The Ameliorating Effect of Jatropha curcas Extract Against CCl₄-Induced Cardiac Toxicity and Genotoxicity in Albino Rats

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ABSTRACT

This study characterizes the effective role of Jatropha curcas methanolic extract against the toxic impact of carbon tetrachloride (CCl₄)-induced cardiac dysfunction in rats. The methanolic leaf extract at a dose 250 mg/kg was administered orally for 30 days. Blood and heart tissues were collected for the assessment of serum adhesion molecules biomarkers; intercellular adhesion molecule-1 (ICAM) and vascular cell adhesion molecule-1 (VCAM), micronucleus formation, DNA damage, gene expression analyses and the possible protective effect of J. curcas against CCl₄ induced cardiac toxicity. Moreover, the histopathological investigation was carried out on cardiac tissue to ascertain the biochemical results. The obtained results declared that, injection of rats with CCl₄ significantly increased the levels of ICAM, VCAM, the micronucleus formation, DNA damage besides significant alterations in the genes expression encoding oxidative (NOS), antioxidant enzymes (GST) as well as genes related to lipid metabolism (H-FABP and C-FABP) in heart tissues. On the other hand, treating of rats with J. curcas extract exhibited decrease in the elevated levels of ICAM, VCAM, the rate of the micronucleus formation, DNA damage and inhibited the alterations in the gene expression of rats induced by CCl₄. In addition, the histopathological investigation in the rat’s heart supported that J. curcas extract markedly reduced the toxicity of CCl₄ and preserved the architecture of the cardiac tissue to near normal level. Hence, the present results suggest that J. curcas extract acts as a potent therapeutic agent against CCl₄-induced cardiotoxicity.

Keywords: Jatropha curcas, CCl₄, cardiac dysfunction, adhesion molecules, DNA damage, gene expression, silymarin.

INTRODUCTION

Cardiovascular diseases, which include stroke, coronary artery diseases (CAD) and myocardial infarction, are the leading cause of morbidity and mortality in the world. Atherosclerosis, the complex interaction of serum free and esterified cholesterol with the cellular components of the arterial wall, is known to be the primary underlying factor of these cardiovascular events, it is a chronic inflammatory disease started by endothelial injury, followed by sub-intimal focal recruitment of circulating monocytes and T-lymphocytes that heals by fibrosis and calcification and is the leading cause of cardiovascular disease worldwide. Inflammation plays a crucial role in atherogenesis either by local cellular mechanisms or humoral consequences easily measurable in plasma. In most cases inflammation and endothelial dysfunction are triggered by cardiovascular risk factors: hypercholesterolemia, hypertension, toxicity, smoking or diabetes. Irrespective of its cause systemic inflammation is correlated with cardiovascular events, but currently there are controversial results regarding inflammatory markers and early atherosclerotic process. The selected inflammatory markers are associated with different pathogenic steps in atherosclerosis, endothelial activation markers (soluble VCAM-1, ICAM-1).

Carbon tetrachloride (CCl₄) induces liver damage in experimental models. Chronic liver damage in rats produces liver fibrosis and biochemical and histological patterns that resemble human liver cirrhosis. Various studies have demonstrated that CCl₄ intoxication causes free radical generation in many tissues such as liver, kidney, heart, lung, testis, brain and blood. Free radicals are found to be responsible for various pathological conditions including, dyslipidemia leading to atheroma formation, the oxidation of LDL, endothelial dysfunction, plaque rupture, myocardial ischemic injury, and recurrent thrombosis. Oxidative stress has been implicated as well in diabetic cardiomyopathy, congestive cardiomyopathy and hypertensive heart disease. CCl₄ metabolism in the liver induces hepatotoxicity by the stimulation of lipid peroxidation and the production of free radicals which causes necrosis of hepatocytes, induces inflammation and further promotes progression of hepatic fibrogenesis. To overcome from such deleterious effects of free radicals, an effective natural product with excellent antioxidant potential may be the one of solution.

Herbal products, supported by their safety, cost-effectiveness and versatility, are enjoying growing worldwide popularity to treat different inflammatory conditions. Jatropha curcas, belonging to Euphorbiaceae family, cures many diseases, such as arthritis, jaundice, dental complaints, tumours, allergies, burns, cut wound, leprosy scabies, and smallpox. J. curcas leaves extracts are reported to have hepatoprotective activity against CCl₄-induced hepatic damage. Methanolic fraction of J. curcas showed hepatoprotective activity on aflatoxin B1 induced hepatic carcinoma in animals. 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. The leaves of J. curcas could serve as a promising source of drug for the treatment of liver related complications of oxidative stress.

