Hepatoprotective activity of *Hyphaene thebaica* extract against mercuric chloride-induced hepatotoxicity in adult male albino rats

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**Summary.** Doum, *Hyphaene thebaica* (*H. thebaica*), known as a famine food, is an African palm tree common in Upper Egypt. The present study was performed to evaluate the protective effect of *H. thebaica* (the fruits of doum) extract on liver/kidney functions in mercuric chloride (*HgCl₂*) treated rats. Rats were randomly classified into healthy control, and 5 treated groups which were *H. thebaica* extract alone with either 500 or 1000 mg/kg b.w, *HgCl₂* alone, *H. thebaica* extract (low dose) plus *HgCl₂* and *H. thebaica* extract (high dose) plus *HgCl₂* groups. Results clearly revealed that consumption of *H. thebaica* alone (especially high dose) showed improvement of healthy status. The pre-treatments of *H. thebaica* extract with high dose which had the best modulatory results as increasing hepatic non-enzymic/enzymic antioxidant system as well as decreasing pro-inflammatory cytokines compared with healthy control group with decreasing lipid peroxidation. Our results indicate that consumption of *H. thebaica* extract has a beneficial role against hepatotoxicity induced by *HgCl₂* in male albino rats.

**Key words:** Doum, *HgCl₂*, hepatotoxicity, rats

**Introduction**

Heavy metal such as mercury (*Hg*) are considered as the most dangerous substances causing many health hazards to human and animals through progressive irreversible accumulation in their bodies. *Hg* is capable of damaging the organism in several ways due to its high affinity to various tissues such as liver, kidney, brain, reproductive organs, lung, immune system and its tendency to accumulates (1,2). Recent evidences also show that *Hg* causes severe oxidative changes (3), thus *Hg* proved to be a potential oxidant in the category of environmental factories. Therefore, there is a need to provide protection against *Hg* induced toxicity.

*Hyphaene* (*H.*) *thebaica* is one of the plants used in ethno-medicine and belongs to the family Palm and subfamily Borassioideae. It grows commonly in both Sahel and Sahara regions in Upper Africa. The pericarp and the outer coat of the endocarp of the fruits of doum are inedible, while the mesocarp and kernel flesh of the fruits are edible. Research on the fruit pulp of *H. thebaica* showed that it contains nutritional trace minerals, proteins and fatty acids, in particular the nutritionally essential linoleic acid (4). The identification of compounds, by thin-layer chromatography, showed that the fruit pulp of *H. thebaica* contains significant amounts of saponins, coumarins, hydroxycinnamates, essential oils, flavonoids, phenolics acids and oxylipids. In addition, 17 compounds were identified and quantified from the fruit of *H. thebaica*, including 2 cinnamic acid derivatives, 5 flavonoids, 6 fatty acids, 2 sphingolipids, a lignan, and a stilbene. The plant’s sugar composition was characterized and quantified by 1H-nuclear magnetic resonance, with sucrose detected as its major component at a level of 219 mg/g (5, 6). *H. thebaica* also contains crude protein and lipids (7, 8).
Also, the aqueous extract of *H. thebaica* fruits showed an antioxidant activity; this is due to the substantial amount of their water-soluble phenolic contents of flavonoids within it, which represent conjugates of o-glycosides and include quercetin, chrysoeriol, luteolin, and isorhamnetin H (9-11). Also, *H. thebaica* is thought to possess anti-inflammatory property due to its ability to inhibit cyclooxygenase (12). Locally, various extracts and decoction of fruit pulp of *H. thebaica* are used in the treatment of bilharzia, haematuria, hypertension and as a haematinic agent in human and in animal models (4,13). Also, it has a cooling and soothing effect on the digestive system and antioxidant properties which help flush toxins from the body (11,14). In addition, *H. thebaica* may be advised as a good choice that can delay diabetic complications in rats (6). This study was undertaken to study the protective effect of fruit pulp of *H. thebaica* on hepatotoxicity induced by HgCl₂ in male rats and to find its probable mechanism of action including its antioxidant potential.

**Materials and methods**

**Plant material.** The fruit of *H. thebaica* (doum) was obtained from local markets (Cairo, Egypt) and was authenticated by Dr. U.K. Abdel-Hameed, a plant taxonomist in the botany department, faculty of science, Ain Shams University, Cairo, Egypt. A voucher specimen was deposited in the herbarium of the botany department, faculty of science, Ain Shams University.

**Preparation of suspension.** The fruits of *H. thebaica* was cleaned and separated into pulp. The pulp was then dried and ground into fine powder using mortar and pestle. Subsequently, 5g of the powder was extracted by 70% ethanol on cold until exhaustion. In order to obtain the dried extracts, the solvent was distilled in rotary evaporator at 55°C till dryness. Aqueous suspension was performed by suspending in double distilled water for final administration.

**Animals.** Adult male albino rats (*Rattus norvegicus*), weighing about 130-140 g, were obtained from the Animal House Colony of the National Research Center (Dokki, Giza-Egypt). Animals were housed in polypropylene cages with wood dust and given standard rodent food pellets (Agricultural-Industrial Integration Company, Giza, Egypt) and tap water *ad libitum*. The animals were procured, maintained and used in accordance with WHO guideline for animal care and the study design was approved by the Ain Shams University Research Ethics Committee.

