Anti-Cancer Activity of Curcin and Latex Isolated from Jatropha Plant (Jatropha Curcas L.)

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INTRODUCTION

Jatropha curcas L. (Jatropha) is a perennial plant belongs to family Euphorbiaceae and well-known for its great capacity of producing latex exudates from the stem and high content of seed oil used in biodiesel production. J. curcas different extracts and exudates are possess many pharmacological effects as anticancer, hepatoprotective and wound healing. The present study aimed to evaluate the different extracts of Jatropha curcas as anti-cancer activity by in vitro against MCF-7, HepG2 and HCT-116 cancer cell lines. The highest in vitro cytotoxic activity of curcin and methanolic extract of Latex were towards HepG2 cell line (IC50 36.7, and 19.11 µg/ml respectively). In addition, in vivo antitumor activity against chemically induced rat hepatocellular carcinoma (HCC) model showed that curcin showed marked ameliorations in all evaluated liver functions. Moreover, histopathological assessments as well as the tissue levels of certain oxidative stress biomarkers strongly assured the beneficial effects of curcin in fighting DENA-induced HCC.

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ABSTRACT

Jatropha curcas L. (Jatropha) is a perennial plant belongs to family Euphorbiaceae and well-known for its great capacity of producing latex exudates from the stem (Laxane et al, 2013). J. curcas is well-known for its great capacity of producing latex exudates from the stem (Laxane et al, 2013). In addition, it is cultivated in a large scale for biodiesel production from its high content of seed oil (Ashaful et al, 2015).

J. curcas possess various medicinal uses. The recent pharmacological investigation revealed the activity of J. curcas as anti-inflammatory, antioxidant, antimicrobial, anticancer, antiviral, anti-diabetic, analgesic, hepatoprotective, wound healing, anticoagulant and antifertility activities (Abdelgadir and Van Staden, 2013).

The latex from J. curcas was scientifically proved to have significant wound healing activity, also tends to increase capillary permeability (Salim et al, 2018). Curcin is a lectin isolated from the seeds of J. curcas by (Nesseim et al, 2012) as type I RIP (ribosome-inactivating protein), a single chain protein with a molecular weight of about 28.2 kDa, and showed strong inhibition to rabbit reticuloocyte lysate protein synthesis (Assieh et al, 1989).

Primary hepatocellular carcinoma (HCC), the sixth most common cancer worldwide and the third most common cause of death from cancer, is a tumor of considerable epidemiologic, clinical, and pathological interest (Siddiqui et al., 2018).

In the present study, we examined the in vitro cytotoxic activities of different extracts of Jatropha curcas against MCF-7, HepG2 and HCT-116 cancer cell lines and assessed the in vivo antitumor efficacy of the most effective extract of Jatropha curcas on a chemically induced HCC rat model. Histological investigations and assessments of some circulating liver function indices as well as liver tissue levels of some bio-indices of oxidative stress are used to evaluate the therapeutic efficacy.

MATERIALS AND METHODS

Plant collection, identification, and extraction

The fresh Jatropha curcas seeds and latex were collected in December 2017 from the cultivated plants in Faculty of Agriculture, Al-Azhar University, Assiut branch, Assiut, Egypt.

Extraction of Curcin and other protein isolates from Jatropha curcas seeds

The seed coats of Jatropha were removed and then grinded by blender. The oil was extracted from seeds by ice cold acetone. The defatted meal was air-dried to give seedcake. Ten grams of the seedcake was dispersed in 100 ml acetate-buffer solution and the pH was adjusted to pH 4. A similar amount of the seedcake (10 g) was dispersed in 100 ml phosphate-buffer solution then pH value was adjusted to pH 7. A third similar amount of the seedcake (10 g) was dispersed in 100 ml phosphate-buffer solution pH 11. Another fraction of the seedcake (10 g) was used to extract Curcin as previously described in literatures (Lin, et al., 2010). The protein content was calculated and expressed as a percentage of the weight sample used as described in literatures (Walpole, 1914).

