Potential Impact of Jatropha curcas in Combating Liver Dysfunction Induced by Carbon Tetrachloride In Rats

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ABSTRACT

The present study is undertaken to appraise the hepatoprotective effect and antioxidant properties of Jatropha curcas leaves methanolic extract. Hepatoprotective activity was measured by using aminotransferases (AST and ALT), alkaline phosphatase (ALP) enzyme activities, levels of hydrogen peroxide (H$_2$O$_2$), nitric oxide (NO) as well as total antioxidant capacity (TAC). Rats were orally administered J. curcas extract (250 mg/kg of b.w.t.) and silymarin as reference drug (50 mg/kg of b.w.t.), for one month post injection of CCl$_4$. The data indicated that, liver enzyme activities; AST, ALT and ALP were significantly increased in CCl$_4$-intoxicated rats with percentages 148.33, 64.66 and 177.15%, respectively. Treatment with J. curcas and silymarin drug improved the increment in serum AST, ALT and ALP enzyme activities as compared to control rats. On the other hand, oxidative stress markers; H$_2$O$_2$ and NO were significantly increased with percentages 264.53 and 93.16%, respectively in CCl$_4$-intoxicated rats while, TAC was significantly reduced with percentage 38.46% as compared to control rats. The administration of J. curcas extract declared amelioration in the elevated levels of AST, ALT ALP, H$_2$O$_2$ and NO in CCl$_4$-intoxicated rats with improvement percentages 53.75, 74.35, 180.93, 51.41 and 75.87%, respectively. In contrast, extract of J. curcas increased TAC with percentage 38.46%. Histopathological investigation further substantiated the protective effect of J. curcas extract through normalization of hepatic architecture as well as hepatic cells. Because of the broad umbrella term that is used to describe several mechanisms dependent-antioxidant properties of J. curcas, the methanolic extract of J. curcas leaves may be used in health benefits as nutraceuticals and/or a natural ingredient in pharmaceutical industries for curing liver dysfunction.

Keywords: Hepatoprotective, Antioxidant, Jatropha curcas, CCl$_4$, Aminotransferases, Silymarin.

INTRODUCTION

Liver, the largest organ in the human body, is the key organ of metabolism including glycogen storage, decomposition of red blood cells, plasma protein synthesis and detoxification.1 The liver consists of various cell types, including hepatocytes, biliary epithelial cells, sinusoidal endothelial cells, stellate cells and Kupffer cells however, hepatocytes, which carry out most of the metabolic and synthetic functions of the liver, account for about 80% of liver weight and about 70% of all liver cells.2 Hepatocytes are involved in contrastive enzymatic metabolic activities, and any damage occurs to this organ will lead to upset in the body metabolism.3 Hepatotoxicity is defined as injury to the liver that is associated with impaired liver function caused by exposure to a drug or another non-infectious agent.4 Chemical toxins including acetaminophen, carbon tetrachloride (CCl$_4$), galactosamine and thioacetamide are often used as the model substances causing experimental hepatocyte injury in both in vivo and in vitro conditions.5 CCl$_4$, producing reactive free radicals when metabolized, a widely used hepatotoxic agent induces toxicity in rat liver which closely resembles human cirrhosis via the generation of trichloromethyl (CCl$_2$) free radical6. Moreover, CCl$_4$ increases lipid peroxidation in hepatic cells and induces liver damage and necrosis.7 Inspite of tremendous scientific advancement in the field of hepatology in recent years, liver problems are on the rise.8 In addition, numerous side effects are associated with synthetic drugs used in treating hepatic disorders. Consequently traditional herbal drugs, spices, fruits, vegetables, and medicinal plants have gained popularity over the past decades owing to their safety and efficacy.9 Jatropha curcas plant, commonly known as physic nut or purging nut, belongs to the Euphorbiaceae family. J. curcas is used in folklore remedies for treatment of various ailments such as skin infections, gonorrhea, jaundice and fever.10 Ekundayo and Ekewe11 mentioned the scientific validation for the use of J. curcas plant in traditional medicine that serves as a guide for selection of plants with antimicrobial activity due to the presence of compounds with antibacterial activity. The therapeutic effects of the extract of J. curcas leaves have been documented.12 J. curcas is a good source of antioxidant and metal chelating peptides, which may have a positive impact on the economic value of this crop, as a potential source of food functional components.13 Triterpenoids from J. curcas leaves showed extraordinarily strong antibacterial and antifungal activities as well as antioxidant ability, which tallyed with the traditional use of J. curcas leaves in oral and wound treatments.14 Moreover, El-Baz15 has reported that, the methanolic extract of J. curcas leaves is highly valuable source of natural antioxidants that showed high antioxidant activity. There is a relation between antioxidant and hepatoprotective mechanisms that has aroused the present study. Thus, the current study, aims to determine...

