injected intraperitoneal with CCl_4 in dose (2ml/kg) twice a weekly diluted in olive oil (1:1) for a period six weeks. **Group VII** Rats injected with CCl_4 twice weekly and treated orally with curcumin daily. **Group VIII** Rats injected with CCl_4 twice weekly and treated orally with royal jelly daily. **Group IX** Rats injected with CCl_4 twice weekly and treated orally with curcumin and royal jelly daily.

All treatments were given for six weeks. The sign of toxicity were recorded daily during the experimental period. Each group contain 15 rats, five rats from each group were anesthetized and scarified after the 2^{nd}, 4^{th}, and 6^{th} week for biochemical parameters.

Induction of hepatotoxicity:

Hepatotoxicity was induced by Interperetonial injection of CCl_4 (2 ml/kg) diluted in olive oil (50 % v/v) twice weekly for 6 consecutive weeks**.(Tu *et al.* 2012)**.

Sample preparation

All samples should be prepared before reconstitution of the reagents.

1. Wash the liver tissue in physiological saline solution, pH 7.4.
2. Homogenize the tissue in 10 ml cold phosphate buffer pH 7.4.
3. Centrifuge the homogenate tissue at 4,000 rpm for 15 minutes at 4 °C.
4. Collect supernatant, If not assayed immediately store the supernatant at -80°C.

Blood samples were collected without anti-coagulants and centrifuged at 4000 r.p.m for 10 minutes to prepare serum. The sera were frozen at -20 ºC until used.

Biochemical parameters:

The serum levels of transaminases, alkaline phosphatase, total protein and albumin were estimated using kits from Elitech diagnostic Co. France. The concentrations of transaminases (ASAT and ALAT) were determined using the method of **Bergmeyer *et al*. (1986)**. Serum ALP was determined according to the method described by **the German Society for Clinical Chemistry (1972)**. Serum total protein was determined according to the method described by **Gornal *et al* (1949)**. Serum albumin was determined according to the method described by **Doumas *et al* (1971)**.

Lipid peroxidation product, malondialdehyde (MDA), was measured by thiobarbituric acid (TBARS) assay, which is based on the determination of malondialdehyde (MDA), an end product of lipid peroxidation, which can react with thiobarbituric acid to yield a pink colored complex exhibiting a maximum absorption at 534nm **(Yoshioka et al., 1979)**

Glutathione was determined according to the method of **Beutler et al. (1963).** This method is based on the reduction of 5, 5` dithiobis (2 - nitrobenzoic acid) (DTNB) with glutathione (GSH) to produce a stable yellow compound. The reduced chromogen directly proportional to GSH concentration and its observance can be measured at 412 nm.

The activities of CAT and SOD were estimated using kits from bio-diagnostic for research kits, Egypt. The SOD activity was assayed according to the procedure described by Minami and Yoshikawa (1979) and the assay of CAT activity was determined according to the method of **Aebi (1984).**

Statistical analysis

The statistical package for social sciences SPSS/PC computer program (version 19) was used for statistical analysis of the results. Data were analyzed using one-way analysis of variance (ANOVA). The data were expressed as mean ± S.E. Differences were considered statistically significant at ($P < 0.05$).

Results:

CCl_4 induced hepatic damage as reflected by significantly ($p < 0.05$) elevated serum ALAT, ASAT, and ALP enzymes activities when compared to control group after 2^{nd}, 4^{th}, and 6^{th} week. On the other hand, insignificant differences with recorded in cur, RJ and cur+Rj when compared to control groups. Rats treated with CCl_4+CUR, CCl_4+RJ and CCl_4+CUR+RJ observed a significant decrease ($p < 0.05$) when compared with CCl_4 groups after 2^{nd}, 4^{th}, and 6^{th} week. As shown in Table (1, 2 and 3).

Data presented in table (4,5) recorded that a significant decrease ($p < 0.05$) in serum total protein and albumin level in rats injected with CCl_4 when compared with control groups after 2^{nd}, 4^{th} and 6^{th} week. On contrast insignificant differences with recorded in cur, RJ and CUR+RJ when compared to control groups. Rats treated with CCl_4+CUR, CCl_4+RJ and CCl_4+CUR+RJ observed a significant increase ($p < 0.05$) when compared with CCl_4 groups after 2^{nd}, 4^{th}, and 6^{th} week.