From this point of view, the purpose of the present in vivo study is to optimize and evaluate the therapeutic efficacy and the molecular pathway of J. curcas extract and silymarin as reference drug in ameliorating cardiac dysfunction induced by CCl₄ in female rats.

**MATERIALS AND METHODS**

**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.

**Preparation of the plant powder and extraction**

The plant leaves were obtained from the farm of Aromatic and Medicinal Plant Department, Agriculture Research Centre (ARC), Egypt. The plant was authenticated by Mrs. Tres Labib, Herbarium section, El-Orman Botanical Garden, Giza, Egypt. Leaves were washed with tap water then with distilled then dried under shade. Shade dried leaves were milled, homogenized and extracted using methanol. The homogenate was kept on shaker (Heidolph) at room temperature for 48 hrs. at 150 r.p.m. Then, the extract was filtered using Whatman No. 4 filter paper and Buchner then evaporated to dryness by using Rotary evaporator (Heidolph) at 40°C. The extract was stored in refrigerator at (4°C) till biological assay and chemical analysis until used for the experiment.

**Biological experiment**

**Animals**

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 Medical Ethical Committee of National Research Centre in Egypt.

**Experimental design**

The rats were randomly divided into 5 groups of 10 rats each as follows:

**Group (1):** Normal control.

**Group (2):** Control rats administered methanolic extract of J. curcas leaves at the dose 250 mg/kg body weight.

**Group (3):** CCl₄-intoxicated rats intraperitoneally administered a single dose of CCl₄ (0.5 ml/kg body weight) suspended in olive oil (1:9 v/v) twice for six consecutive weeks.

**Group (4):** CCl₄-intoxicated rats orally administered with crude methanolic extract of J. curcas at the dose 250 mg/kg body weight daily for 30 days.

**Group (5):** CCl₄-intoxicated rats orally administered with silymarin drug at the dose 50 mg/kg body weight daily for 30 days.

**Preparation of serum from blood**

Rats were fasted overnight (12-14 hours), 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 separated serum was used for biochemical analysis of ICAM-1 and VCAM-1. The heart of all the experimental animals were removed and processed immediately for histology and genotoxicity investigation.

**Estimation of serum adhesion molecules**

Adhesion molecules (VCAM-1 and ICAM-1) of serum were estimated by ELISA; a sandwich enzyme immunoassay.

**Calculation:**

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

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

**Micronucleus test**

The blood cells of female rats re-suspended in a small volume of fetal calf serum on a glass slide were used for smear preparation. The smear of blood cells was prepared from each rat. After air-drying, the slide was fixed in methyl alcohol for 10 min and stained with 5% Giemsa stain for 10 min. Three slides were prepared for each animal and were coded before observation and one was selected for scoring. From each coded slide, 2,000 polychromatic erythrocytes (PCEs) were scored for the presence or micronuclei under oil immersion at high power magnification. In addition, the percentage of micronucleated polychromatic erythrocytes (%MnPCEs) was calculated on the basis of the ratio of MnPCEs to PCEs.

**Comet Assay**

Heart tissues were homogenized in a density gradient of Gradiisol L (Aqua Medica, Lodz, Poland). A freshly prepared suspension of cells in 0.75% low melting point agarose (Sigma Chemicals) dissolved in phosphate buffer saline (PBS; Sigma chemicals) was cast onto microscope slides precoated with 0.5% normal melting agrose. The cells were then lysed for 1h at 4°C in a buffer consisting of 2.5 M NaCl, 100 mM EDTA, 1% Triton X-100 and 10 mM Tris, pH 10. After the lysis, DNA was allowed to unwind for 40 min in electrophoresis solution consisting of 300
mM NaOH, 1mM EDTA, pH >13. Electrophoresis was conducted at 4°C for 30 min at electric field strength 0.73 V/cm (30mA). The slides were then neutralized with 0.4 M Tris, pH 7.5, stained with 2 µg/ml ethidium bromide (Sigma Chemicals) and covered with cover slips. The slides were examined at 200 x magnification fluorescence microscope (Nikon Tokyo, Japan) connected to a COHU 4910 video camera (Cohu, Inc., SanDiego, CA, USA) equipped with a UV vilter block consisting an excitation filter (359 nm) and barrier filter (461nm) and connected to a personal computer-based image analysis system, Lucia-Comet v. 4.51. Fifty images were randomly selected from each sample and the comet tail DNA was measured. 18 Endogenous DNA damage was measured as the mean comet tail DNA of heart tissues of several rat groups. The number of cells scored for each animal was 100. 18

