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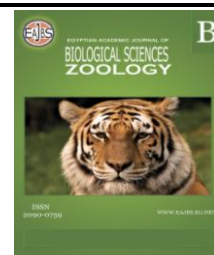


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Therapeutic Effects of Royal Jelly on Some Biochemical Parameters in Nephrotoxicity Induced by Carbon Tetrachloride (CCl₄) In Albino Rats.

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ABSTRACT

The present study was designed to determine the possible therapeutic effects of royal jelly against carbon tetrachloride (CCl₄) induced oxidative stress and biochemical changes in the kidney of albino rats. A patch of 20 male albino rats averaged weights (190±10 g) at the beginning of the experiment were divided into 4 main groups according to the treatment and requirements of the experiment. The rats injected i.p. with CCl₄ at a dose (1 mL/kg, 1:1 in olive oil) twice per week and received, via gavage, royal jelly at a dose (400 mg/kg b. wt) along the experimental period. Each group contains 5 rats that were sacrificed on the 45 days from the start of the experiment. The results refer to a significant elevation of renal parameters (creatinine, blood Urea and Uric acid) on the 6th week in rats injected intraperitoneal with CCl₄ as compared to the control groups. The administration of the royal jelly has beneficial and decrease side effects against the deleterious changes of CCl₄.

In conclusion, according to the results obtained the administration of the royal jelly provides considerable nephroprotective effects against intoxicated with CCl₄ in male Wister albino rats by preventing oxidative stress through ROS scavenger and improvement in the former biochemical parameters.

INTRODUCTION

CCl₄ is an organic compound widely used as a dry-cleaning solvent until it was recognized as a potent carcinogen (Kovacic and Jacintho 2001). Several previous studies have indicated that CCl₄ is the best model for the generation of reactive oxygen species (ROS) in many tissues (Kamisan *et al.*, 2014). The toxicity produced by CCl₄ depends on the production of trichloromethyl radical (CCl₃), which further converts trichloro-methyl into trichloromethyl peroxy radical (CCl₃O₂) in the presence of oxygen that is more toxic than trichloromethyl radicals (Ali and Abdelaziz 2014). Chlorine and trichloromethyl radicals are formed due to the metabolic regeneration of CCl₄ by cyto-chrome P-450 enzymes, which increases the oxidant-antioxidant instability. These Free radicals trigger lipid peroxidation and initiate a prolonged chain reaction (Naz *et al.*, 2014). Excessive free radical production causes massive damage in lipids, proteins, and DNA. Extensive disruption of DNA strands may trigger compensatory cell transformation and cell death because of CCl₄-induced toxicity. It is revealed that CCl₄-induced oxidative stress leads to renal toxicity inciting several uncontrolled disorders by producing acute and chronic kidney failure (Khan and

Zehra 2013). The kidney is one of the extremely vital organs in the body obviously because of its indispensable metabolic roles (excretory and regulatory). Globally, kidney disease of any type is a severe and critical health challenge. With constant exposure to xenobiotics including nephrotoxins through modern-day lifestyle, the organ is susceptible to acute injury capable of compromising its physiological state and metabolic functions (Schetz *et al.*, 2005), (Ighodaro and Akinloye 2018). Carbon tetrachloride (CCl_4) is a typical xenobiotic and potent nephrotoxic agent commonly used in experimental studies to assess the ability of a test compound to prevent or protect against tissue derangements (Ali and Abdelaziz 2014). Its toxicity is underlined by the production of tri-chloromethyl radical (CCl_3) during its metabolic activation by cytochrome p 450. Worse still, (CCl_3) is converted to a more hazardous radical known as trichloromethyl peroxy radical (CCl_3O_2) under aerobic cellular conditions (Zager 1997). Both radicals synergistically attack essential biomolecules in the kidney and other body tissues and are responsible for kidney damage associated with CCl_4 , a phenomenon which may occur through altered intraglomerular hemodynamic, chronic inflammation, rhabdomyolysis, microangiopathy, or tubular cell toxicity (Schetz *et al.*, 2005, Ighodaro and Akinloye 2018).

Acute and chronic renal damage is also very common pathophysiologic disturbances caused by CCl_4 (Ogeturk *et al.*, 2005). Administration of CCl_4 causes an increase in lipid peroxidation products (Daniels *et al.*, 1995) and a decrease in the activity of enzymes protecting lipid peroxidation in the kidney. The trichloromethyl and trichloromethyl peroxy radicals are reported to enhance lipid peroxidation and protein oxidation, resulting in widespread membrane damage and a decrease in the activity of enzymes protecting lipid peroxidation in the kidney (Doğukan *et al.*, 2003). The antioxidant enzymes include superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px) and indirectly glutathione reductase. The role of these enzymes is well known and has been investigated extensively both in vivo and in vitro in model systems. The nephrotoxic effect of CCl_4 is associated with free radical formation (Ozturk *et al.*, 2003) which leads to lipid peroxidation and break down of the membrane structure. Characteristic renal injuries from carbon tetrachloride exposure are nephritis, nephrosis, and renal failure. Kidney failure is one of the leading causes of death in CCl_4 intoxication. The consequence of oxidative damage is serious and, in many cases, apparent in increased activities of enzymes involved in oxygen detoxification. The negative effects of free radicals, oxidative stress may well be overcome by antioxidants (Larson 1995).