Resulted data which found in table (6) showed a significantly increase ($p < 0.05$) in MDA level in CCl_4 groups, as compared with control group after 2^{nd},4^{th}, and 6^{th} week. On the other hand, insignificant differences with recorded in cur, RJ and CUR+RJ when compared to control groups. Rats treated with CCl_4+CURr, CCl_4+RJ and CCl_4+CUR+RJ observed a significant decrease ($p < 0.05$) when compared with CCl_4 groups after 2^{nd}, 4^{th}, and 6^{th} week.

Statistical data in table (7, 8 and 9) recorded a significant decrease ($p < 0.05$) in hepatic CAT, GSH and SOD activities in rats injected with CCl_4 when compared with control groups after 2^{nd}, 4^{th} and 6^{th} week. On contrast insignificant differences with recorded in cur, RJ and CUR+RJ when compared to control groups. Rats treated with CCl_4+CUR, CCl_4+RJ and CCl_4+CUR+RJ observed a significant increase ($p < 0.05$) when compared with CCl_4 groups after 2^{nd}, 4^{th}, and 6^{th} week.

Table (1): Serum ALAT activity (IU/L)of male albino rates subjected to carbon tetrachloride (CCl_4) toxicity and treated with curcumin and royal jelly for 2,4 and 6 weeks.

	ALAT			
Groups		2nd week	4th week	6th week
Control	Mean± S.E	58.00 ± 2.7a	54.00 ± 7.7a	54.80±2.0a
Olive oil	Mean± S.E	43.00 ± 6.1a	35.80 ± 3.4a	45.50±2.4a
	%	-25.9	-33.7	-17
CUR	Mean± S.E	52.20 ± 2.7a	59.20 ± 7.3a,g	54.00±7.1a
	%	-10	9.6	-1.6
RJ	Mean± S.E	46.80 ± 3.9a	42.20±4.5a	52.20±2.3a
	%	-19.3	-21.9	-4.7
CUR+RJ	Mean± S.E	45.6 ± 4.4a	46.0±3.3a	56.2±3.2a,g
	%	-21.4	-14.8	2.6
CCl_4	Mean± S.E	110.40 ± 4.4b	338.80±33.7c	494.00±42.4d
	%	89.7	527.4	801.5
CCl_4+CUR	Mean± S.E	96.60 ± 2.8b	291.80±8.8e	159.80±6.6f
	%	66.6	438.9	191.6
CCl_4+RJ	Mean± S.E	88.20 ± 6.8b,g	225.20±9.0h	177.00±7.9f
	%	52.1	316.7	223
CCl_4+CUR+RJ	Mean± S.E	93.40 ± 4.7b	151.40±15.3f	98.00±2.6b
	%	61	180.4	78.8
F value		83.727***		

Each value represented means of 5 records ± S.E.
$^{a,b,c,d.e}$ means comparison between all groups which the groups have the same letter mean there is no significance difference and which have different letter mean there is a significance change.
%: Percent of changes from control values. – CCl_4: carbon tetrachloride. –CUR: curcumin - RJ: royal jelly.

Table (2): Serum ASAT enzyme activity (IU/L) of male albino rates subjected to carbon tetrachloride (CCl_4) toxicity and treated with curcumin and royal jelly for 2,4 and 6 weeks.