Gene expression analysis

RNA extraction

Total RNA was isolated from 100 µg of heart tissues of female rats by the standard TRizol extraction method (Invitrogen, Paisley, UK) and recovered in 100 µl molecular biology grade water. In order to remove any possible genomic DNA contamination, the total RNA samples were pre-treated using DNA-free™ DNase removal reagents kit (Ambion, Austin, TX, USA) following the manufacturer’s protocol.

Reverse transcription

The complete Poly(A)+ RNA samples were reverse transcribed into cDNA in a total volume of 20 µl using 1 µl oligo(dT) primer. The composition of the reaction mixture, termed as master mix (MM), consisted of 50 mM MgCl2, 10x reverse transcription (RT) buffer (50 mM KCl; 10 mM Tris-HCl; pH 8.3; Perkin-Elmer), 10 mM of each dNTP (Amersham, Brunswick, Germany), and 50 µM of oligo(dT) primer. The RT reaction was carried out at 25°C for 10 min, followed by 1 h at 42°C, and finished with denaturation step at 99°C for 5 min. Afterwards the reaction tubes containing RT preparations were flash-cooled in an ice chamber until being used for DNA amplification through polymerase chain reaction (PCR).

Quantitative Real Time-PCR

The first strand cDNA from different samples was used as templates for RT-PCR with a pair of specific. The sequences of specific primer and product sizes are listed in Table 1. β-actin was used as a housekeeping gene for normalizing mRNA levels of the target genes. 19 PCR reactions were set up in 25 µl reaction mixtures containing 12.5 µl 1× SYBR® Premix Ex TaqTM (TaKaRa, Biotech. Co. Ltd., Germany), 0.5 µl 0.2 µM sense primers, 0.5 µl 0.2 µM antisense primer, 6.5 µl distilled water, and 5 µl of cDNA template. The reaction program was allocated to 3 steps. First step was at 95.0°C for 3 min. Second step consisted of 40 cycles in which each cycle divided to 3 steps: (a) at 95.0°C for 15 sec; (b) at 55.0°C for 30 sec; and (c) at 72.0°C for 30 sec. The third step consisted of 71 cycles which started at 60.0°C and then increased about 0.5°C every 10 sec up to 95.0°C. At the end of each qRT-PCR a melting curve analysis was performed at 95.0°C to check the quality of the used primers. Each experiment included a distilled water control. The quantitative values of RT-PCR (qRT-PCR) of oxidative (inducible nitric oxide synthase, iNOS) and antioxidant enzymes genes (glutathione S-transferase, GST) and lipid metabolism related-genes (H-FABP and C-FABP) were normalized on the bases β-actin expression (Table 1). At the end of each qRT-PCR a melting curve analysis was performed at 95.0°C to check the quality of the used primers.

<table>
<thead>
<tr>
<th>Target cDNA</th>
<th>Primer name</th>
<th>Primer sequence (5' –3')</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-Actin</td>
<td>F</td>
<td>GTG GCC CGC TCT AGG CAC CAA</td>
<td>Rawal20</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>CTC TTT GAT GTC AGG CAC GAT TTC</td>
<td></td>
</tr>
<tr>
<td>INOS</td>
<td>F</td>
<td>gtt tgt cac cag gag atg tgt</td>
<td>Rawal20</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>tgg ggc agt ctc cat tgc ca</td>
<td></td>
</tr>
<tr>
<td>GST</td>
<td>F</td>
<td>CTG AAC TCA GGT AGT CCA GC</td>
<td>Khalli and Booles21</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>GGA GGT AGA AGT GCA AGA</td>
<td></td>
</tr>
<tr>
<td>H-FABP</td>
<td>F</td>
<td>CTA GCA TGA GGG AAG CAA GG</td>
<td>Eshak22</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>TGC TTC ATC CAG ACA AGT GG</td>
<td></td>
</tr>
<tr>
<td>C-FABP</td>
<td>F</td>
<td>GGG CTG GCT CTT AGG AAG AT</td>
<td>Eshak22</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>AAA ACA CGG TCG TCT TCA CC</td>
<td></td>
</tr>
</tbody>
</table>