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the potential impact of \textit{J. curcas} methanolic extract in combating liver dysfunction induced by \textit{CCl}	extsubscript{4} in rats throughout measuring liver function tests, oxidative stress, antioxidant activity as well as the histopathological examination of liver.

\textbf{MATERIALS AND METHODS}

\textbf{Chemicals and drugs}

Solvents of analytical grade were purchased from Merck and Aldrich. All the chemicals including silymarin were purchased from Sigma Aldrich, USA. All the biochemical kits were purchased from Biosystems (Alcobendas, Madrid, Spain), Sigma Chemical Company (St. Louis, MO, USA), Biodiagnostic Company (Cairo, Egypt).

\textbf{Collection and identification of plant material}

The fresh leaves of \textit{J. curcas} plant were collected from the farm of Aromatic and Medicinal Plant Department, Agriculture Research Centre (ARC), Egypt. The plant was identified and authenticated by Mrs Treas Labib, Herbarium section, El-Orman Botanical Garden, Giza, Egypt. The leaves were washed thoroughly in tap water then distilled water to remove dust and dirt, shade dried, powdered and stored in opaque screw tight jars prior to further use.

\textbf{Preparation of methanolic extract}

Leaves of \textit{J. curcas} were extracted by maceration in methanol with a ratio (3:1) at room temperature for 48 hours on shaker (Heidolph) at 150 rpm. The macerate was filtered successively on Whatman No. 4 filter paper and Buchner. The filtrate was evaporated to dryness under low-pressure vacuum evaporation of the methanol in Rotary evaporator (Heidolph) at 40°C then stored in refrigerator (4°C) till biological assay and chemical analysis.

\textbf{In vivo estimation of hepatoprotective and antioxidant activities}

\textbf{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 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 the Medical Ethical Committee of National Research Centre, Egypt.

The rats were divided into five groups of ten animals each as follows

- Group 1: Normal control rats
- Group 2: Normal control rats administered methanolic extract of \textit{J. curcas} leaves (250 mg/kg body weight).
- Group 3: \textit{CCl}	extsubscript{4}-intoxicated rats intraperitoneally (ip) injected with \textit{CCl}	extsubscript{4} (0.5 ml/kg body weight) suspended in olive oil (1:9 v/v) twice a week for six consecutive weeks.\textsuperscript{16}
- Group 4: \textit{CCl}	extsubscript{4}-intoxicated rats orally administered with crude methanolic extract of \textit{J. curcas} (250 mg/kg body weight) daily for 30 days.
- Group 5: \textit{CCl}	extsubscript{4}-intoxicated rats orally administered with silymarin drug (50 mg/kg body weight) daily for 30 days.\textsuperscript{17}

\textbf{Blood sampling}

After 30 days of treatments, 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 rpm for serum separation. The liver of all the experimental animals were removed and processed immediately for histological investigation.

\textbf{Assessment of liver function}

Serum enzyme activities of AST, ALT and ALP were measured using colorimetric kits to assess the hepatotoxicity according to the method of Reitman and Frankel\textsuperscript{18} and Belfield and Goldberg.\textsuperscript{19}

\textbf{Assessment of oxidative stress and antioxidant biomarkers}

Liver in each exponential group weighed and homogenized in 5-10 volumes of appropriate medium using electrical homogenizer, centrifuged at 3000 rpm for 15 min, the supernatants (10%) were collected and placed in Eppendorff tubes then stored at -80°C and used for determination of oxidative stress and antioxidant biomarkers (H\textsubscript{2}O\textsubscript{2}, NO and TA). H\textsubscript{2}O\textsubscript{2} was determined according to the method of Aebi.\textsuperscript{20} While, NO was determined in liver homogenate according to the method of Moshage.\textsuperscript{21} Moreover, TAC was determined in liver homogenate according to the method described by Koracevic.\textsuperscript{22}

