www.ijpbs.net

INTERNATIONAL JOURNAL OF PHARMA AND BIO SCIENCES

Internationally indexed journal

Indexed in Chemical Abstract Services (USA), Index coppernicus, Ulrichs Directory of Periodicals, Google scholar, CABI ,DOAJ , PSOAR, EBSCO , Open J gate , Proquest , SCOPUS , EMBASE ,etc.

Rapid and Easy Publishing

The "International Journal of Pharma and Bio Sciences" (IJPBS) is an international journal in English published quarterly. The aim of IJPBS is to publish peer reviewed research and review articles rapidly without delay in the developing field of pharmaceutical and biological sciences

Pharmaceutical Sciences

- Pharmaceutics
- Novel drug delivery system
- Nanotechnology
- Pharmacology
- Pharmacognosy
- Analytical chemistry
- Pharmacy practice
- Pharmacogenomics
- Polymer sciences
- Biomaterial sciences
- Medicinal chemistry
- Natural chemistry
- Biotechnology
- Pharmacoinformatics
- Biopharmaceutics

Biological Sciences

- Biochemistry
- Biotechnology
- Bioinformatics
- Cell biology
- Microbiology
- Molecular biology
- Neurobiology
- Cytology
- Pathology
- Immunobiology

Indexed in Elsevier Bibliographic Database

(Scopus and EMBASE)

SCImago Journal Rank 0.288

Impact factor 5.121*

ISSN 6299 -0975

Chemical Abstracts Service (www.cas.org)

A division of the American Chemical Society

CODEN IJPBJ2

Google Scholar BETA

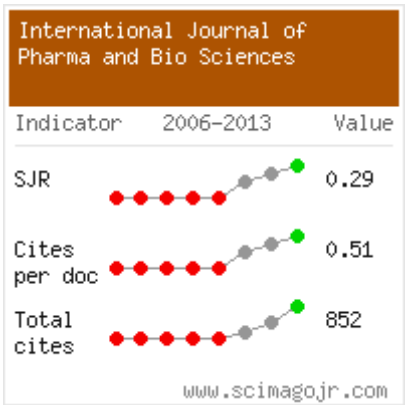
Elsevier Bibliographic databases (Scopus & Embase)

SNIP value – 0.77

SJR - 0.288

IPP - 0.479

SNIP – Source normalised impact per paper
SJR – SCImago Journal rank
IPP –Impact per publication
Source –www.journalmetrics.com
(Powered by scopus (ELSEVIER)



ProQuest

LUND UNIVERSITY

JSU JACKSONVILLE STATE UNIVERSITY

Jacksonville State University
Houston Cole Library
USA (Alabama)

UNIVERSITY OF OXFORD

Oxford, United Kingdom

INDEX COPERNICUS INTERNATIONAL

And indexed/catalogued in many more university

*Instruction to Authors visit **www.ijpbs.net**

For any Queries, visit "contact" of www.ijpbs.net

Research Article **Biochemistry**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

JATROPHA CURCAS EXTRACT IMPROVES CARBON TETRACHLORIDE-INDUCED NEPHROTOXICITY AND INFLAMMATION IN RATS

FAROUK K. EL-BAZ *[1], HANAN F. ALY[2] AND SAFAA A. SAAD[1]

[1]Plant Biochemistry Department, National Research Centre (NRC), 33 EL Bohouth st. (former EL Tahrir st.), Dokki, Giza, Egypt, P.O.12622
[2]Therapeutic Chemistry Department, National Research Centre (NRC), 33 EL Bohouth st. (former EL Tahrir st.), Dokki, Giza, Egypt, P.O.12622

ABSTRACT

The aim of this research is to throw light on the therapeutic properties of *Jatropha curcas* leaves methanolic extract in CCl_4-intoxicated female rats as compared to silymarin drug. Female rats were injected with CCl_4 (0.5 ml/kg body weight) suspended in olive oil (1:9 v/v) twice a week for six consecutive weeks induced hepatic toxicity. *J. curcas* methanolic extract and silymarin drug were orally administrated at doses of 250 and 50 mg/kg body weight, daily for one month. Creatinine, urea, inflammatory biomarkers; C-reactive protein (C-RP), tumor necrosis factor alpha (TNF-α) and interlukin-10 (IL-10) levels were measured in rats blood serum. Moreover, histopathological investigation of kidney was performed. The present results reveal that creatinine, urea, C-RP and TNF-α levels were significantly increased in CCl_4-intoxicated rats with percentages 49.54, 76.83, 90.59 and 62.73%, respectively. However, IL-10 level showed a significant decrease with percentage 49.05%. However, the current results declare that administration of intoxicated rats with 250 mg/kg b.wt. methanolic extract of *J. curcas* showed amelioration in the levels of creatinine, urea, C-RP, TNF-α and IL-10 with percentages of improvement reached to and 56.36, 73.99, 78.39, 54.34 and 39.43%, respectively which were documented with the ameliorative effect of *J. curcas* extract on renal architectures. Thus, the present results clearly indicated that, oral administration of *J. curcas* extract might provide an alternative approach for ameliorating nephrotoxicity associated with CCl_4 toxicity.

KEYWORDS: ***Jatropha curcas,*** **CCl_4, Inflammatory biomarkers, Histopathology, Silymarin**

FAROUK K. EL-BAZ
Plant Biochemistry Department, National Research Centre (NRC), 33 EL Bohouth st. (former EL Tahrir st.), Dokki, Giza, Egypt, P.O.12622