Table 1: Primers and reaction parameters in RT-PCR

Calculation of Gene Expression

First the amplification efficiency (Ef) was calculated from the slope of the standard curve using the following formula found in the manufacturer’s instruction pamphlet:

\[ Ef = 10^{\frac{1}{slope}} \]

Efficiency (%) = \( \frac{Ef - 1}{1} \times 100 \)

The relative quantification of the target to the reference was determined by using the \( 2^{-\Delta\Delta Ct} \) method if Ef for the target (iNOS, GST, H-FABP and C-FABP) and the reference primers (β-Actin) as follows:

\[ \Delta Ct_{(test)} = Ct_{(target, test)} - Ct_{(reference, test)} \]

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\[ \Delta \text{CT(calibrator)} = C_{\text{target, calibrator}} - C_{\text{reference, calibrator}} \]

\[ \Delta \Delta \text{CT} = \Delta C_{\text{T(test)}} - \Delta C_{\text{T(calibrator)}} \]

The relative expression was calculated by \(2^{-\Delta \Delta \text{CT}}\).

**Histopathological Investigation of cardiac tissue**

Anatomy of the heart was studied immediately after sacrificing rats. A fresh piece of the heart was obtained from each group for light microscopic investigations. The heart specimens were fixed in 10% buffered formalin for 24 h. 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.\(^{23}\) The cytoplasm stained shades of pink and red and the nuclei gave blue colour. The slides were examined and photographed under a light microscope (×400 magnification).

**Statistics**

Statistical analysis was performed using the SPSS for Windows statistical package, version 10.0 (SPSS Inc., Chicago, IL, USA). Data 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 \(p < 0.05\). All data of gene expression were analyzed using the General Liner Models (GLM) procedure of Statistical Analysis System\(^{24}\) followed by Scheffé-test to assess significant differences between groups. The values are expressed as mean±SEM. All statements of significance were based on probability of (Ps 0.05).

**RESULTS**

**Soluble endothelial adhesion molecules level in different experimental groups**

\(\text{CCL}_4\)-intoxicated rats showed significant increment in \(\text{ICAM-1}\) and \(\text{VCAM-1}\) with percentages reached to 115.73 and 62.46%, respectively as compared to normal control (Figure. 1).

However, treatment of intoxicated rats with \(\text{J. curcas}\) methanolic extract as well as silymarin drug revealed percentages of improvement in \(\text{ICAM-1}\) and \(\text{VCAM-1}\) levels 91.13 and 41.63%, for \(\text{J. curcas}\) and 94.75 and 48.46%, for silymarin respectively. Thus, it could be deduced that, silymarin drug revealed the highest percentages of improvement in \(\text{ICAM-1}\) and \(\text{VCAM-1}\) levels (94.75 and 48.46%, respectively), followed by \(\text{J. curcas}\) methanolic extract which recorded 91.13 and 41.63%, respectively.

**Micronucleus formation**

The effect of \(\text{J. curcas}\) and silymarin against \(\text{CCL}_4\) induced MnPCEs formation in the blood cells of female rats is summarized in Figure 1. The results revealed that MnPCEs formation in control and \(\text{J. curcas}\) treated rats was lower than those in all treated groups. Treatment of female rats with \(\text{CCL}_4\) increased significantly the formation of MnPCEs as compared to control group (Figure. 2). Where, the increase rates of MnPCEs formation were 349% of \(\text{CCL}_4\) in comparison to control rats.

On the other hand, treatment of female rats with \(\text{J. curcas}\) alone revealed similar rate of MnPCEs formation to that in control group (Figure 2). Moreover, treatment of female rats with \(\text{J. curcas}\) post \(\text{CCL}_4\) injection decreased significantly the incidence of MnPCEs in comparison to \(\text{CCL}_4\) alone. Moreover, similar protective effect was observed with silymarin treatment. Treatment of female rats with silymarin after \(\text{CCL}_4\)-injection demonstrated significantly reduction in the incidence of MnPCEs as compared to \(\text{CCL}_4\) alone. Treatment with \(\text{J. curcas}\) or silymarin decreased the rates of MnPCEs formation by 39.7% and 34.6%, respectively comparing with those in rats treated with \(\text{CCL}_4\) alone (Figure 2).