\textbf{Calculation}

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

\[
\text{% of Improvement} = \frac{\text{Mean of treated} - \text{Mean of disease}}{\text{Mean of control}} \times 100
\]

\textbf{Histopathological examination}

One day post the thirty day of the experiment, all the animals were sacrificed and the liver was dissected out. The animal liver specimen obtained from different groups were fixed in 10% buffered formalin for 24 hr 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.\textsuperscript{23} The cytoplasm stained shades of pink and red and the nuclei gave blue color. The slides were examined and photographed under a light microscope (x100, 200 magnification).
Statistics

All values are expressed as means ± SD. Biochemical results were subjected to one-way analysis of variance (ANOVA) and the significance differences between means was tested using co-state computer program. Unshared letters are statistically significant at p ≤ 0.05.

RESULTS

Liver function tests in different experimental groups

Liver function enzyme activities showed significant increase in AST, ALT and ALP levels in CCl₄-intoxicated rats with percentages 75.89, 65.79 and 167.59%, respectively as compared to normal control rats (Figure 1).

These elevated values returned more or less to normal levels post administration of J. curcas extract as well as silymarin reference drug with improvement percentages 53.75 and 99.21%, respectively for AST, 74.35 and 84.61%, respectively for ALT, 180.93 and 164.60%, respectively for ALP enzyme activities.

Figure 1: Effect of J. curcas extract supplementation on liver enzyme activities in different experimental groups

Oxidative stress biomarkers and antioxidant activity in different experimental groups

Regarding to intoxicated rats, H₂O₂ and NO levels were found to be significantly increased with percentages 264.53 and 93.16%, respectively as compared to normal control group (Figure 2). However, TAC was significantly decreased in liver injected with CCl₄ with percentage 38.46%. Administration of J. curcas extract and silymarin drug remarkably ameliorated CCl₄-induced hepatotoxicity by reducing the levels of oxidative stress markers (H₂O₂ and NO) with percentages 51.40 and 49.38%, respectively for H₂O₂, 75.87 and 75.87%, respectively for NO. While, TAC was elevated post J. curcas extract as well as silymarin treatments with improvement percentages 38.46 and 46.15%, respectively.

Figure 2: Effect of J. curcas extract supplementation on oxidative stress and antioxidant biomarkers in different experimental groups

Histopathological examination of liver

The liver of control rats showed normal hepatic cells and normal lobular architecture (Photo1). Portal tract, radiating fibrous strands, fibrous stands, ballooning degenerative hepatic cells and compressed blood vessels were observed in CCl₄-intoxicated group (Photo2). The histological appearance of J. curcas extract and silymarin-treated rats was quite similar to that of control rats, so that less extent of tissue damage in these two groups was observed than CCl₄-intoxicated one (Photos 3&4).

Photo 1: A photomicrography of control group showing normal hepatic tissue. The liver is divided histologically into lobules. The center of the lobule is the central vein (thin arrow) and at the periphery of the lobule there are the portal triads (thick arrow) (H&E 100)

Photo 2: A photomicrography of intoxicated rats hepatic tissue, the left one showing portal tract (P.T) and radiating fibrous strands (arrows), while the right one with highly magnification showing the fibrous stands (thin arrow) and ballooning degenerative hepatic cells (arrow heads) all this lead to compressed blood vessels (thick arrow (H&E 100)
Photo 3: A photomicrography of J. curcas treated-intoxicated rats showing portal tract (P.T) in the center with well improved hepatic architecture, the vessels walls looking normal (straight arrow) surrounded by retracted fibrous band (curved arrow), also the hepatic cells (arrow heads) looks well improved (H&E100)

Photo 4: Aphotomicrography of silymarin treated intoxicated rats showing the central veins (arrows) and the hepatic cells (arrow heads) are improved (H&E 100)