	ASAT			
Groups		**2nd week**	**4th week**	**6th week**
Control	Mean± S.E	123.00 ± 2.6[a]	122.00±1.4[a]	120.80±3.0[a]
Olive oil	Mean± S.E	112.20 ± 2.4[a]	110.60±1.3[a]	111.60±2.8[a]
	%	-8.78	-9.34	-7.62
CUR	Mean± S.E	124.60 ± 0.9[a]	111.00±2.0[a]	115.20±1.6[a]
	%	1.3	-9.02	-4.64
RJ	Mean± S.E	116.00 ± 1.1[a]	112.80±2.7[a]	111.40±2.6[a]
	%	-5.69	-7.54	-7.78
CUR+RJ	Mean± S.E	119.60 ± 2.1[a]	108.60±1.9[a]	106.60±2.4a
	%	-2.76	-10.98	-11.8
CCl_4	Mean± S.E	247.20 ± 4.6b	464.20±16.5c	1208.40±30.8d
	%	100.8	280.5	900.3
CCl_4+CUR	Mean± S.E	214.00 ± 7.0[e,g]	265.60±5.4b	373.40±5.8f
	%	75.8	117.7	209.1
CCl_4+RJ	Mean± S.E	196.80 ± 6.1[e,h]	304.80±8.0i	353.40±7.7k
	%	74	149.8	192.5
CCl_4+CUR+RJ	Mean± S.E	187.80 ± 5.3h	223.00±4.7g	182.00±3.6h
	%	52.7	82.8	50.7
F value		791.073***		

Each value represented means of 5 records ± S.E.
[a,b,c,d.e] means comparison between all groups which the groups have the same letter mean there is no significance difference and which have different letter mean there is a significance change.
%: Percent of changes from control values. – CCl_4: carbon tetrachloride. –CUR: curcumin - RJ: royal jelly.

Table (3): Serum alkaline phosphatase (ALP) enzyme activity (IU/L) of male albino rates subjected to carbon tetrachloride (CCl_4) toxicity and treated with curcumin and royal jelly for 2,4 and 6 weeks.

	ALP			
Groups		**2nd week**	**4th week**	**6th week**
Control	Mean± S.E	83.00 ± 1.5a	77.60±1.4a,b	78.80±1.9a,b
Olive oil	Mean± S.E	78.00 ± 4.1a,b	76.20±2.3a,b	75.80±2.0a,b
	%	-6.0	-1.8	-3.8
CUR	Mean± S.E	74.80 ±2.1a,b	74.00±2.8b	76.00±3.6[a],b
	%	-10	-4.6	-3.6
RJ	Mean± S.E	73.40 ± 3.5[b]	73.00±1.9b	76.60±3.5a,b
	%	-11.6	-5.9	-2.8
CUR+RJ	Mean± S.E	71.60 ± 2.7[b]	70.80±3.6[b]	74.80±2.4a,b
	%	-13.7	-8.8	-5.1
CCl_4	Mean± S.E	171.20 ± 4.6c	217.40±6.1d	274.60±3.9e
	%	106.3	180.2	248.5
CCl_4+CUR	Mean± S.E	154.40 ± 2.4f,i	168.60±1.6c,g	185.80±3.6h
	%	86.0	117.3	135.8
CCl_4+RJ	Mean± S.E	146.60 ± 2.3f,k	160.20±2.4f,g,i	190.00±5.8h
	%	76.6	106.4	141.1
CCl_4+CUR+RJ	Mean± S.E	142.00 ± 2.1k	156.20±2.5i	131.00±5.1L
	%	71.1	101.3	66.2
F value		318.451***		

Each value represented means of 5 records ± S.E.
[a,b,c,d.e] means comparison between all groups which the groups have the same letter mean there is no significance difference and which have different letter mean there is a significance change.
%: Percent of changes from control values. – ccl4: carbon tetrachloride. –cur: curcumin - Rj : royal jelly.

Table (4): Serum total proteins level(g/dl) of male albino rates subjected to carbon tetrachloride (CCl_4) toxicity and treated with curcumin and royal jelly for 2,4 and 6 week.