**Assessment of DNA damage using Comet Assay**

The results in Table 2 show the effect of \(\text{CCL}_4\) on DNA damage and the therapeutic role of \(\text{J. curcas}\) extract and silymarin against its effect using comet assay. The results revealed that \(\text{J. curcas}\) treatment had positive effect on the DNA structure, where the rate of DNA damage in this group was similar to control group. However, the proportion of DNA damage significantly increased in

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*Figure 1: Effect of *J. curcas* methanolic extract on soluble endothelial adhesion molecules level in different experimental groups*

*Figure 2: Micronucleated polychromatic erythrocytes (MnPCEs) of female rats exposed to *CCL*_4 and *J. curcas* or silymarin (mean ± SD).*
female rats intoxicated with CCl₄ as compared to control group. This damage of DNA decreased using J. curcas extract or silymarin as ameliorative products. DNA damage was significantly reduced using J. curcas extract and silymarin in rats injected with CCl₄. Thus, J. curcas extract and silymarin greatly ameliorated the genetic materials, and gave the low proportion of DNA damage in respect to CCl₄ treatment.

Table 2: Visual score of DNA damage in female rats injected with CCl₄ and treated with J. curcas or silymarin using comet assay.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Number of animals</th>
<th>No. of cells Analyzed(*)</th>
<th>No. of cells</th>
<th>Class [Range]</th>
<th>DNA damaged cells (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5</td>
<td>500</td>
<td>32</td>
<td>0</td>
<td>6.4</td>
</tr>
<tr>
<td>J. curcas</td>
<td>5</td>
<td>500</td>
<td>34</td>
<td>1</td>
<td>6.8</td>
</tr>
<tr>
<td>CCl₄</td>
<td>5</td>
<td>500</td>
<td>129</td>
<td>1</td>
<td>25.8</td>
</tr>
<tr>
<td>CCl₄+J. curcas</td>
<td>5</td>
<td>500</td>
<td>78</td>
<td>2</td>
<td>15.6</td>
</tr>
<tr>
<td>CCl₄+silymarin</td>
<td>5</td>
<td>500</td>
<td>71</td>
<td>2</td>
<td>14.2</td>
</tr>
</tbody>
</table>

* Class 0= no tail; 1= tail length < diameter of nucleus; 2= tail length between 1X and 2X the diameter of nucleus; and 3= tail length > 2X the diameter of nucleus. (*): No of cells analyzed were 100 per an animal.

The results revealed that treatment with J. curcas post injection of CCl₄ prohibited significantly the alterations induced by CCl₄ of the studied genes including INOS and GST (Figures 3&4).

Genes expression encoding oxidative and antioxidant enzymes as well as genes related to lipid metabolism in cardiac tissue

Expression analysis using quantitative RT-PCR was conducted to verify the potential protective role of J. curcas and silymarin against CCl₄ induced alteration in the expression of genes encoding oxidative (iNOS) and antioxidant enzymes (GST) and genes related to lipid metabolism (H-FABP and C-FABP) in heart tissues (Figures 3-6).

However, J. curcas or silymarin exhibited an ineffective role against the expression alterations of H-FABP and C-FABP genes induced by CCl₄ (Figures 5&6).
The results revealed that injection with CCl₄ induced significant alterations in the iNOS and GST genes in heart tissues of female rats compared with normal control rats. Intoxication with CCl₄ exhibited also alterations in the expression of H-FABP and C-FABP genes compared with control rats. On the other hand, treatment of intoxicated rats with J. Curcas declared protective effect on the gene expression alteration against CCl₄ treatment.

**Histopathological examination of cardiac tissue**

Histopathological investigation of normal cardiac tissue showed normal cardiac wall thickness with normal muscle and vein (Photo 1a). In contrast, intoxicated rats showed fibrotic strands in the cardiac wall, collagen deposition on the fibrotic cardiac wall and ballooning degeneration of the cardiac muscle cells (Photo 1b).

**Photo 1a:** A photomicrography of normal control rats showing normal cardiac tissue which showing average cardiac wall thickness (arrow) and the cardiac muscle normally appeared (arrow head) also the cardiac vein (v) looks normal (H&E100).