INTRODUCTION

Acute and chronic liver diseases are the evidence of oxidative stress, which represents the dysfunction or death of hepatocytes and other liver cell types, participating in disease pathogenesis Mari et al. [1]. The authors added that, reactive oxygen species (ROS) are normally produced by metabolism of normal cells and play a pinpointed role in cell signaling but, in liver diseases an over production of free radicals occurs, which is not counter balanced by an increase in antioxidant defenses, damaging the hepatic tissue. Carbon tetrachloride (CCl_4) is used as a model to study hepatotoxic effects and causes liver damage through a number of mechanisms [2]. Elhag et al. [2] added that, liver cell injury induced by CCl_4 involves initially the metabolism of CCl_4 to trichloromethyl free radical by the mixed function oxidase system of the endoplasmic reticulum. In addition, it is postulated that secondary mechanisms link CCl_4 metabolism to the widespread disturbances in hepatocyte function [3]. The advantage of this CCl_4 model is that it can fulminate hepatitis within a few hours, which specifically leads to necrosis and fatty liver, in a similar way as what happens in the cases of acute hepatitis [3]. Liver fibrosis is a significant health problem resulting from response of the liver to injury [4,5]. Hepatocytes are the major targets in liver fibrosis [6]. Next injury, hepatocytes respond to create an inflammatory consequently, tainted hepatocytes release ROS as well as fibrogenic mediators and induce the recruitment of white blood cells by inflammatory cells [7,8]. In the absence of reliable hepatoprotective drugs in modern medicine, a large number of phytochemicals and extracts prepared from folk medicinal plants with proven hepatoprotective properties, could be an alternative in the treatment of liver diseases resulting from high alcohol consumption, exposure to xenobiotics and therapeutic agents as well as other factors leading to the onset of chronic liver diseases which are very often related to oxidative stress [9,10]. Moreover, concerns over harmful side effects of synthetic compounds have shifted the focus to natural plant resources where, many plants have a long history of traditional use in revitalizing the liver and treating liver dysfunction and disease [11]. Many active plant extracts are frequently utilized to treat a wide variety of clinical diseases including liver disease [12]. *Jatropha curcas* (physic nut or purging nut) is a species of flowering plant in the spurge family Euphorbiaceae. *Jatropha* species are used in traditional medicine to cure various ailments in Africa, Asia, and Latin America or as ornamental plants [13]. Several known species from genus *Jatropha* have been reported for their medicinal uses, chemical constituents, and biological activities such as *J. curcas*, *Jatropha elliptica*, *Jatropha gossypiifolia*, and *Jatropha mollissima*, among others [13]. All parts of *J. curcas* plant (seeds, leaves and bark) have been used in traditional medicine and for veterinary purposes for several centuries [14]. Some of the known medicinal properties of *J. curcas* include antitumor activities, molluscicidal, insecticidal and fungicidal properties [15]. The successive methanolic extract of *J. curcas* leaves is highly valuable source of natural antioxidants that showed high antioxidant activity [16]. So, the present study is designed to evaluate the therapeutic characters of *J. curcas* extract against CCl_4-induced nephrotoxicity in rats and inflammatory biomarkers. Beside, the histopathological examination of rats' kidney was examined to confirm the protective role of *J. curcas* on renal cells architectures.

MATERIALS AND METHODS

(i) Chemicals and reagents

Silymarin was obtained from the Sigma Chemical Company. All kits were the products of Biosystems (Alcobendas, Madrid, Spain), Sigma Chemical Company (St. Louis, MO, USA), Biodiagnostic Company (Cairo, Egypt). All chemicals in the present study are of analytical grade, products of Sigma, Merck and Aldrich.

(ii) Plant collection and crude methanolic extract preparation

J. curcas leaves were collected from the farm of Aromatic and Medicinal Plant Department, Agriculture Research Centre (ARC), Egypt. The plant was authenticated by Mrs Treas Labib, Herbarium section, El-Orman Botanical Garden, Giza, Egypt. The leaves were washed with tap water then with distilled water to

remove dust and dirt. Leaves were dried under shade, powdered and stored in opaque screw tight jars prior to further use. The powdered leaves of *J. curcas* (300 g) were homogenized with methanol (900 ml) and the homogenate was kept on shaker (Heidolph) at room temperature for 48 hr. at 150 rpm. The extract was filtered using Whatman No. 4 filter paper and Buchner. The filtrate was evaporated to dryness under reduced pressure by using Rotary evaporator (Heidolph) at 40°C and stored in refrigerator (4°C) till biological assay and chemical analysis.

(iii) Biological experiment

1. Animals and treatments

Fifty female adult rats of the albino strain (130-150 g), bred in the Animal House, National Research Centre (NRC), Egypt were maintained and kept in the controlled environment of air and temperature (26-29°C) with access of water and diet. Anesthetic procedures and handling with animals complied with the ethical guidelines of Medical Ethical Committee of National Research Centre in Egypt. The rats were divided into five groups of ten rats each as follows:

Group 1: Normal control rats, Group 2: Normal rats administered methanolic extract of *J. curcas* leaves at a dose 250 mg/kg body weight. Group 3: CCl_4-intoxicated rats intraperitoneally injected a single dose of CCl_4 (0.5 ml/kg body weight) suspended in olive oil (1:9 v/v) twice a week for six consecutive weeks [17]. Group 4: Intoxicated rats orally administered with crude methanolic extract of *J. curcas* at a dose 250 mg/kg body weight daily for 30 days. Group 5: Intoxicated rats orally administered with silymarin drug at a dose 50 mg/kg body weight daily for 30 days.

2. Preparation of serum from blood

Rats were fasted overnight (12-14 hr), anesthetized by diethyl ether and blood collected by puncture of the sublingual vein in clean and dry test tube, left 10 minutes to clot and centrifuged at 3000 r.p.m for serum separation. The separated serum was used for biochemical analysis of creatinine, urea, C-RP, TNF-α and IL-10. The kidney of all the experimental animals were removed and processed immediately for histological examination.

3. Serum markers of kidney damage

Serum level of creatinine was measured using colorimetrical kits to assess the nephrotoxicity [18]. Total urea level was estimated using colorimetric kit [19].

4. Inflammatory markers

Estimation of serum inflammatory markers; CRP, TNF-α as well as IL-10 was performed by ELISA; a sandwich enzyme immunoassay.