Preparation of Latex

To prevent oxidation of latex, it was collected into vials containing some drops of ethanol (95%) (Osoguchi and Onajobi 2003). The freshly collected latex was directly soaked in methanol and then filtered after being left in the dark for three days. The filtrate was dried at temperature <50°C to avoid heat-induced destruction of the bioactive
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phytochemicals. The obtained dried fraction is called crude methanolic extract (Aiyehaagbe et al., 2007).

**Qualitative examination of secondary metabolites of Jatropha:**

Secondary metabolites such as alkaloids, tannins and saponins in the latex extracts were determined as described in literatures (Atherder, 1969; Ibrahim et al., 2013; Sofowora, 1993; Harborne, 1973).

**Extraction and determination of total phenolic compounds (TPCs):**

The total phenolic compounds were extracted from sample (0.5 g) by refluxing with 30 ml of methanol containing 1.0% HCl for 10 minutes and centrifugation at 5,000 rpm for 10 minutes. The concentration of total phenolic compounds in the methanolic extracts expressed as gallic acid equivalents according to a standard method (Singleton and Rossi, 1965).

**Total saponin content:**

The total saponin content was determined as previously described (Makkar et al., 2009). It was expressed as mg diosgenin equivalents per g dry matter.

**Determination of molecular weight of protein by Sodium Dodecyl Sulphate- Polyacrylamid Gel Electrophoresis (SDS-PAGE) of the Jatropha protein:**

Determination of the protein molecular weight was performed by SDS-PAGE according to a published method (Laemmli 1970).

**In vitro cytotoxicity studies using viability assay:**

The *Jatropha curcas* extracts were tested for cytotoxic activity on human HCC (HepG-2), human colon carcinoma (HCT-116), and human breast carcinoma (MCF-7) cancer cell lines that were obtained from VACSERA Tissue Culture Unit, Cairo, Egypt. Cisplatin and Doxorubicin were used as reference compounds. The 50% inhibitory concentration (IC50) was estimated from graphic plots of the dose response curve for each concentration using Graphpad Prism software (San Diego, CA, USA) (Mosmann, 1983 and Gomha et al., 2015).

**In vivo anticancer activity**

**Experimental animal model design:**

All animal experiments were carried out after approval of the Institutional Animal Care and Use Committee of the Faculty of Medicine, Assiut University, Egypt. All experiments were fulfilled using 15-16-week-old male Wistar rats (110-130 g). These rats were obtained from the laboratory animal colony, Assiut University, Assiut, Egypt. The animals were acclimatized in the laboratory for two weeks. Rats were housed (4 per cage) in a regulated condition (temperature, 20-22°C; humidity, 50±5%; night/day cycle, 12 hours) with free access to standard rodent diet pellets and water *ad libitum*.

At the end of the two weeks’ acclimatization period, rats were randomly divided into 2 groups of 8 and 40 rats, respectively. The small group served as a negative control and rats in the large group received orally 100 mg/L DENA (Sigma-Aldrich GmbH, Munich, Germany) for 8 weeks (Di Stefano et al., 2008). Four weeks after the end of DENA administration, the rats of the large group were subdivided into 5 groups of 8 rats each. The first subgroup (DENA group) received 5% glucose solution (i.v., weekly for 4 consecutive weeks). The second subgroup (DOX group) received doxorubicin (Yick-Vic Chemicals & Pharmaceuticals, HK, China), 2.5 mg/kg (i.v., weekly for 4 consecutive weeks). The third subgroup (ME group) received 400 mg/kg of the methanolic extract by oral gavages daily for one month. The fourth subgroup (curcin group) received curcin 3 mg/kg (i.p., weekly for 4 consecutive weeks). The fifth subgroup received a combination of both DOX and ME as previously described (Mittal et al., 2014).

One week after the last treatment, a blood sample was collected from each rat for biochemical assays and the rat was subsequently sacrificed by cervical decapitation under isoflurane anesthesia.

After autopsy, the liver was excised, purified from adhering tissues, washed in ice-cold saline solution, inspected, photographed, and subdivided into two parts. One part was kept in 10% formalin solution for histopathological investigations. The other part was homogenized using Potter-Elvehjem rotor-stator homogenizer, fitted with a Teflon pestle (Omi International, Kennesaw, GA, USA). Samples were divided into aliquots and kept frozen at -80°C until use.