**Photo 1b:** A photomicrography of CCl₄-intoxicated rats, the cardiac tissue the left one showing fibrotic strands in the cardiac wall (arrows), while on the right one with higher magnification showing collagen deposition on the fibrotic cardiac wall (arrows), also there are ballooning degeneration of the cardiac muscle cells (H&E 100 & 200).

Treating of J. curcas methanolic extract as well as silymarin drug revealed improvement in cardiac architecture with minimal hyaline deposition with minimal fibrotic strands and improved cardiac muscle cells (Photos 1c&1d).

**DISCUSSION**

Cell adhesion molecules (CAMs), which are glycoproteins on cell surface, are involved in cell-cell and cell-substrate interactions and related to the development of liver illnesses. The current results indicated that, the induction of CCl₄ caused significant increment in the levels of ICAM-1 and VCAM-1 (115.73 and 62.46%, respectively) as compared to control group. These high levels of both CAMs declared the initiation of inflammatory process; inflammation emerges to be independent risk factor for the development of atherosclerosis.

The over-expression of ICAM-1 in CCl₄ induced cardiac injury most likely involves TNF-α. Expression of VCAM-1 on endothelial cells is also induced by inflammatory cytokines, and in certain pathological conditions. Thus, adhesion molecules may be associated with the initiation of cardiac injury during CCl₄ intoxication. As we know previously the adhesion of circulating leukocytes to the vascular endothelium is a critical early event in the development of atherosclerosis. This localized accumulation of leukocytes which is mediated by endothelial expression of specific adhesion molecules, such as vascular cell adhesion molecule-1 (VCAM-1), intercellular cell adhesion molecule-1 (ICAM-1), and endothelial cell selectin (E-selectin). Increased expression of adhesion molecules in human atherosclerotic lesions may lead to further recruitment of leukocytes to atherosclerotic sites. Regulation of adhesion molecule expression occurs at the transcriptional level and is mediated, at least in part, by the redox-sensitive transcription factor, nuclear factor-κB (NF-κB). Inducers of NF-κB activation, such as TNF-α, phorbol 12-myristate 13-acetate, UV radiation or toxicity cause oxidative stress, suggesting that the induction of radical oxygen species (ROS) is a signal common to a wide variety of NF-κB-inducing conditions. Although, these inducible molecules have received considerable attention,
little is known about the effects of J. curcas on adhesion molecule expression, and a better understanding of this might provide important insights into the prevention of atherogenesis. A key component of TNF-α-inducible adhesion molecule expression is the redox-sensitive transcription factor, NF-κB. 33 TNF-α activates NF-κB expression in endothelial cells, suggesting that the up-regulation of VCAM-1 expression in response to TNF-α is mediated by this transcriptional factor. Thus, NF-κB factors are necessary to activate VCAM-1 gene expression. Several lines of evidence indicate that ROS are implicated in the activation of NF-κB and act as a common second messenger in various stimulus-specific pathways leading to NF-κB activation. 35 So, the potent antioxidants, such as J. curcas extract may block NF-κB activation, not only by H₂O₂ and ionizing radiation, but also by nonoxidizing stimuli. 34 Cominacini 35, 36 reported that ox-LDL or TNF-α increases the expression of adhesion molecules on endothelial cells via NF-κB activation, and that the effect is inhibited by the presence of compounds with radical scavenging activity, and propose that NF-κB-mediated adhesion molecule expression follows activation by multiple radical-generating systems. The current study shows that J. curcas extract can directly scavenge H₂O₂ and significantly decrease the amount of H₂O₂ generated by CCl₄ under steady state or oxidative stress conditions. Based on the results of the present study, we propose that the inhibitory effect of J. curcas on VCAM-1 expression and NF-κB activation may be due to its antioxidant properties and that it acts by directly scavenging H₂O₂. In addition, reduction of lipid peroxidation by J. curcas may also contribute to NF-κB inactivation. However, the critical steps in the signal transduction cascade of NF-κB activation by ROS remain to be determined.