Calculation:

$$\%\ \text{Change} = \frac{\text{Mean of control} - \text{Mean of treated}}{\text{Mean of control}} \times 100$$

$$\%\ \text{of improvement} = \frac{\text{Mean of treated} - \text{Mean of disease}}{\text{Mean of control}} \times 100$$

5. Histopathological examination

For light microscopic investigations. The kidney specimens obtained from the control and treated groups of animals were fixed in 10% buffered formalin for 24 h for fixation. Then processed in automatic processors, embedded in paraffin wax (melting point 55-60 °C) and paraffin blocks were obtained. Sections of 6 µm thicknesses were prepared and stained with Haematoxylin and Eosin (H & E) stain [20]. The cytoplasm stained shades of pink and red and the nuclei gave blue color. The slides were examined and photographed under a light microscope (x400 magnification).

6. Statistics

All values are expressed as means ± SD. Biochemical results were subjected to one-way analysis of variance (ANOVA) and the significance of the differences between means was tested using Co-state computer program. Statistically significant differences between groups were defined as $p < 0.05$.

RESULTS

1. Renal function of different experimental groups

Kidney function tests showed a significant increase in creatinine and urea levels in CCl_4-intoxicated rats with percentages 49.54 and 76.83%, respectively as compared to normal control (Table 1). These values returned to the normal levels post administration of *J. curcas* methanolic extract as well as silymarin standard drug with improvement percentages reached to 56.36 and 44.09%, for creatinine, 73.99 and 79.97%, respectively for urea level. From the obtained results it could be concluded that, the highest increase in creatinine and urea levels was observed in CCl_4-intoxicated rats. However, the highest percentage of improvement in creatinine level was obtained throughout treatment of intoxicated rats with - methanolic extract (56.36%) followed by silymarin drug (44.09%). While treatment of intoxicated rats with silymarin drug and crude methanolic extract declared approximate ameliorative percentage in urea (79.97, 73.99%, respectively)

2. Inflammatory and anti-inflammatory biomarkers level of different experimental groups

Table (2) showed the levels of inflammatory biomarkers (C-RP and TNF-α) as they recorded significant elevation in intoxicated rats with percentages reached to 90.59 and 62.73%, respectively as compared to normal control rats (Table 2). While, the anti-inflammatory marker (IL-10) level was decreased significantly with percentage 49.05%. Treatment of intoxicated rats with methanolic extract of *J. curcas* leaves as well as silymarin drug attenuated the levels of these biomarkers and restored them more or less to their corresponding normal value. So, *J. curcas* methanolic extract treated-intoxicated rats showed an insignificant increase in CRP level with improvement percentage 78.39%, while silymarin showed significant increase in CRP level with improvement percentage 68.55%. Regarding to, TNF-α level, methanolic extract and silymarin drug showed significant increase with improvement percentages 54.34 and 52.17%, respectively. However, *J. curcas* extract and silymarin drug showed increase in IL-10 level with improvement percentages 39.43 and 44.03%, respectively. From the obtained results it could be suggested that, *J. curcas* extract revealed higher ameliorative percentage in CRP and TNF-α levels (78.39 and 54.34%, respectively) than silymarin drug (68.55 and 52.17%, respectively). In contrast, silymarin drug showed higher improvement percentage in IL-10 level (44.03%) than *J. curcas* extract (39.43%).

3. Histopathological examination of kidney

The kidney of the normal control group showed normal histological structure (Photo 1). However, intoxicated rats showed degenerated glomeruli, others are completely necrotic, the tubules showing edematous epithelial lining. In addition, disrupted epithelial lining with debris tubular lumen (Photo 2). On the other hand, treatment of intoxicated rats with methanolic extract of *J. curcas* as well as silymarin drug reversed these changes and appearing more or less normal (Photos 3 & 4).

Table 1
Effect of J. curcas methanolic extract on kidney function of different experimental groups

Groups	Parameters	Creatinine (mg/dl)	Urea (mg/dl)
Normal control	Mean± S.D.	2.20 ± 0.01^{bc}	34.41 ± 2.65^{b}
Normal control rats treated with *J. curcas*	Mean± S.D.	2.10 ± 0.03^{bc}	35.07 ± 2.33^{b}
	% Change to control	4.54	1.91
CCl_4-intoxicated rats	Mean ±S.D.	3.29 ± 0.13^{a}	60.85 ± 1.48^{a}
	% Change to control	49.54	76.83
Intoxicated rats treated with *J. curcas* methanolic extract	Mean ±S.D.	2.05 ± 0.05^{c}	35.39 ± 2.70^{b}
	% Change to control	6.81	2.84
	% of improvement	56.36	73.99
Intoxicated rats treated with silymarin	Mean ±S.D.	2.32 ± 0.04^{b}	33.33 ± 1.87^{b}
	% Change to control	5.45	3.13
	% of improvement	44.09	79.97

-All values are means ± SD of 10 rats in each group
-Data were analyzed using analysis of variance (ANOVA) combined with Co-state computer program, where unshared letter is significant at p≤0.05

Table 2

Effect of J. curcas methanolic extract on inflammatory and anti-inflammatory biomarkers level of different experimental groups

Groups	Parameters	C-RP (ηg/ml)	TNF-α (ρg/ml)	IL-10 (ρg/ml)
Normal control	Mean± S.D.	29.35±1.01^{c}	47.44±2.14^{c}	85.62±1.49^{a}
Normal control rats treated with *J. curcas*	Mean± S.D. % Change to control	29.87±0.96^{c} 1.77	49.07±3.11^{c} 3.43	86.55±.66^{a} 1.08
CCl_4-intoxicated rats	Mean ±S.D. % Change to control	55.94±0.61^{a} 90.59	77.20±2.24^{a} 62.73	43.62±0.54^{c} 49.05
Intoxicated rats treated with *J. curcas* methanolic extract	Mean ±S.D. % Change to control % of improvement	32.93±2.04^{bc} 12.19 78.39	51.42±1.09^{b} 8.38 54.34	77.38±2.95^{b} 9.62 39.43
Intoxicated rats treated with silymarin	Mean ±S.D. % Change to control % of improvement	35.82±3.65^{b} 22.04 68.55	52.45±2.13^{b} 10.56 52.17	81.32±3.07^{ab} 5.02 44.03

-All values are means ± SD of 10 rats in each group
-Data were analyzed using analysis of variance (ANOVA) combined with Co-state computer program, where unshared letter is significant at p≤0.05.