	Total protein			
Groups		**2nd week**	**4th week**	**6th week**
Control	Mean± S.E	7.24 ±0.08[a]	7.18±0.09[a]	7.10±0.05[a]
Olive oil	Mean± S.E	7.14±0.07a	7.19±0.08a	7.28±0.04a
	%	-1.4	0.14	2.5
CUR	Mean± S.E	7.22±0.06a	7.12±0.11a	7.08±0.11a
	%	-0.28	-0.84	-0.3
RJ	Mean± S.E	7.14 ±0.10a	7.14±0.11a	7.34±0.08a
	%	-1.4	-0.56	3.4
CUR+RJ	Mean± S.E	7.18 ±0.08a	7.17±0.09a	7.20±0.15a
	%	-0.83	-0.14	1.4
CCl_4	Mean± S.E	4.96±0.07b	3.52±0.40c	2.82±0.42d
	%	-31.5	-51.0	-60.3
CCl_4+CUR	Mean± S.E	4.98 ±0.07b,e	5.26±0.32b,e	6.20±0.24f
	%	-31.2	-26.7	-12.7
CCl_4+RJ	Mean± S.E	4.92 ±0.16b	5.48±0.22e,g	5.80±0.18f,e
	%	-32.0	-23.7	-18.3
CCl_4+CUR+RJ	Mean± S.E	5.18 ±.15b,g	6.26±0.36f	6.08±0.09f
	%	-28.5	-12.8	-14.4
F value		46.701***		

Each value represented means of 5 records ± S.E.
[a,b,c,d.e] means comparison between all groups which the groups have the same letter mean there is no significance difference and which have different letter mean there is a significance change.
%: Percent of changes from control values. -CCl_4: carbon tetrachloride. –CUR: curcumin - RJ : royal jelly.

Table (5): Serum albumin level (g/dl) of male albino rates subjected to carbon tetrachloride (CCl_4) toxicity and treated with curcumin and royal jelly for 2,4 and 6 weeks.

	Albumin			
Groups		**2nd week**	**4th week**	**6th week**
Control	Mean± S.E	4.22 ± 0.06a,c	4.16±0.04c	4.14±0.07c
Olive oil	Mean± S.E	4.24 ± 0.05a,c,b	4.18±0.10a,c	4.46±0.05b
	%	0.5	0.5	7.7
CUR	Mean± S.E	4.24 ± 0.11a,c,b	4.20±0.10a,c	4.30±0.05a,b,c
	%	0.5	1.0	3.9
RJ	Mean± S.E	4.40 ± 0.07a,b	4.14±0.06c	4.22±0.05a,c
	%	4.3	-0.5	1.9
CUR+RJ	Mean± S.E	4.32 ± 0.06a,b,c	4.18±0.10a,c	4.45±0.07b
	%	2.4	0.5	7.5
CCl_4	Mean± S.E	3.12 ± 0.07e,i	2.22±0.07f	1.56±0.16g
	%	-26.1	-46.6	-62.3
CCl_4+CUR	Mean± S.E	3.36 ± 0.05h,k,L	3.06±0.07e	3.54±0.10d,h,L
	%	-20.4	-26.4	-14.5
CCl_4+RJ	Mean± S.E	3.26 ± 0.08e,k,i	3.16±0.08e,k,i	3.32±0.07i,h
	%	-22.7	-24.0	-19.8
CCl_4+CUR+RJ	Mean± S.E	3.28 ± 0.06e,k,i	3.56±0.16d,L	3.74±0.08d
	%	-22.3	-14.4	-9.7
F value		73.398***		

Each value represented means of 5 records ± S.E.
a,b,c,d.e means comparison between all groups which the groups have the same letter mean there is no significance difference and which have different letter mean there is a significance change.
%: Percent of changes from control values. -CCl_4: carbon tetrachloride. –CUR: curcumin - RJ: royal jelly.

Table (6): Hepatic malondialdehyde (MDA) concentration in tissues (nmole/gm) of male albino rates subjected to carbon tetrachloride (CCl_4) toxicity and treated with curcumin and royal jelly for 2,4 and 6 weeks.