In comparison with CCl₄ intoxicated rats, the treatment with J. curcas extracts as well as silymarin, both CAMs were significantly reduced. On the basis of the presented data, both treatments were observed to inhibit the expression of VCAM-1 and ICAM-1 as they known to be protective against the progression of atherosclerosis, Borai 36 declared that, this effect may be attributed to anti-oxidative effects of both treatment that reduced the oxidation of LDL-C to ox-LDL-C. Furthermore, VCAM-1 can also mediate the adhesion and migration of monocytes. These cells, located under the endothelium, become activated and differentiated into macrophages. Finally, these monocytes become foam cells via the aggregation of lipids. Additionally, the vascular smooth muscle cells gradually proliferate and migrate from the media to intima, promoting further development of atherosclerotic lesions. Ox-LDL-C can also stimulate endothelial cells to produce adhesion molecules, increasing the atherogenicity. 37 The cells involved in the atherogenesis secretion are activated by soluble factors; the cytokines. The immune inflammatory response in atherosclerosis is modulated by regulatory pathways, in which the balance between anti-inflammatory and pro-inflammatory cytokines plays a crucial role as a major determinant of plaque stability. 38

Intoxicated rats orally administered J. curcas methanol extract possessed enhanced in ICAM-1 and VCAM-1 levels with percentages of improvement 91.13 and 41.63%, respectively. ROS increases the adhesiveness of endothelial cells via activation of nuclear factor-kappa B (NF-κB), a transcriptional factor for regulating the expression of genes including CAMs. 39 The inhibition of ROS overproduction and NF-κB signal pathway activation may be useful in preventing vascular injury. 40 J. curcas plant, a potential source of natural antioxidants, including bioactive compounds such as; phenol, flavonoids, flavonols and proanthocyanidin and may be a good candidate for pharmaceutical plant based products. 41 Hence, the ameliorating effect of J. curcas methanolic extract may be due to the antioxidant effect of this plant.

Injection of CCl₄ induced damage in the heart of rats as proved by estimation of antioxidant enzymes such as, superoxide dismutase (SOD), catalase (CAT), glutathione Peroxiase (GPx), GR (glutathione reductase), glutathione-S- transferase (GST). 42 Regarding to the present results, the histopathological examination of intoxicated rats showed fibrotic strands in the cardiac wall, collagen deposition on the fibrotic cardiac wall and ballooning degeneration of the cardiac muscle cells (Photo 1b). On a similar basis Chang 43 stated that, the CCl₄-treated rats induced cardiac-fibrosis via TGF-β-p-Smad2/3-CTGF pathway. The authors hypothesized that, CCl₄ intoxication may induce the activation of TGF-β and its various downstream pathways and performed a Western blotting analysis to confirm this. Treatments of intoxicated cardiac tissue with methanolic extract of J. curcas and silymarin showed improvement in its architecture with minimal hyaline deposition. Also, the cardiac wall showing minimal fibrotic strands as well as the cardiac muscle cells are improve (Photos 1c and 1d). The cardiac ameliorating effect of J. curcas may be explained on the basis of J. curcas is considered as a powerful source of natural antioxidants, including bioactive compounds, phenol, flavonoids, flavonols and proanthocyanidin 41.

This work showed that the injection of female rats with CCl₄ significantly increased the formation of micronucleus, DNA damage and induction of alterations in the expression of genes encoding oxidative (INOS) and antioxidant enzymes (GST) as well as lipid metabolism related genes (H-FABP and C-FABP) in heart tissues. The current results are run in the same line with those reported by Abdou 44 as They reported that, rats injected with CCl₄ exhibited significant increase in the DNA damage as compared to control rats. DNA damage can originate from the direct modification of DNA throughout chemical agents or their metabolites; the processes of DNA excision repair, replication and recombination; or the process of apoptosis. 45 Therefore, CCl₄ could be one of
the mutagens in which they are inducing DNA damage due to the inhibition of the repair process of the DNA.

CCL₄ induces DNA damage or alter the gene expression patterns depending on Cytochrome P₄₅₀ system that activates CCL₄ to form trichloromethyl (CCL₃) radical. CCL₄ radical binds to cellular molecules (nucleic acids, proteins and lipids) thereby impairing crucial cellular processes such as lipid metabolism, with the potential outcome of fatty degeneration. Therefore, the effect of CCL₄ in induction of heart disease in the present study might be due to its role in lipid metabolism.

The current results show that, CCL₄ altered the expression of lipid metabolism related genes (H-FABP and C-FABP) in heart tissues. Furthermore, this radical can also react with oxygen to form the trichloromethylperoxy (CCL₃O₂⁻) radical, a highly reactive species initiates the chain reaction of lipid peroxidation, which attacks and destroys polyunsaturated fatty acids, in particular those associated with phospholipids. The authors added that, this affects the permeability of mitochondrial, endoplasmic reticulum, and plasma membranes, resulting in the loss of cellular calcium sequestration and homeostasis, which can contribute heavily to subsequent cell damage. Based on these findings and suggestions the reaction between CCL₄ radical and lipid pathway is thought to function as initiator of cardiovascular disease.