**Biochemical estimations:**

Serum Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), gamma glutamyl transferase (GGT), and Alkaline Phosphatase (ALP) activities were assayed by kinetic procedures using corresponding commercially available kits according to the manufacturer's instructions. Serum albumin and total bilirubin were assayed using commercially available colorimetric kits according to the manufacturer's instructions (Elzadek et al., 2017).

**Histopathological examinations**

Formalin fixed liver samples were embedded in paraffin and stained with hematoxylin and eosin (H&E) according to a slandered protocol (Banchroft et al., 1996).

**Statistical analysis**

Experiment design was laid in a randomized complete block. The obtained data were subject to the analysis of variance procedure and treatment means were compared to the LSD test according standard method (Gomez and Gomez 1984).

**RESULTS AND DISCUSSION**

**Yield of Jatropha seeds protein isolates (JPIs):**

As described in Table-1, the obtained data showed that a high protein yield (~11.13%) was obtained from the alkaline fraction, while lower corresponding yields were obtained in both the acid and the neutral fractions (~8.74% and ~6.46%), respectively. On the other hand, a relatively minute yield of curcin was obtained (~0.20%). These results indicate a poor water solubility of *Jatropha curcas* proteins.

**Table 1. Yield of Jatropha curcas protein isolates extracted seeds (JCPIS) at different pH values**

<table>
<thead>
<tr>
<th>pH-value</th>
<th>Acidic (pH4)</th>
<th>Neutral (pH7)</th>
<th>Alkaline (pH11)</th>
<th>Curcin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yield of JCPIS (%)</td>
<td>8.74±0.39</td>
<td>6.46±0.25</td>
<td>11.13±0.11</td>
<td>0.20±0.011</td>
</tr>
</tbody>
</table>

Data are expressed as a percentage of the used seedcake weight.

**SDS-PAGE for molecular weight confirmation:**

The molecular weight of the protein was determined with the help of relative position of a specific molecular marker on SDS-PAGE. As obvious in Figure-I, the partially
purified curcin fraction showed an intense single band with a relatively a molecular weight of ~26 kDa that belong to curcin. On the other hand, the corresponding band in the total ME-fraction is hardly observed because of the low curcin abundance in the total extract. These results are in accordance with previous studies in which curcin has been isolated and characterized as a single peptide chain in the same molecular weight level (Lin et al., 2010).

**Qualitative examination of the secondary metabolites of latex from Jatropha curcas:**

The latex of *J. curcas* was examined for qualitative detection of secondary metabolites. As illustrated in Table 2, it could be observed that the latex of *J. curcas* contains four different groups of secondary metabolites including saponins, tannins, and phenolic compounds.

**Figure 1.** SDS-PAGE pattern of extracted proteins from *J. curcas*

**Table 2.** Screening of secondary metabolites in the latex of *J. curcas*.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Total phenolic</th>
<th>Tannins</th>
<th>Alkaloids</th>
<th>Total saponin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Latex</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

**Quantitative determination of the secondary metabolites in the Jatropha curcas**

As described in Figure 2, each gram of the *Jatropha curcas* latex was found to contain appreciable amounts of TPCs (88.0±1.83 mg) and saponins (80.7±1.73 mg) in addition to a minute amounts of tannins (3.95±0.93 mg).

These compounds are widely distributed in the plant kingdom to help in the defense against predators and pathogens and have been documented to exhibit activities towards a wide range of organisms (Upadhyay, 2011). These phytochemicals were also extensively reported to exhibit a good antioxidant activity towards DPPH and NO radical scavenging activity. These results agree with previous researchers who reported the presence of such compounds in a relatively equivalent abundance in Jatropha (Namuli et al., 2011).

**Figure 2.** Levels of total phenolic compounds (TPCs), saponins, and tannins of Jatropha latex.

**Cytotoxicity assessment of the Jatropha curcas extracts on MCF-7, HepG2 and HCT-116 cancer cell lines**

Isolation and elucidation of newly biologically active agent have become an essential part of cancer research for the recognition of novel anticancer agents (Ma and Wang, 2009). As illustrated in Table-3, the total phenolic, Nonpolar, and total proteins fractions did not exhibit a remarkable cytotoxicity behavior towards MCF-7, HepG-2 or HCT-116 cell lines with IC₅₀ of more than 500 μg/ml. According to the US National Cancer Institute’s plant screening program (US NCI), a crude extract is generally considered to have in *vivo* cytotoxic activity only if the IC₅₀ value is less than 30 μg/ml (Boik, 2001).