Nowadays, more and more natural products are used for health problems. These products have been proved to reduce the risk of chronic diseases and reduce the investment in health care (Jenketkan *et al.*, 2017). In recent years, natural bee products have been widely used in traditional and modern medicine. Royal jelly (RJ), as a bee product, is also used to maintain human health (Pasupuleti *et al.*, 2017). RJ is the creamy substance secreted by the mandibular and hypopharyngeal glands of worker bees, which is the food of all honeybee larvae in the first three days after birth; after three days, worker bee larvae begin to eat worker jelly, which is mainly composed of honey and pollen. The queen bee larvae continue to eat RJ (Yang *et al.*, 2017). And royalactin may be a specific factor in RJ to induce bee larvae to differentiate into the queen. (RJ is a kind of widely used functional food and dietary supplement, which has many biological activities, such as anti-tumor, anti-allergy, anti-inflammatory, and immune regulation (Pasupuleti *et al.*, 2017).

RJ is a superfood secreted from the mandibular and hypopharyngeal salivary glands of young nurse bees aged 5–14 days. It is a unique nutrient for all larvae in the first three days, but only the queen bees feed on it all their lives (Fratini *et al.*, 2016). RJ is a functional food with antimicrobial, anti-aging, antioxidant, and immunomodulatory effects, as well as other health benefits for diabetes, cancer, Alzheimer's, and cardiovascular disease (Ahmad *et*

al., 2020). These activities are related to the several functional constituents of RJ, such as proteins, lipids, carbohydrates, vitamins, and minerals, among others (Fratini *et al.*, 2016). The majority of RJ proteins (about 90%), comprising nine members (MRJP1-MRJP9), are water-soluble and have many physiological functions. Among these MRJPs, we have recently proven the antiviral, anticancer, and anti-hepatic damage potential of MRJP2 and MRJP2 X1 (Habashy and Abu-Serie 2019).

Research has shown that RJ has many pharmacological activities, among them antioxidants, neurotrophic, anti-inflammatory, immunomodulatory, hypoglycemic, ant allergic, general tonic and antiaging (Pavel *et al.*, 2011). Moreover, RJ long-term administration can affect the brain neurotransmitters in naturally aged rats (Honda *et al.*, 2011). In addition to experimental data, references indicate the medical use of RJ (Kocot *et al.*, 2018). In Cuba, bee products, including RJ, are used within the official system of Natural and Traditional Medicine. In Russia, positive results have been found in a local hospital following treatment for several diseases with RJ (Bogdanov 2011).

MATERIALS AND METHODS

20 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 placed in regularly 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 *ad libitum* to the animals.

The rats were classified into main four groups as follow: Group 1: Normal control, Group II: Rats treated orally with royal jelly in dose (400 mg/kg) suspended in distilled water daily, Group III Rats injected intraperitoneal with CCl_4 in dose (1ml/kg) twice a weekly diluted in olive oil (1:1) for a period six weeks. Group IV Rats injected with CCl_4 twice weekly and treated orally with royal jelly daily. All treatments were given for six weeks. The sign of toxicity was recorded daily during the experimental period. Each group contains 5 rats, five rats were anesthetized and sacrificed after the 6th week for biochemical parameters.

Induction of Nephrotoxicity:

Nephrotoxicity was induced by Intraperitoneal injection of CCl_4 (1 ml/kg) diluted in olive oil (50 % v/v) twice weekly for 45 consecutive days (Tu *et al.*, 2012).

Biochemical Parameters:

The levels of urea, uric acid and creatinine in plasma were estimated spectrophotometrically using commercial diagnostic kits, Serum urea concentration was determined according to the method of (Salem *et al.*, 2018).; Serum creatinine level was determined according to the method described by (Newman and Price 2001) and Serum creatinine level was determined according to the method described by (Barham and Trinder 1972). using commercial kit purchased from Bio-diagnostic Company Egypt.

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 \pm S.E. Differences were considered statistically significant at ($P < 0.05$).