Photo 1

A photomicrograph of normal renal tissue. The glomeruli (G) normally appeared and tubules lined by thin epithelial layer (thin arrow). (H & E 100)

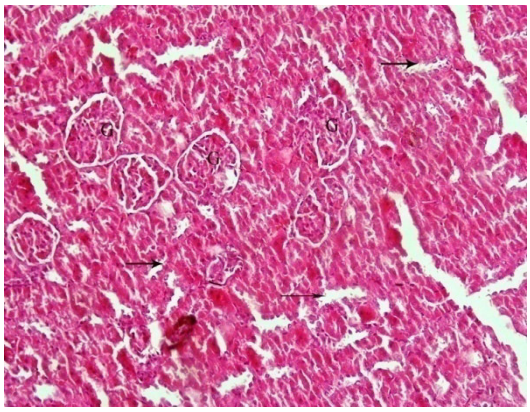


Photo 2

A photomicrograph of CCl_4-intoxicated renal tissue showing moderate degenerated glomeruli (G), others are completely necrotic (G*), the tubules showing edematous epithelial lining (thick arrow) and others with disrupted epithelial lining and tubular lumen showing debris (thin arrow). (H&E 100)

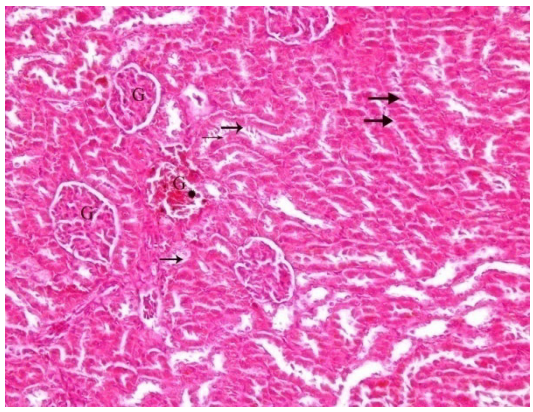


Photo 3

A photomicrograph of CCl_4-intoxicated rats kidney treated with J. curcas extract showing well improved glomeruli (G) with minimal deposition of hyaline material (arrow) also the tubules have been improved with intact their epithelial lining (arrow heads). (H&E 100).

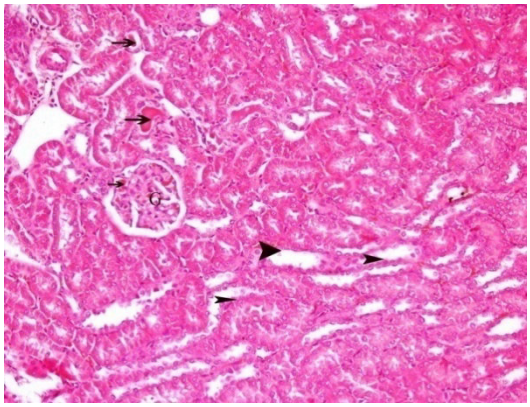
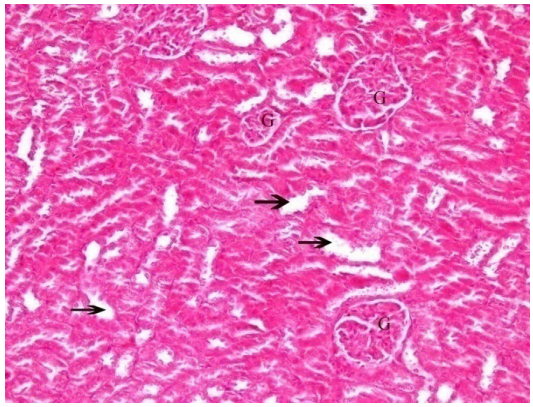


Photo 4

A photomicrograph of CCl_4- intoxicated rats kidney treated with silymarin drug showing well improved glomeruli (G) also the tubules have been improved with intact their epithelial lining (arrow). (H&E 100)



DISCUSSION

Liver is the first organ to metabolize all foreign compounds and hence it is susceptible to injury that can result in different diseases such as hepatitis, cirrhosis or hepatocellular carcinoma [21]. Hamid et al. [21] added that, liver disorders can rise from major causes such as exposure to different environmental pollutants and chemicals including, paracetamol, CCl_4, thioacetamide, alcohol, etc. Worldwide, hepatitis is an important liver disease with a staggering incidence of 550 million [22]. Carbon tetrachloride (CCl_4) has been used in animal model to induce liver damage similar to that of acute viral hepatitis in human patients [23]. The principle causes of CCl_4 in inducing the hepatic damage are lipid peroxidation, decreased activities of antioxidant enzymes and generation of free radicals [23]. The concentration of creatinine and urea are well known indicators of nephrotoxicity. While, the low clearance of creatinine and/or urea indicates a decrease in the ability of filtering and elimination of blood excretion products in urine at the kidneys level [24]. On the other hand, creatinine clearance decreases, the level in the blood increases and high creatinine level is an element in the diagnosis of renal insufficiency. Administration of CCl_4 causes nephrotoxicity as indicated by elevation in urine and serum level of urea, creatinine and urobilinogen while it decreased the creatinine clearance [25]. These pathological changes signify the potential damage to liver and kidney cells induced with CCl_4 treatment [26]. The present results reveal that, intoxicated rats showed a significant increase in creatinine and urea levels as compared to normal control rats. These elevations are