	Liver TBARS (MDA)			
Groups		**2nd week**	**4th week**	**6th week**
Control	Mean± S.E	202.34±2.7a	199.95±2.0a	200.97±2.0a
Olive oil	Mean± S.E	199.60±3.1a	198.49±2.7a	202.22±3.5a
	%	-1.35	-0.73	0.62
CUR	Mean± S.E	199.16±3.4a	196.96±2.4a	195.49±4.0a
	%	-1.57	-1.49	-2.73
RJ	Mean± S.E	194.99±2.6a	195.91±5.3a	200.29±3.7a
	%	-3.63	-2.02	-0.34
CUR+RJ	Mean± S.E	196.84±3.8a	197.45±2.5a	201.64±3.7a
	%	-2.72	-1.25	0.33
CCl_4	Mean± S.E	280.99±3.3b	333.37±2.8c	411.68±4.1d
	%	38.9	66.7	104.8
CCl_4+CUR	Mean± S.E	263.64±1.9e	298.44±2.5f	329.35±1.2c
	%	30.3	49.3	63.9
CCl_4+RJ	Mean± S.E	262.52±1.5e	299.64±2.5f	327.44±1.7c
	%	29.7	49.9	62.9
CCl_4+CUR+RJ	Mean± S.E	260.86±2.1e	278.43±2.2b	293.79±5.0f
	%	28.9	39.2	46.2
F value		381.618***		

Each value represented means of 5 records ± S.E.
a,b,c,d.e means comparison between all groups which the groups have the same letter mean there is no significance difference and which have different letter mean there is a significance change.
%: Percent of changes from control values. -CCl_4: carbon tetrachloride. –CUR: curcumin - RJ: royal jelly.

Table (7): Liver catalase (CAT) activity (Unit/mg wet tissue)of male albino rates subjected to carbon tetrachloride (CCl_4) toxicity and treated with curcumin and royal jelly for 2,4 and 6 weeks

	CAT			
Groups		2nd week	4th week	6th week
Control	Mean± S.E	1.49±0.03a,b	1.56±0.04 a,b	1.52±0.01 a,b
Olive oil	Mean± S.E	1.53±0.03 a,b	1.57±0.03b	1.60±0.01b
	%	2.68	0.64	5.26
CUR	Mean± S.E	1.52±0.03 a,b	1.59±0.03b	1.61±0.03b
	%	2.01	1.92	5.92
RJ	Mean± S.E	1.59±0.03b	1.58±0.02b	1.63±0.02b
	%	6.71	1.28	7.24
CUR+RJ	Mean± S.E	1.55±0.04a,b	1.54±0.03a,b	1.59±0.02b
	%	4.03	-1.28	4.61
CCl_4	Mean± S.E	1.12±0.02c,k	0.74±0.06d	0.49±0.03e
	%	-24.8	-52.6	-67.8
CCl_4+CUR	Mean± S.E	1.41±0.03a,f,h	0.86±0.10d,g	0.97±0.08c,g
	%	-5.4	-44.9	-36.2
CCl_4+RJ	Mean± S.E	1.33±0.02f,h,L	0.81±0.14d,g	0.92±0.05g
	%	-10.7	-48.1	-39.5
CCl_4+CUR+RJ	Mean± S.E	1.46±0.04a,b,h	0.92±0.14g	1.18±0.13k,L
	%	-2.01	-41.0	-22.4
F value		31.671***		

Each value represented means of 5 records ± S.E.
a,b,c,d.e means comparison between all groups which the groups have the same letter mean there is no significance difference and which have different letter mean there is a significance change.
%: Percent of changes from control values. -CCl_4: carbon tetrachloride. –CUR: curcumin - RJ: royal jelly.

Table (8): Hepatic reduced glutathione (GSH) concentration (n mole/gm wet tissue) of male albino rates subjected to carbon tetrachloride (CCl_4) toxicity and treated with curcumin and royal jelly for 2,4 and 6 weeks.