RESULTS

CCl₄ induced renal damage as reflected by significantly ($p < 0.05$) elevated serum creatinine, Urea, and uric acid levels when compared to the control group after the 6th week. On the other hand, insignificant differences with recorded in RJ when compared to control groups. Rats treated with CCl₄+RJ observed a significant decrease ($p < 0.05$) when compared with CCl₄ groups after the 6th week. As shown in Tables (1, 2 and 3).

Table 1: Serum Creatinine concentration (mg/dL) in adult male albino rats subjected to carbon tetrachloride (CCl₄) toxicity and treated with royal jelly for 45 days.

Creatinine		
Groups		45 days
Control	Mean± S. E	0.50±0.09a
	%	
Royal jelly	Mean± S. E	0.44±0.05a
	%	-12.0
CCl ₄	Mean± S. E	2.25±0.16b
	%	350.0
CCl ₄ + Royal jelly	Mean± S. E	1.67±0.09c
	%	234.0

Each value represented means of 5 records ± S.E.
^{a, b, c} 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.

Table 2: Serum urea concentration (mg/dl) in adult male albino rats subjected to carbon tetrachloride (CCl₄) toxicity and treated with royal jelly for 45 days

Urea		
Groups		45 days
Control	Mean± S. E	29.58±1.3 ^a
	%	
Royal jelly	Mean± S. E	31.20±1.5 ^a
	%	5.48
CCl ₄	Mean± S. E	100.8±4.5 ^b
	%	240.8
CCl ₄ + Royal jelly	Mean± S. E	59.5±4.1 ^c
	%	101.1

Each value represented means of 5 records ± S.E.
^{a, b, c} 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.

Table 3: Serum Uric acid concentration (mg/dl) in adult male albino rats subjected to carbon tetrachloride (CCl₄) toxicity and treated with royal jelly for 45 days.

Uric acid		
Groups		45 days
Control	Mean± S. E	1.45±0.16a
	%	
Royal jelly	Mean± S. E	1.59±0.11a
	%	9.66
CCl ₄	Mean± S. E	5.47±0.29b
	%	277.2
CCl ₄ + Royal jelly	Mean± S. E	2.91±0.26b
	%	100.7

Each value represented means of 5 records ± S.E.
^{a, b, c} 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.

DISCUSSION

The present study was conducted to evaluate the effect of the royal jelly against CCl₄-induced kidney disorders in the rat. Chronic renal injury by carbon tetrachloride is a well-established animal model of kidney disorders.

Urea and creatinine are nitrogenous waste products that are eliminated by the kidneys, whereas excretion is suppressed in renal insufficiency (Lall *et al.*, 1997, Ozturk *et al.*, 2003). The rise in urea and creatinine concentrations in serum is used as an indicator of CCl₄-induced nephrotoxicity.

The obtained data showed a significant increase in serum urea, creatinine, and uric acid. in CCl₄, and CCl₄ + RJ groups when compared to the corresponding values in the control group because the administration of CCl₄ causes nephrotoxicity as indicated by elevation of urea and creatinine level in serum. These pathological changes signify the potential damage in hepatic and/or kidneys induced with CCl₄ treatment (Khan and Siddique 2012). The increased serum level of creatinine can be attributed to the damaged nephron structure (Ogeturk *et al.*, 2005). These results are in agreement with previous studies that recorded those high levels of urea, uric acid and creatinine in serum are possible indicators of hepatic and/or kidney injuries induced through CCl₄ treatment (Khan *et al.*, 2009, Venkatanarayana *et al.*, 2012). Also, a previous study reported that chronic renal injuries and urea elevations developed in rats after CCl₄ intoxication (Mahmoud 2013).

On other hand recent studies recorded that administration of CCl₄ causes nephrotoxicity as indicated by an elevation in urine and serum level of urea, creatinine this elevation in serum urea and creatinine levels can be attributed to the damage of nephron structural integrity (Mohamed *et al.*, 2014).

Urea is the end-product of protein catabolism, and this is confirmed by the decrease in serum proteins and /or referred to as liver dysfunction as proven by the increase in serum ASAT, ALAT and ALP activities.

The obtained data showed a significant decrease in serum urea, creatinine and uric acid. in CCl₄+RJ groups when compared to the corresponding values in CCl₄ group because a decrease in urea, creatinine and uric acid may also be attributed to RJ normalized the functions of the kidney. Which is in agreement with similar studies such as (Yapar *et al.*, 2009). Data reported that treatment the rats with AlCl₃ plus royal jelly decreased serum urea.

administration of two different doses of RJ together with lambda-cyhalothrin (LCT) significantly decreased the levels of BUN, and creatinine.

Results showed the administration of RJ prior to cisplatin significantly prevented the increase in serum urea and creatinine compared to the cisplatin-alone group. Moreover, histological findings showed that RJ administration protected kidney tissues from cisplatin-induced free radical damage.

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