coinciding with the results of Rahmat et al. [27] and Khan and Siddique [28] as they attributed these elevations to the damage of nephron structural integrity. Our results are parallel also with those reported by Lotfy [29], who attributed these elevations to the damage in kidney glomeruli and the pathological changes observed in the kidney tissue as a result of CCl_4 injection for 6 weeks. Moreover, the increase in serum urea could be attributed to the reduction in glomerular filtration rate as well as impairment of renal blood flow after CCl_4 injection [30]. Treatment of intoxicated rats with methanolic extract of *J. curcas* reduced the elevated levels of creatinine and urea with percentages of improvement 56.36 and 73.99%, respectively. These results may be illustrated on the basis of, the therapeutic ability of the bioactive compounds, lingonberry to normalize the renal function and prevent the development of kidney-related diseases [31]. On a similar base, treatment with vitamin C resulted in less deterioration in the biochemical variables most likely because it exerts the cytoprotective effect through the inhibition of free radical production [32]. The present findings are in concomitant with Diab et al. [30] who stated that, the antioxidative, scavenging free radicals and inhibiting lipid peroxidation might be probably the cause of decreased urea and creatinine concentration. Further support to the current results is that, curcumin treatment decreased serum creatinine and urea concentrations in cyclosporine induced renal injury in rats in a dose dependent [33]. This effect may be related to the antioxidant properties of curcumin since it has been found that reactive oxygen species (ROS) may be involved in the impairment of glomerular filtration rate [34]. Extracts of *J. curcas* different parts contained various levels of phenolics, flavonoids, saponins and latex which showed the highest antioxidant activity [35]. So, the ameliorative effect of *J. curcas* methanolic extract on kidney biomarkers may be related to the presence of these bioactive compounds that have antioxidant activity. On the other hand, silymarin have possessed ameliorative effect on creatinine and urea level in CCl_4-intoxicated rats. It could be relies on the basis of, silymarin has been shown to have potential impact in many liver disorders, including oxidative stress, injury and fibrosis induced by CCl_4 [36]. It also has a protective effect against reperfusion-induced myocardial infarction in rats [37]. Silymarin can prevent lipid peroxidation, inhibit low-density lipoprotein oxidation and scavenge reactive oxygen species [38]. It was found that, CCl_4 at a high dose often rapidly causes cellular necrosis, oxidative stress and inflammation which leads to acute tissue injury and apoptotic organ failure [39]. The second phase involves the activation of tissue macrophages which is accompanied by the production of inflammatory and profibrogenic mediators [40]. Hepatic fibrosis is usually initiated by hepatocyte damage, leading to the recruitment of inflammatory cells and platelets with the subsequent release of cytokines, chemokines, and growth factors [41]. It was reported that factors released by these inflammatory cells lead to activation of Hematopoietic Stem Cells (HSCs) and their transformation into a myofibroblast-like phenotype. Chronically activated HSCs produce large amounts of extracellular matrix proteins (ECM) and enhance fibrosis by secreting a broad spectrum of cytokines such as TGF-β1 [42]. This exerts pro-fibrotic actions in other cells and in an autocrine manner perpetuates their own activation **(**and this is accompanied by a decrease in anti-inflammatory cytokines such as IL-10 and an increase in IL-2 and IL-6 [43]. TNF-α, a pro-inflammatory cytokine elevated in acute and chronic diseases like the induction of CCl_4 [44]. TNF-α was found to mediate the induction of hepatotoxicity and fibrogenesis in the bile duct ligation model [45]. The present study reveal that, the induction of CCl_4, led to increment in pro-inflammatory fibrogenic cytokine, CRP and TNF-α with percentages reached to 90.59 and 62.73%, respectively as compared to normal control rats. These results were supported by the results of Lee et al. [46], who confirmed the alterations in these cytokines in response to liver fibrosis. Moreover, Mohamed et al. [42] declared, significant increase in inflammatory markers TNF- α and CRP in liver injury induced by CCl_4. IL-10 is a potent anti-inflammatory cytokine that inhibits the synthesis of pro-inflammatory cytokines by T helper type1 T cells, mono/macrophages, and neutrophils [47]. Exogenous IL-10 can reverse CCl_4-induced hepatic fibrosis in rats. IL-10 may exert its reversible effects on hepatic fibrosis by blocking CCl_4-induced

inflammation, inhibiting expression of MMP-2 and tissue inhibitor of metalloproteinase-1 (TIMP-1) and promoting resolution of collagen types I and III [48]. In the present study, the level of IL-10 exhibited significantly decrease with percentage reached to 49.05% as compared to normal control rats. These results are supported by Hou et al. [49] who found that, hepatic IL-10 level, was significantly decreased in the CCl_4 injected rats. The current results markedly indicated that, the administration of *J. curcas* methanolic extract possessed an ameliorative effect on pro-inflammatory cytokines (CRP and TNF-α) levels as well as the anti-inflammatory IL-10 level. The inhibition of pro-inflammatory cytokines, enzymes, transcription nuclear factor, and free radicals could offer a new therapeutic strategy against inflammatory liver disease [50]. Phytochemicals have been shown to inhibit inflammation throughout blocking inflammatory pathways downstream of cytokine release and also by reducing macrophage production of pro-inflammatory factors [51,52]. The methanolic extract of *J. curcas* leaves contained useful active phytochemicals including alkaloids, cardiac glycosides, cyanogenic glycosides, phlobatannins, tannins, flavonoids and saponins which may serve as potential drug for the treatment of diseases [53]. Saponins, triterpenes, sterols and bitter principles might possess hepatoprotective agents against CCl_4-induced hepatotoxicity [54]. Thus, *J. curcas* extract may produce hepatoprotective activity *via* reducing the inflammatory effect in CCl_4-intoxicated rats. The most remarkable pathological characteristics of CCl_4-induced hepatotoxicity are fatty liver, cirrhosis and necrosis, which have been thought to result from the formation of reactive intermediates such as trichloromethyl (CCl_3^+) free radicals metabolized by the mixed function cytochrome P450 in the endoplasmic reticulum [55]. According to the study of Ozturk et al. [56], kidney failure is one of the leading causes of death in CCl_4 intoxication and its toxic effect of CCl_4 on hepatocyte is due to its metabolic conversion by the NADPH-cytochrome P450 metabolizing enzyme system to the highly reactive toxic free radical CCl_3. Consequently, the free radicals cause the peroxidation of the polyenoic lipids of the endoplasmic reticulum and the generation of secondary free radicals derived from these lipids that lead to breakdown of membrane structure and function [57,58]. Histopathological examination of kidney architecture in intoxicated rats, revealed degeneration and necrotic glomeruli, the tubules showed edematous epithelial lining and others with disrupted epithelial lining as well as tubular lumen showed debris. These results are in agreement with the results obtained by Mohamed et al. [23] and Ozturk et al. [56] who detected glomerular necrosis and histological alterations in proximal and distal tubules. Besides, tubular epithelial cell alterations, including vacuolization, atrophy, and detachment of the epithelial cells, indicated tubular necrosis. These findings are in parallel with those reported by Venkatanarayana et al. [25], who showed degenerative changes in glomerulus, renal tubules and vacuolization of cells in CCl4 induced renal damge. *J. curcas* leaves were found to contain bioactive compounds including; flavonoids, tannins, phenolics, saponins, coumarins, sterols and triterpenes [16]. Previous report showed that high concentrations of the methanolic extract of *J. curcas* have been reported to be more effective in quenching free radicals in the system [59]. So, the possible mechanism of *J. curcas* protection against CCl_4-induced nephrotoxicity may be explained through antioxidant and/or free radical scavenging activities of extract [60]. In a parallel results, several of medicinal plants exerts their nephroprotective effects mediate *via* antioxidant and/or free radical scavenging activities due to they having high concentration of flavonoids and alkaloids [61,62]. In addition, saponins have been reported to protect liver and kidney against CCl_4 intoxication [63]. Moreover, silymarin had been reported to protect liver cells from a wide variety of toxins including CCl_4. The mechanisms which provide silymarin hepatoprotective effect are many and varied, including antioxidant activity and lipid peroxidation inhibition [64,65]. Also, silymarin ameliorated the level of inflammatory markers and returned it more or less to normal level. This is may be due to silymarin has anti-inflammatory effects related to its ability to inhibit the transcription factor nuclear factor-kB (NF-kB), which contributes to the production of pro-inflammatory mediators such as