On the contrary, Curcin, the total methanolic extract, and saponins appeared to significantly reduce the cell viabilities in a dose-dependent manner in the three used cell lines with a relatively low IC₅₀ as presented in Table-3. It is obvious that, the HepG2 cells are more sensitive to the Curcin and the total methanolic extract than the MCF-7 and HCT-116 cell lines. Of-notes, DOX was strongly cytotoxic against the three used cell lines as mentioned in Table-3. Our results are in good agreement with previous reports who found that some extracted Jatropha fractions exhibit cytotoxic activities against HT-29 and Chang liver cell lines (Oskouiean et al., 2011).

**Table 3.** The IC₅₀ values of the tested fractions against MCF-7, HepG2 and HCT-116 cancer cell lines

<table>
<thead>
<tr>
<th>Sample</th>
<th>MCF-7 IC₅₀ (μg/ml)</th>
<th>HepG-2 IC₅₀ (μg/ml)</th>
<th>HCT-116 IC₅₀ (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Curcin</td>
<td>113.0± 2.9</td>
<td>36.7± 2.1</td>
<td>114.0± 3.2</td>
</tr>
<tr>
<td>Total phenolic</td>
<td>&gt;500</td>
<td>&gt;500</td>
<td>&gt;500</td>
</tr>
<tr>
<td>Saponin</td>
<td>358± 6.8</td>
<td>449.0± 7.9</td>
<td>276.0± 9.1</td>
</tr>
<tr>
<td>Methanolic</td>
<td>236.0± 4.1</td>
<td>19.11± 1.21</td>
<td>114.0± 4.6</td>
</tr>
<tr>
<td>Nonpolar</td>
<td>&gt;500</td>
<td>&gt;500</td>
<td>&gt;500</td>
</tr>
<tr>
<td>Total protein</td>
<td>&gt;500</td>
<td>&gt;500</td>
<td>&gt;500</td>
</tr>
<tr>
<td>Doxorubicin</td>
<td>0.35</td>
<td>8.62</td>
<td>0.49</td>
</tr>
</tbody>
</table>

**In vivo antitumor activity of the tested compounds**

**Morphological changes in the liver features**

Naked-eye screening of the rat livers in the DENA group revealed unhealthy morphological characteristics such as faint, irregular rough surfaces with several HCC nodules (Figure-3). Similar abnormal features have previously been reported in several DENA-induced HCC rat models (Elsadek et al., 2017). Similarly, livers in the DOX group...
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exhibited abnormal morphological changes in the form of irregular, rough and pale surfaces, with some scattered micronodules. Conversely, the livers of rats in the other three groups that were treated with Jatropha extracted products, especially that in the combination group, showed relatively healthy morphological appearance with a normal reddish brown view, normal steady consistency, smooth surface, and margins without any assigned lesions as those observed in the normal control group, except for very limited number of small nodules in few cases as appear in Figure 3.

Liver function tests

Compared to the normal control group, rats in the DENA and DOX groups showed features of deteriorated liver functions, as presented in Table 4. This pronounced DENA-induced liver insufficiency could be due to secondary proceedings following lipid peroxidation of hepatocyte membranes with a resultant increase in the escape of the liver function indices from injured liver tissues, as has been formerly described in other models of DENA-induced liver carcinogenesis (Elsadek et al., 2017). Noticeably, these recorded signs of unwell liver function were well corrected in the in the other three groups that were treated with Jatropha extracted products, especially in the Curcin-treated group that comparatively parallel the corresponding levels in the normal control group, as presented in Table 4.

Histological examinations of liver tissues

The liver tissue sections of normal control animals exhibited a firm hepatic lobular architecture, steady hepatocyte cording, maintained central veins and recognizable portal tracts. Minutely, the hepatocytes are polyhedral and symmetrical in size and shape with monotonous nuclei revealing no signs of cytological or nuclear atypia (Figure 4A).