DISCUSSION

The magnitude of hepatic damage is usually assessed by measuring the level of released cytosolic transaminases including ALT and AST enzyme activities in the circulation. ALP enzyme activity is excreted by liver via bile in the liver injury due to hepatotoxins, which result in a defective excretion of bile by the liver and is reflected in their increased levels in serum. Regarding to the present study, the CCl₄-intoxicated rats revealed significant increment in AST, ALT and ALP enzymes activities with percentages 75.89, 65.79 and 167.59%, respectively. These results run in parallel with those reported by Adewale and Mohamed, as they demonstrated increase in the levels of serum enzymes, (AST, ALT and ALP) in CCl₄-treated rats. These increments could be rely on the basis of, damaged structural integrity of the liver alter permeability of the membrane causes releasing the enzymes from the cells into the circulation. Administration of J. curcas extract led to decrease in the elevated levels of AST, ALT and ALP enzyme activities with improvement percentages 53.75, 74.35 and 180.93%, respectively. While, improvement percentages using silymarin reached to 99.21, 84.61 and 164.60%, respectively. These results were supported by the results of Imtiyaz who reported that, petroleum ether, alcholic and aqueous extracts of J. curcas leaves (200mg/kg) showed a protective effect through lowering serum enzymes AST, ALT and ALP in CCl₄-induced hepatotoxicity. The coordinate action of antioxidant system is very critical for the detoxification of free radicals. J. curcas plant has promising phytochemicals such as; polyphenol and flavonoid compounds that have antioxidant properties. El-Baz reported that, successive methanolic extract of J. curcas leaves had bioactive compounds including; gallic acid, catechin, rutin, coumaric acid, ferulic, benzoic acid, acacetin, coumarin, luteolin and genistein with high antioxidant activities. The protective effect on the deteriorated hepatic function may be due to the presence of polyphenols and other antioxidants, as the toxicity produced by CCl₄ is due to oxidative stress. The methanolic extract of J. curcas stem bark had proanthocyanidins compounds. Dai hypothesized that, the antioxidant properties of proanthocyanidins would relieve the oxidative stress initiated by CCl₄-induced free radicals and subsequently prevent liver injury. Thus, the enhancement effect of J. curcas extract on liver function enzymes may be due to its antioxidant activity. Taken together, reactive oxygen species (ROS) and reactive nitrogen species (RNS) generated in our body are quite reactive and harmful to the cells. Most ROS are generated as by-products during mitochondrial electron transport and the sequential reduction of oxygen by the addition of electrons leads to the formation of a number of ROS including; superoxide (O₂⁻), hydrogen peroxide (H₂O₂), hydroxyl radical (OH•), hydroxyl ion (OH⁻) and nitric oxide (NO). Generated ROS and RNS can damage important molecules such as, proteins, DNA and lipids which lead to the development of a variety of diseases including aging, mutagenesis, carcinogenesis, coronary heart disease, diabetes and neuro-degeneration. According to the present results, CCl₄-intoxicated rats revealed significant increase in the H₂O₂ and NO levels with percentages 264.53 and 93.16 %, respectively. In contrast, TAC recorded significant decrease with percentage 38.46%, in CCl₄-intoxicated rats. These results were supported by the results of Abd El aziz and Mohamed, who found that, CCl₄-intoxicated rats revealed an increase in the level of NO. Treating of CCl₄-intoxicated rats with the methanolic extract of J. curcas resulted in reduction of H₂O₂ and NO levels with improvement percentages 51.40 and 75.87%, respectively. While, TAC exhibited improvement post J. curcas extract with percentage 38.46%. Regarding to silymarin reference drug, H₂O₂ and NO levels declared amelioration with percentages 49.38 and 75.87%, respectively. However, TAC demonstrated improvement percentage 46.15%. Igbinosa reported that, J. curcas plant contained polyphenolic components which could be attributed to its pharmacological activities associated with scavenging free radicals. The authors added that, the plant could play an important role in the prevention of oxidative stress dependent diseases. Moreover, natural antioxidants strengthen the endogenous antioxidant defenses, by ROS scavenging, restoring the optimal balance by neutralizing...
the reactive species and gaining immense importance by virtue of their critical role in disease prevention.\textsuperscript{38} The antioxidant activity of \textit{J. curcas} extract may be related to lycopene which has a considerable reactive oxygen species (ROS) scavenging activity, allows to prevent lipid peroxidation, DNA damage, inducing enzymes of the cellular antioxidant defense systems by activating the antioxidant response element transcription system.\textsuperscript{39} The liver is expected not only to perform physiological functions but also to protect against the hazards of harmful drugs and chemicals.\textsuperscript{40} The prescription of multiple drugs is related to increase the risk of adverse drug-related events, among these, hepatic injury.