		GSH		
Groups		2nd week	4th week	6th week
Control	Mean± S.E	2.918±0.04a	2.915±0.02a	2.926±0.02a
Olive oil	Mean± S.E	2.924±0.03a	3.016±0.04a	2.938±0.04a
	%	0.20	3.46	0.41
CUR	Mean± S.E	2.936±0.02a	2.970±0.03a	2.920±0.02a
	%	0.62	1.89	-0.21
RJ	Mean± S.E	2.964±0.03a	2.948±0.05a	3.014±0.04a
	%	1.58	1.13	3.01
CUR+RJ	Mean± S.E	2.945±0.03a	2.982±0.04a	2.982±0.3a
	%	0.93	2.30	1.91
CCl_4	Mean± S.E	2.418±0.04b	1.958±0.02c	1.164±0.06d
	%	-17.14	-32.83	-60.22
CCl_4+CUR	Mean± S.E	2.562±0.05e	2.120±0.05f,h	1.832±0.02g
	%	-12.20	-27.27	-37.39
CCl_4+RJ	Mean± S.E	2.518±0.03e	2.106±0.04f	1.822±0.03g
	%	-13.71	-27.75	-37.73
CCl_4+CUR+RJ	Mean± S.E	2.540±0.02e	2.220±0.03h	2.120±0.06f,h
	%	-12.95	-23.84	-27.55
F value		187.937***		

Each value represented means of 5 records ± S.E.
a,b,c,d.e means comparison between all groups which the groups have the same letter mean there is no significance difference and which have different letter mean there is a significance change.
%: Percent of changes from control values.-CCl_4: carbon tetrachloride. –CUR: curcumin -RJ: royal jelly.

Table (9): Liver superoxide dismutase (SOD) activity (Unit/mg wet tissue) of male albino rates subjected to carbon tetrachloride (CCl_4) toxicity and treated with curcumin and royal jelly for 2,4 and 6 weeks.

	Hepatic SOD			
Groups		2nd week	4th week	6th week
Control	Mean± S.E	56.28±2.0a,b	56.50±1.6a,b	55.14±2.4a,b
Olive oil	Mean± S.E	53.84±1.9a	57.62±2.0a,b	56.58±1.3a,b
	%	-4.34	1.98	2.61
CUR	Mean± S.E	55.99±2.6a,b	54.38±2.2a,b	54.73±1.8a,b
	%	-0.52	-3.75	-0.74
RJ	Mean± S.E	56.11±2.4a,b	57.05±1.7a,b	54.72±2.2a,b
	%	-0.30	0.97	-0.76
CUR+RJ	Mean± S.E	58.64±1.6a	57.99±1.8a,b	56.15±1.7a,b
	%	4.19	2.64	1.83
CCl_4	Mean± S.E	43.79±0.75c,f,h	30.78±0.74d	21.44±0.69e
	%	-22.19	-45.52	-61.12
CCl_4+CUR	Mean± S.E	44.28±0.68c,h	36.34±0.62g	40.64±0.69c,g
	%	-21.32	-35.68	-26.30
CCl_4+RJ	Mean± S.E	47.00±0.88h	38.98±0.63g,i	40.43±0.59c,g
	%	-16.49	-31.01	-26.68
CCl_4+CUR+RJ	Mean± S.E	47.10±1.15h	39.70±0.51f,g,i	43.03±0.70c,h,i
	%	-16.31	-29.73	-21.96
F value	40.113***			

Each value represented means of 5 records ± S.E.
a,b,c,d.e means comparison between all groups which the groups have the same letter mean there is no significance difference and which have different letter mean there is a significance change.
%: Percent of changes from control values. -CCl_4: carbon tetrachloride. –CUR: curcumin - RJ: royal jelly.

Discussion:

The Present study was conducted to evaluate the combined effect of the curcumin and royal jelly against CCl_4-induced hepatic disorders in rat. Chronic hepatic injury by carbon tetrachloride is a well-established animal model of liver fibrosis. Reactive oxygen species and oxidative stress have been shown to play an important role in the etiopathogenesis of the hepatic fibrotic changes, and antioxidant treatment in vivo seems to be effective in preventing or reducing chronic liver damage and fibrosis **(Wang *et al.*, 2013). The results refer to a significant increase in serum ALAT, ASAT and ALP enzymes these increase may be due to** the release of hepatocyte cytosolic enzymes such as ALAT and ASAT in the blood Their appearance in blood does not necessarily indicate cell death and also that enzyme release during reversible cell damage occurs with an apparent lack of histological evidence of necrosis **(Solter , 2005).** Inadequacy of treatment with conventional drugs and possible hazards associated with their use prompted our search for better and safer hepatoprotective of herbal origin **(Ozbek *et al.*, 1991)** As measurement of serum levels of enzymes such as ALAT and ASAT renders a reliable means of assessment of liver damage **(Gupita *et al.*, 2004).**