In contrast, liver sections in the DENA-treated animals showed malformed lobulation, thick hepatic cording with trabeculae and focal acini formation. The cells appear pleomorphic with increased nucleocytoplasmic ratio and hyperchromatism (Figure 4B). These signs match several previously published histological observations in which DENA-exposure caused severe histopathological ailments in the liver architecture (Elsadek et al., 2017). These recorded histopathological observations concluded neoplastic alterations in hepatocytes with unlimited and uncontrolled malignant cell proliferation that induced abnormal mitotic Figures, giant tumor cells and genetic alterations associated with malignant turnover.

Figure 3. Representative photographs of livers from different groups

Figure 4. Histological examinations of liver tissue sections (H&E) obtained from untreated control (A), DENA (B), DOX-treated (C), ME-treated (D), Curcin-treated (E), and combination-treated groups (F). The magnification power is x400.
Table 4. Circulating levels of liver function biomarker in different groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>Control</th>
<th>DENA</th>
<th>DENA+ ME</th>
<th>DENA+ CU</th>
<th>DENA+ DOX</th>
<th>DENA+ DOX-ME</th>
<th>LSD 5%</th>
<th>LSD 1%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumin (g/dL)</td>
<td>4.36±0.23</td>
<td>3.68±0.14</td>
<td>3.73±0.08</td>
<td>3.37±0.12</td>
<td>4.06±0.14</td>
<td>4.29±0.21</td>
<td>0.86</td>
<td>1.16</td>
</tr>
<tr>
<td>sALT (IU/L)</td>
<td>19.26±3.33</td>
<td>50.35±3.86</td>
<td>43.33±4.10</td>
<td>39.77±6.21</td>
<td>51.84±2.43</td>
<td>42.94±4.41</td>
<td>26.64</td>
<td>38.93</td>
</tr>
<tr>
<td>sAST (IU/L)</td>
<td>135.93±24.6</td>
<td>178.66±16.75</td>
<td>178.96±29.48</td>
<td>159.8±12.13</td>
<td>212.92±23.08</td>
<td>278.75±1.26</td>
<td>89.75</td>
<td>122</td>
</tr>
<tr>
<td>sGPT (IU/L)</td>
<td>17.8±1.01</td>
<td>17.9±2.11</td>
<td>14.5±1.66</td>
<td>20.05±1.27</td>
<td>20.08±1.91</td>
<td>18.85±1.07</td>
<td>10.31</td>
<td>14.02</td>
</tr>
<tr>
<td>ALP (IU/L)</td>
<td>203.36±13.59</td>
<td>485.85±73.40</td>
<td>453.78±37.73</td>
<td>317.05±47.31</td>
<td>486.89±57.47</td>
<td>604.15±115.60</td>
<td>355.94</td>
<td>483.80</td>
</tr>
<tr>
<td>Total Bilirubin (mg/dL)</td>
<td>0.45±0.08</td>
<td>0.33±0.07</td>
<td>0.45±0.06</td>
<td>0.37±0.032</td>
<td>0.52±0.04</td>
<td>0.52±0.012</td>
<td>0.33</td>
<td>0.45</td>
</tr>
</tbody>
</table>

Data are presented as the mean ± SEM (n = 8).

The liver sections in the DOX group showed pseudolobulation with residual nodules and low-grade HCC. Notably, these residual histopathological alterations reason the negligible efficacy of DOX in fighting DENA-enforced liver carcinogenesis. In rats treated with the methanolic extract of Jatropha, liver sections revealed neoplastic changes in form of eosinophilic hyperplastic foci with enlarged and disarranged hepatocytes. Liver sections in the curcin treated group showed almost intact hepatocytes except for some eosinophilic foci.

In the combination group, hepatic tissue sections revealed multifoci of eosinophilic hepatocytes that compress the surrounding tissue without features of anaplasia.

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Conflict of interest: We confirm no known conflicts of interest associated with this work.

REFERENCES


النشاط المضاد للسرطان من الكيورسين والملتسب المتعزز من نبات الجاتروفا

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