interleukin (IL)-1 and IL -6, TNF-a, lymphotoxin, granulocyte macrophage, colony-stimulating factor (GM-CSF) and interferon (IFN)-c [66].

CONCLUSION

It could be supposed that *J. curcas* methanolic extract may play an important role in medicine by scavenging free radicals and arresting the production of inflammatory cytokine, subsequently protecting kidney against CCl_4-induced damage. Also, the bioactive components present in *J. curcas* methanolic extract might be responsible for the amelioration of CCl_4 induced nephrototoxicity in rats.

Conflict of Interest

Conflict of Interest declared none.

REFERENCES

1. Mari M, Colell A, Morales A, von Montfort C, Garcia-Ruiz C and Fernandez-Checa JC, Redox control of liver function in health and disease. Antioxid Redox Signal, 11, 1295-1331, (2010).
2. Elhag RAM, El Badwi SMA, Bakhiet AO and Galal M, Hepatoprotective activity of *Solanum nigrum* extracts on chemically induced liver damage in rats. Journal of Veterinary Medicine and Animal Health, 3, 45-50, (2011).
3. Huang HL, Wang YJ, Zhang QY, Liu B, Wang FY, Li JJ and Zhu RZ, Hepatoprotective effects of baicalein against CCl4-induced acute liver injury in mice. World Journal of Gastroenterology, 18, 6605-6613, (2012).
4. Friedman SL, Liver fibrosisefrom bench to bedside. Journal of Hepatology, 38, 38-53, (2003).
5. Shigeki TA, Parsons CJ and Rippe RA, Mechanisms of liver fibrosis. Clinica Chimica Acta, 364, 33-60, (2006).
6. Higuchi H and Gores GJ, Mechanisms of liver injury: an overview. Current Molecular Medicine, 3, 483-490, (2003).
7. Bataller R and Brenner DA, Liver fibrosis. Journal of Clinical Investigation, 115, 209-218, (2005).
8. Deng YR, Ma HD, Tsuneyama K, Yang W, Wang YH, Lu FT, Liu CH, Liu P, He XS, Diehl AM, Gershwin ME and Lian ZX, STAT3-mediated attenuation of CCl_4-induced mouse liver fibrosis by the protein kinase inhibitor sorafenib. Journal of Autoimmunity, 46, 25-34, (2013).
9. Novo E and Parola M, Redox mechanisms in hepatic chronic wound healing and fibrogenesis. Fibrogenesis Tissue Repair, 1:5, 1-58, (2008).
10. Gurtsevitch VE, Human oncogenic viruses: hepatitis B and hepatitis C viruses and their role in hepatocarcinogenesis. Biochemistry, 73, 504-513, (2008).
11. Singh CR, Nelson R, Krishnan PM and Mahesh K, Hepatoprotective and anti-oxidant effect of root and root callus extract of *Premna serratifolia* L. in paracetamol induced liver damage in male albino rats. International Journal of Pharma and Bio Sciences, 2, 244-252, (2011).
12. Chattopadhyay RR, Possible mechanism of hepatoprotective activity of *Azadirachta indica* leaf extract: part II. Journal of Ethanopharmacology, 89, 217-219, (2003).
13. Sabandar CW, Ahmat N, Jaafar FM and Sahidin I, Medicinal property, phytochemistry and pharmacology of several *Jatropha* species (Euphorbiaceae): a review. Phytochemistry, 85, 7-29, (2013).
14. Prasad DMR, Izam A and Khan MR, *Jatropha curcas*: Plant of medical benefits. Journal of Medicinal Plants Research, 6, 2691-2699, (2012).
15. Dahake R, Roy S, Patil D, Rajopadhye S, Chowdhary A and Deshmukh RA, Potential anti-hiv activity of *Jatropha curcas* Linn. leaf extracts. Journal of Antivirals & Antiretrovirals, 5:7, 160-165, (2013).
16. El-Baz F K, Ali FF, Abd El-Rahman AA, Aly HF, Saad SA and Mohamed AA, HPLC evaluation of phenolic profile, and antioxidant activity of different extracts of *Jatropha curcas* leaves. International Journal of Pharmaceutical Sciences