The levels of marker enzymes such as ASAT, ALAT, and ALP increase in serum. This increase is mitigated by treatment with curcumin and royal jelly. Curcumin, by scavenging or neutralizing free radicals, inhibits peroxidation of membrane lipids and maintains cell membrane integrity and their function **(Rukkumani *et al.*, 2003).** royal jelly have antioxidant effect and its ability to act as a free radical scavenger in case the cisplatin hepatotoxicity, thereby protecting membrane permeability **(Ashry and Elkady, 2014)**. Curcumin and royal jelly stabilize cell membrane integrity and prevent the increase of these marker enzymes.

Liver injury induced by CCl_4 is a classical system of xenobiotic-induced hepatotoxicity and has been used extensively for decades for the screening of antihepatotoxic/hepatoprotective activities of different drugs (Alqasoumi, 2010). Oxidative stress has been postulated as a major molecular mechanism involved in experimental animal models. It is well known that CCl_4 is activated by the cytochrome P450 system. The initial metabolite is the trichloromethyl free radical, which is believed to stimulate the biochemical events that ultimately culminate in liver cell necrosis **(Lin *et al.*, 2000).** In response to hepatocellular injury initiated by the biotransformation of CCl_4 into reactive radicals, "activated" Kupffer cells respond by releasing increased amount of active oxygen species and other bioactive agents **(Yam *et al.*, 2007).** Radical formation and lipid peroxidation are the predominant cellular mechanisms involved in the development of fatty liver caused by CCl_4 **(Tribble *et al.*,1987).**

Serum hepatobilliary enzymes such as ASAT, ALAT and ALP are present in high concentrations in the liver under normal conditions. When there is hepatocyte necrosis or membrane damage, these enzymes will be released into the circulation, as indicated by elevated serum enzyme levels **(Drotman and Lawhorn, 1978)** In the present study, the elevated levels of all these marker enzymes observed in CCl_4-treated rats indicate liver damage induced by hepatotoxins. Treatment of curcumin and royal jelly to CCl_4-induced rats ameliorated the toxic effects of CCl_4 and the above markers restored towards the normal level. This effect may be free radical scavenging activity of curcumin and results obtained in this study are in agreement with earlier findings **(Parola and Robino 2001; Acharya, 2004 and Naik *et al.*, 2011)** and the royal jelly have a role in protect the liver from damage **(Uzbekova *et al,* 1998). These results are in agreement with El-Nekeety *et al.* (2007)** reported that RJ resulted in a significant improvement in ALAT, ASAT, cholesterol and triglyceride levels toward to normal values of the control rats in fumonisin intoxication study. The reason why RJ administered irradiation decreased hepatotoxicity could be related to the fact that hepatocyte-stimulating substance and glutathione precursor cysteine which is important role in the liver detoxification system. Also **Andritoiu *et al.*, (2014)** recorded that RJ with CCl_4-induced hepatopathy determined a decrease of the liver enzymes activity ASAT and ALAT in serum.

Hepatic malondialdehyde (MDA) levels were significantly increased in CCl_4 treated group, showing an increased oxidative stress compared to control group. Increased oxidative stress has been attributed to the formation of reactive metabolites due to biotransformation by Cytochrome P450 2E1. Once formed, free radicals trigger a cascade of reactions that culminate in lipoperoxidation **(Pereira *et al.*, 2008 and Nissar et al., 2013).** The significant decline in the concentration of these constituents in the liver of CCl_4+CUR and royal jelly administered rats indicates anti-lipid peroxidative effects. Recent studies suggest that curcumin inhibits CYP2E1 activity **(Guangwei *et al.*, 2010). Azab *et al,* (2011)** recorded that the RJ administration to irradiated rats decreased the serum TBARS (MDA) level when compared to irradiated rats and the results is agreement with (**Kanbura *et al,* 2009**) thus limiting ROS production from microsomes, and activates NF-E2-related factor 2 (Nrf2) translocation to the nucleus, where it activates the expression of antioxidant enzymes **(Farombi *et al.*, 2008 and Charoensuk *et al.*, 2011).** In this study it is also evidenced by CMN and royal jelly treatment significantly restoring the antioxindant enzymes (SOD and CAT) activities as well as non-enzymatic