The present study demonstrates that CCL₄ stimulated the expression of oxidative enzyme (iNOS) and inhibited the expression of the antioxidant enzyme (GST). In the same line with the present observations, CCL₄ increased the expression of tumor necrosis factor-α (TNF-α), and transforming growth factors (TGF-α and β) in the cell, processes that appear to direct the cell toward self-destruction or fibrosis. Our previous study also found that CCL₄ induced significantly alterations of the genes expression encoding antioxidant enzymes; superoxide dismutase (SOD), CAT and GPx as well as HSP70 proteins in liver tissues as compared to control rats.

The American Heart Association (AHA) has had a longstanding commitment to provide information about the role of nutrition in risk reduction of cardiovascular disease (CVD) besides, all dietary guidelines have promotes the consumption of dieters rich in fruits, vegetables, low-fat or nonfat dairy products including a lot of antioxidants. Antioxidants are closely related to their bio-functionalities, such as the reduction of cellular abnormalities like DNA damage, mutagenesis, carcinogenesis and which is also associated with free radical propagation in biological systems. The current study declared, treatment of CCL₄-intoxicated rats with J. curcas decreased the formation of micronucleus, DNA damage and induction of alterations in the expression of iNOS, GST, H-FABP and C-FABP in heart tissues. Relying on Sundari results, this study suggested that extracts of J. curcas were capable of scavenging hydroxyl and may have a stronger hydroxyl radical scavenging activity. Also, the present study reveals, J. curcas and silymarin had a prominent effect on hydroxyl radical/and or super oxide scavenging. In concomitant with our results, Sundari reported that J. curcas fractions decreased the DNA damage in human peripheral blood lymphocytes exposed to UVB-irradiation. Moreover, Katiyar reported that silymarin protects epidermal keratinocytes from ultraviolet radiation-induced apoptosis and DNA damage by nucleotide excision repair mechanism. In addition, a recent study of Linjawi revealed, detoxified J. curcas seed meal decreased the DNA fragmentation induced by benzene exposure in male rats.

Antimutagen can reveal its role throughout several ways including; preventing the transformation of a mutagenic compound into mutagen, inactivating the mutagen or otherwise prevent the reaction between mutagen and DNA, inducing repress or inactivating directly or indirectly the enzymes of the DNA repair recombination and replication pathways (Bhattacharya). The genetic toxicity induced by CCL₄ is attributed to the hydrogen donating ability. J. curcas showed the presence of bioactive compounds such as steroids and terpenoids that play an important role in free radical scavenging and quenching activity in which it might be suppress the initiation of CVD. So, the depressing of genotoxicity induced by CCl₄ may be due to the presence of bioactive compounds in the methanolic extract.

Respected to CCL₄-intoxicated rats treated with J. curcas methanolic extract and silymarin drug, the cardiac tissue showed improvement in the cardiac architecture with minimal hyaline deposition, minimal fibrotic strands, besides the cardiac muscle cells were improved. This improvement may rely on the fact that, the over expression of transforming growth factor beta (TGF-β), p-Smad 2/3, and connective tissue growth factor (CTGF) in CCL₄ induced cardiac damage may be reversed by Ocimum gratissimum extract or silymarin treatment thus lowering liver cirrhosis, which confirms the importance of the growth factors TGF-β and CTGF.

CONCLUSION

Relying on the experimental results here, it could be hypothesized that J. curcas methanolic extract and silymarin drug may play an important role in medicine by scavenging free radicals, stimulating activity of antioxidant enzymes, and arresting production of adhesion molecules, subsequently protecting the heart against CCL₄-induced damage. The current study is a step to increase the phytochemical and pharmacological information about using J. curcas in the medical applications. The high content of antioxidants in J. curcas which exhibited it as an effective drug to treat cardiomyopathy should encourage us for further investigation.
REFERENCES


44. Abdou HS, Salah SH, Boole H, Abdel Rahim EA. Effect of pomegranate pretreatment on genotoxicity and hepatotoxicity induced by carbon tetrachloride (CCL4) in male rats. Journal of Medicinal Plants Research, 6(17), 2012, 3370-3380.


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