Review and Research, 29, 203-210, (2014).
17. Marsillach J, Camps J, Ferre N, Beltran R, Rul A, Mackness B, Michael Mackness and Jorge Joven, Paraoxonase-1 is related to inflammation, fibrosis and PPAR delta in experimental liver disease. BMC Gastroenterol, 9, 1-13, (2009).
18. Schirmeister J, Determination of creatinine level. Deutsche Medizinische Wochenschrift, 89, 1940-1947, (1964).
19. Fawcett JK and Scott JE, A rapid and precise method for the determination of urea. Journal of Clinical Pathology, 13, 156-159, (1960).
20. Drury RA, Wallington EA, Carleton's Histology Technique. (4th Edn.). Oxford University Press, New York, (1980).
21. Hamid A, Kabira N, Muhammad A, Shah M R, Musharrafa S G, Iqbal N and Nadeem S, Hautriwaic acid as one of the hepatoprotective constituent of *Dodonaea viscose*. Phytomedicine, 21, 131-140, (2014).
22. Alter MJ, Epidemiology of viral hepatitis and HIV co-infection. Journal of Hepatology, 44, S6-S9, (2006).
23. Kumar PV, Sivaraj A, Elumalai EK and Kumar BS, Carbon tetrachloride-induced hepatotoxicity in rats - protective role of aqueous leaf extracts of *Coccinia grandis*. International Journal of Pharm. Tech. Research, 1, 1612-1615, (2009).
24. Saka WA, Akhigbe RE, Popoola OT and Oyekunle OS, Changes in serum electrolytes, urea, and creatinine in *Aloe vera*-treated rats. Pharmacology, 4, 78-81, (2012).
25. Venkatanarayana G, Sudhakara G, Sivajyothi P, Indira P, Protective effects of curcumin and vitamin E on carbon tetrachloride-induced nephrotoxicity in rats. EXCLI Journal, 11, 641-650, (2012).
26. Ogeturk M, Kus I, Colakoglu N, Zararsiz I, Ilhan N and Sarsilmaz M, Caffeic acid henethyl ester protects kidneys against carbon tetrachloride toxicity in rats. Journal of Ethnopharmacology, 28, 273-280, (2005).
27. Rahmat AA, Dar FA, Choudhary IM Protection of CCl_4-Induced Liver and Kidney damage by phenolic compounds in leaf extracts of *Cnestis ferruginea* (de Candolle). Pharmacognosy Research, 6, 19-28, (2014).
28. Khan MR and Siddique F, Antioxidant effects of *Citharexylum spinosum* in CCl4 induced nephrotoxicity in rat. Experimental and Toxicologic Pathology, 64, 349-55, (2012).
29. Lotfy, MM, Ph.D. Thesis (Pharmacology) presented to Faculty of Veterniary Medicine, Zagazig University, (2009).
30. Diab AA, Aziz SA, Hendawy AA and Salim DMM, The ameliorative effect of l-carnitine on experimentally induced liver cirrhosis in male albino rats. Journal of American Science, 10, 8-18, (2014).
31. Roman I, Puica C and Toma VA, The effect of *vaccinium vitis-idaea* L. extract administration on kidney structure and function in alcohol intoxicated rats. Studia Universitatis "Vasile Goldiş", Seria Ştiinţele Vieţii, 24, 363-367, (2014).
32. Maged E, Abdelhamid F, Risha E, Salama M and El-Sebaei M, Vitamin C ameliorates gentamicin-induced acute kidney injury in equines: An experimental study. Journal of Equine Veterinary Science, 35, 238-243, (2015).
33. Tirkey N, Kaur G, Vij G and Chopra K, Curcumin, a diferuloylmethane, attenuates cyclosporine-induced renal dysfunction and oxidative stress in rat kidneys. BMC Pharmacology, 5, 1-10, (2005).
34. Farombi EO and Ekor M, Curcumin attenuates gentamicin induced renal oxidative damage in rats. Food and Chemical Toxicology, 44, 1443-1448, (2006).
35. Oskoueian E, Abdullah N, Saad W Z, Omar A, Ahmad S, Kuan WB, Zolkifli NA, Hendra R and Ho YW, Antioxidant, anti-inflammatory and anticancer activities of methanolic extracts from *Jatropha curcas* Linn. Journal of Medicinal Plants Research, 5, 49-57, (2011).
36. Shaker ME, Zalata KR, Mehal WZ, Shiha GE and Ibrahim TM, Comparison of imatinib, nilotinib and silymarin in the treatment of carbon tetrachloride-induced hepatic oxidative stress, injury and fibrosis. Toxicology and Applied Pharmacology, 252, 165-175, (2011).