antioxidant GSH concentration in the liver. However, Curcumin supplementation improves liver function by decreasing superoxide production and by suppressing pro-inflammatory mediators and activating anti-inflammatory signaling pathways (**Armendariz-Borunda *et al.* 1993**) CMN reduces the oxidative stress in animals, by its high ROS scavenging capacity and by protecting the antioxidant enzymes from being denatured **(Madkour 2012).** Curcumin elevates the level of cellular GSH and induces de novo synthesis of GSH in HSC by stimulating the activity and gene expression of glutamatecysteine ligase, a key rate-limiting enzyme in GSH synthesis. These effects may recommend curcumin as a hepatoprotective agent (**Zheng *et al.* 2007**). **EL-Ashry and Elkady (2014)** recorded that the treatment with RJ ameliorated the cisplatin-induced liver damages due to free radical production. Meanwhile, the elevated GSH level and activities of GSH-Px and CAT enzymes in the CP plus RJ group implied a decrease in the number of free radicals after cisplatin administration and reflected that these enzymes played important roles in scavenging free radical. this findings are similar to results of other investigators such as (**Al-Majed *et al.* 2006 and Arafa 2008)** they recorded that liver tissues in which cisplatin injections caused low GSH, GSH-Px. Furthermore, in this study, it was observed that levels of GSH, GPx and CAT in CP plus RJ-treated group were higher than in the CP group. These results suggested that RJ has a supporting effect on the antioxidant system because of increases in GSH, GPx, and CAT activities.

Obtained data showed a significant decrease in liver catalase (CAT), superoxide dismutase (SOD) and reduced glutathione (GSH)in rats group intoxicated with CCl_4 when compared to the corresponding values in control group due to Reduced glutathione is the most abundant thiol in mammalian tissues involved in the protection of the cell against damage from electrophiles free radicals and ROS formed during xenobiotic metabolism **(Meister, 1991).** A reduction in SOD and GSH is associated with the accumulation of high-living free radical, leading to injury of cell function **(Okamoto and Colepicolo, 1998**). Curcumin significantly modulated the activities of SOD and GPx after CCl_4 withdrawal. These results were in accordance with (**Nabavi *et al.* 2014**) CMN reduces the oxidative stress in animals, by its high ROS scavenging capacity and by protecting the antioxidant enzymes from being denatured **(Madkour 2012).** These results are in agreement with (**Hyeong-Seon *et al.* (2010)**.

$CC1_4$ intoxication produced a significant reduction in plasma total protein and albumin level. This may be due to release of these parameter from the cytoplasm into the blood rapidly after cellular damage and a reduction in hepatic protein synthesis. These effects were further confirmed by histopathological observations of liver, where variable forms of hepatocytes degeneration, fatty changes, apoptosis and necrosis were observed **(Fu *et al.*, 2008). (Khedr and Khedr, 2014)** reported that plasma total protein and albumin level was also increased by curcumin administration compared to CCl_4 treated group. **Andritoiu et al. (2014)** who noticed that total protein decrease in rats with CCl_4-induced hepatopathy that lead to severe liver damage correlated with tissue histoarchitecture. By treating with apidiet and apidiet + RJ the lab animals with CCl_4-induced hepatopathy and comparing against the group with CCl_4-induced hepatopathy on standard diet it was noticed total protein and albumin increase, that proved the recovery of liver synthesis function, because it is well known that albumin increase represented a crucial factor in restoring liver function. one of the main targets of the treatment of liver damage is the rise of albumin levels.

Conclusion

Curcumin and royal jelly have a promising hepatoprotective effect; this action could be attributed to antioxidant, anti-fibrotic and anti-apoptotic activities. This may constitute a novel target for hepatoprotective modality utilizing natural products

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