Hepatic fibrosis represents a ubiquitous response of the hepatic acute or chronic injury.\textsuperscript{41} Hepatic fibrosis is a common pathological process in invertebrate rife liver diseases.\textsuperscript{42} According to the present results, \textit{CCl}$\textsubscript{4}$-intoxicated rats showed portal tract with radiating fibrous strands, ballooning degenerative hepatic cells and compressed blood vessels (Photo 2). These findings were reinforced by the results of Abd Al-Zahra\textsuperscript{44} who found that, necrotic damage, ballooning degeneration, cholestasis, steatosis and inflammatory cell infiltration in the liver of \textit{CCl}$\textsubscript{4}$-treated rats. Further support to the present findings, Reza\textsuperscript{45}, observed necrotized tissue scar and ballooning of the hepatocytes in liver of \textit{CCl}$\textsubscript{4}$-treated rats. The current results coincide also with Zameer\textsuperscript{46}, who observed necrosis and degeneration in liver of rats intoxicated with \textit{CCl}$\textsubscript{4}$. A single dose of \textit{CCl}$\textsubscript{4}$ leads to necrosis, while prolonged administration leads to liver fibrosis besides, \textit{CCl}$\textsubscript{4}$ impairs hepatocytes directly throughout altering the permeability of the plasma, lysosomal, and mitochondrial membranes.\textsuperscript{46} Liver fibrosis is the pathological result of ongoing chronic which is characterized by hepatic stellate cell (HSC) proliferation and differentiation to myofibroblast-like cells, which deposit extracellular matrix (ECM) and collagen.\textsuperscript{47} The authors added that, the activated HSC produces large amounts of ECM components such as, laminin and collagen type IV resulting in fibrotic change of the liver. The hepatic architecture well improved and looked normal after treating of \textit{CCl}$\textsubscript{4}$-intoxicated rats with \textit{J. curcas} methanolic extract and silymarin standard drug (photos 3 and 4). The extracts of \textit{Phyllanthus niruri} Linn and \textit{Phyllanthus emblica} Linn (Euphorbiaceae) revealed hepatoprotective activity in \textit{CCl}$\textsubscript{4}$-induced hepatotoxicity in rats which confirmed by improvement in the histopathological observation.\textsuperscript{25} The authors suggested that, the hepatoprotective activity of these plants could be attributed to phytochemicals like phyllanthin, embellin and rographolide. So, the enhancement effect of \textit{J. curcas} extract in hepatic architecture may be due to the phytochemical compounds. Petroleum ether, ethyl acetate, successive and crude methanolic extracts of \textit{J. curcas} leaves showed the presence of flavonoid compounds.\textsuperscript{35} The presence of flavonoids may be responsible for \textit{J. curcas} antioxidant and hepatoprotective activities.\textsuperscript{47} Numerous studies have suggested that flavonoids commonly function as antioxidants and may protect against oxidative stress.\textsuperscript{48,49}

Huo\textsuperscript{50} hypothesized that, licorice extract may play an important role in medicine by scavenging free radicals and stimulating activities of antioxidant enzymes, subsequently protecting the liver against \textit{CCl}$\textsubscript{4}$-induced damage. Hence, it could be suggested that, the hepatoprotective effect of \textit{J. curcas} extract may be due to the presence of flavonoids.

**CONCLUSION**

Findings of the present study strongly suggested that \textit{J. curcas} extract have protective effects similar in most aspects to silymarin drug via antioxidant effects. So, \textit{J. curcas} products may be used as adjunctive therapy in liver damage. However, the present study is a primary platform for further phytochemical, pharmacological studies and mechanism/s of ameliorating action of \textit{J. curcas} plant.

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