37. Rao PR and Viswanath RK, Cardioprotective activity of silymarin in ischemia-reperfusion-induced myocardial infarction in albino rats. Experimental and clinical cardiology, 12, 178-187, (2007).
38. Post-White J, Ladas EJ, Kelly KM, Advances in the use of milk thistle (Silybum marianum). Integrative Cancer Therapies, 6, 104-109, (2007).
39. Karakus E, Karadeniz A, Simsek N, Can I, Kara A, Yildirim S, Kalkan Y, Kisa F, Protective effect of Panax ginseng against serum biochemical changes and apoptosis in liver of rats treated with carbon tetrachloride (CCl4). Journal of *Hazardous* Materials, 195, 208-213, (2011).
40. Shi H, Dong L, Jiang J, Zhao J, Zhao G, Dang X, Lu X and Jia M, Chlorogenic acid reduces liver inflammation and fibrosis through inhibition of toll-like receptor 4 signaling pathway. Toxicology, 303, 107-114, (2013).
41. Khan RA, Khan MR and Sahreen S, CCl4-induced hepatotoxicity: protective effect of rutin on p53, CYP2E1 and the antioxidative status in rat. BMC Complement Alternative Medicine, 178, 1-6, (2012).
42. Mohamed NZ, Abd-Alla HI, Aly HF, Mantawy M, Ibrahim N and Hassan SA, CCl4-induced hepatonephrotoxicity: protective effect of nutraceuticals on inflammatory factors and antioxidative status in rat. Journal of Applied Pharmaceutical Science, 4, 087-100, (2014).
43. Xu MS, Wang ZY and Kang YH, Recent advance on the chemistry and bioactivity of genus *Rumex*. Chin Arch Tradi Chin Med, 22, 417-420, (2004).
44. Zhong HH, Wang B, Liang YK, Bao YY and Gu Y, Hepatoprotective and antioxidant effects of licorice extract against CCl4-induced oxidative damage in rats. International Journal of Molecular Sciences, 12, 6529-6543, (2011).
45. Gabele E, Fron M, Aeteel GE, Uesugi T, Hellerbrand C and Scholmerich J, TNF-alpha is required for cholestasis-induced liver fibrosis in the mouse. Biochemical and Biophysical Research Communications, 378, 348-353, (2009).
46. Lee SS, Kim, DH and Lee S, Anti-inflammatory, analgesic and hepatoprotective effect of semen of *Rumex crispus.* Korean Journal of Pharmacognosy, 38, 334-338, (2007).
47. Moore KW, O'Gaara A, deWaal MR, Vieira P and Mosman T, Interleukin-10. Annual Review of Immunology, 11,165-190, (1993).
48. Huang YH, Shi MN, Zheng WD, Zhang LJ, Chen ZX and Wang XZ, Therapeutic effect of interleukin-10 on CCl4-induced hepatic fibrosis in rats. World Journal of Gastroenterology, 7, 1386-1391, (2006).
49. Hou YL, Tsai YH, Lin YH and Chao JCJ, Ginseng extract and ginsenoside Rb1 attenuate carbon tetrachloride-induced liver fibrosis in rats. BMC Complementary and Alternative Medicine, 414, 415, (2014).
50. Rocha SWS, de França MER, Rodrigues GB, Barbosa KPS, Nunes AKS, Pastor AF, Oliveira AGV, Oliveira WH, Luna RLA, and Peixoto CA, Diethylcarbamazine reduces chronic inflammation and fibrosis in carbon tetrachloride-(CCl4-) induced liver injury in mice. Hindawi Publishing Corporation Mediators of Inflammation, 2014, 1-15, (2014).
51. Frondoza CG, Sohrabi A, Polotsky A, Pan P.A, Hungerford D.S and Lindmark, L. An *in vitro* screening assay for inhibitors of proinflammatory mediators in herbal extracts using human synoviocyte cultures. In Vitro Cellular & Developmental Biology – Animal, 40, 95-101, (2004).
52. Tripathi S, Maier K.G, Bruch D and Kittur DS, Effect of 6-gingerol on pro-inflammatory cytokine production and costimulatory molecule expression in murine peritoneal macrophages. Journal of Surgical Research, 138, 209-213, (2007).
53. Ebuehi OA and Okorie NA, Phytochemical screening and quantification of flavonoids from leaf extract of *Jatropha curcas* Linn. Nigerian Quarterly Journal of Hospital Medicine, 19, 200-205, (2009).
54. Wills PJ and Asha VV, Preventive and curative effect of *Lygodium flexuosum* (L.) Sw. on carbon tetrachloride induced

hepatic fibrosis in rats. Journal of Ethnopharmacology, 107, 7-11, (2006).
55. Recknagel RO, Glende, EA, Dolak JA, Waller RL, Mechanism of carbon tetrachloride toxicity. Pharmacology & Therapeutics, 43, 139-154, (1989).
56. Ozturk f, Ucar M, Ozturk IC, Vardi N and Batcioglu K, Carbon tetrachloride-induced nephrotoxicity and protective effect of betaine in sprague-dawley rats. Urology, 62, 353-356, (2003).
57. Cotran RS, Kumar V and Collins T, Cellular pathology. I: cell injury and cell death, in Cotran RS, Kumar V, and Collins T (Eds): Robbins' Pathologic Basis of Disease, 6th ed. Philadelphia, WB Saunders, 13-14, (1999).
58. Sheweita SA, Abd El-Gabar M and Bastawy M, Carbon tetrachloride changes the activity of cytochrome P450 system in the liver of male rats: role of antioxidants. Toxicology, 169, 83-92, (2001).
59. Ligangli YH, Scott M, Jonathan M, John W and Minq Q, Free radical scavenging properties of wheat extracts. Journal of Agricultural and Food Chemistry, 50, 1619-1624, (2002).
60. Olagunju JA, Adeneyeb AA, Fagbohunkac BS, Bisugac NA, Ketikuc AO, Benebod AS, Olufowobic OM, Adeoyec AG, Alimic MA and Adeleke AG, Nephroprotective activities of the aqueous seed extract of *Carica papaya* Linn. in carbon tetrachloride induced renal injured Wistar rats: a dose-and time-dependent study. Biology and Medicine, 1, 11-19, (2009).
61. Miller NJ and Rice-Evans CA, The relative contribution of ascorbic acid and phenolics antioxidants to the total antioxidant activity of orange and apple fruit juices and blackcurrant drink. Food Chemistry, 60, 331-337, (1997).
62. Adeneye AA and Benebo AS, Protective effect of the aqueous leaf and seed extract of *Phyllanthus amarus* on gentamicin-and acetaminophen-induced nephrotoxic rats. Journal of Ethnopharmacology, 188, 318-323, (2008).
63. Jeong TC, Kim HJ, Park J, Ha CS, Park JD, Kim S and Roh JK, Protective effects of red ginseng saponins against carbon tetrachloride induced hepatotoxicity in Sprague-Dawley rats. Planta Medica, 63, 136-140, (1996).
64. Halim, AB, El-Ahmady O, Hassab-Allah S, Abdel-Galil F, Hafez Y and Darwish A, Biochemical effect of antioxidants on lipids and liver function in experimentally-induced liver damage. Annals of Clinical Biochemistry, 34, 656-663, (1997).
65. Rui YC, Advances in pharmacological studies of silymarin. Memórias do Instituto Oswaldo Cruz, 85, 79-85, (1991).
66. Deep G and Agarwal R, Chemopreventive efficacy of silymarin in skin and prostate cancer. Integrative Cancer Therapies, 6, 130-145, (2007).