**Experimental design.** After one week of acclimatization, all animals were randomly divided into six groups of five rats each:

**Group I** served as healthy control and orally received 1 ml double distilled water once daily for 28 days, then on the 28 day and after 2 hours from last dose of distilled water administration, rats were subcutaneously injected with 0.5 ml normal physiological saline at once.

**Group II** served as control treated and orally received 1 ml of *H. thebaica* extract at dose of 500 mg/kg b.w once daily for 28 days.

**Group III** served as control treated and orally received 1 ml of *H. thebaica* extract at dose of 1000 mg/kg b.w once daily for 28 days.

**Group IV** (Hg-treated) rats were given a single subcutaneously injection of Hg in the form of HgCl₂ at dose of 5 mg/kg b.w dissolved in normal physiological saline (15) on the 28 day of the experiment.

**Group V** (*H. thebaica* + Hg-treated group) received orally 1ml of *H. thebaica* extract (dissolved in double distilled water) at dose of 500 mg/kg b.w/day (13) for 28 days, then, after 2 hours from last dose of extract administration, rats were treated with HgCl₂ (dose same as that in the Hg-treated group).

**Group VI** (*H. thebaica* + Hg-treated group) received orally 1ml of *H. thebaica* extract (dissolved in double distilled water) at dose of 1000 mg/kg b.w/day for 28 days, then, after 2 hours from last dose of extract administration, rats were treated with HgCl₂ (dose same as that in the Hg-treated group).

At the end of the experimental period (day 29), the rats were sacrificed; blood, liver and kidney samples were collected for different analyses.

**Measurements.** Body weight (gain or loss) was calculated by the following equation: body weight (gain or loss) = body weight at the end of the experiment –

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body weight at the beginning of the experiment. The relative organs weight (liver and kidney) was calculated according to the following equation: relative weight (g/100 g b.w) = [(organ weight at the end of the experiment × 100) / (animal body weight at the end of the experiment)].

**Serum Preparation.** Blood samples were taken by cutting the neck at the jugulars by a sharp razor blade after the rats were subjected to light diethyl ether anaesthesia without anticoagulant to separate serum by centrifugation in a cooling centrifuge (IEC centrifuge 4R; International Equipment Co., Needham Heights, MA, USA) for 30 min at 3000 rpm and 4°C. The serum was separated and divided into samples and preserved at -80°C for the subsequent estimation of biochemical parameters. The appropriate kits (Bio-diagnostic kits) were used for determination of the activities of serum alanine and aspartate aminotransferases (ALAT, ASAT) (16), total protein level (17), albumin level (18), urea (19) and creatinine level (20). Serum pro-inflammatory cytokines (TNF-α and IL-1β) levels were measured by sandwich enzyme-linked immunosorbent assay (ELISA) using a commercially available ELISA kit for rat IL-1β (RayBiotech Inc, Norcross, GA, USA) and anti-rat TNF-α (R&D Systems, Minneapolis, MN, USA) according to the manufacturers’ recommendations.

**Liver Tissue Preparation.** About 0.5 g of liver was homogenized in 5 ml of phosphate buffer solution (w/v: 0.5 g tissue with 5 ml PBS, PH 7.4). Homogenates were centrifuged at 10.000 rpm for 15 min at 4°C. The resultant supernatant was used for determination of the activities of liver catalase (CAT) (21), glutathione peroxidase (GSH-Px) (22) and glutathione-S-transferase (GST) (23), as well as the levels of reduced glutathione (GSH) (24) and malondialdehyde (MDA) (25) using Bio-diagnostic kits (Bio-diagnostic Company, Giza, Egypt).

**Statistics.** All numeric variables were presented as mean with ± standard error (SE). Statistical comparisons were performed using one-way ANOVA using Graph Pad Prism version 4.03 for Windows (Graph Pad Software Inc., San Diego, CA, USA). The difference between means was assessed by Tukey’s multiple comparison test (26), in which *P* values of < 0.05, < 0.01 and < 0.001 were considered statistically significant, highly significant and very highly significant, respectively.

### Results

The present study showed that the body weight gain, organs relative weight (liver and kidney), serum globulin level and A/G ratio did not significantly alter (*P*<0.05) by 0.5%, 11% and 28.3%, respectively in HgCl₂ intoxicated rats compared with the control animals (Table 1 and 2). On the other hand, serum total protein, albumin levels and hepatic GSH concentration were significant-

<table>
<thead>
<tr>
<th>Groups</th>
<th>Control</th>
<th>H. thebaica 500</th>
<th>H. thebaica 1000</th>
<th>HgCl₂</th>
<th>H. thebaica 500 + HgCl₂</th>
<th>H. thebaica 1000 + HgCl₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parameters</td>
<td></td>
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</tr>
<tr>
<td>Initial body weight (g)</td>
<td>137.10 ± 0.94</td>
<td>139.40 ± 2.05</td>
<td>137.60 ± 1.23</td>
<td>137.00 ± 1.70</td>
<td>137.60 ± 1.14</td>
<td>137.30 ± 0.81</td>
</tr>
<tr>
<td>Final body weight (g)</td>
<td>190.40 ± 1.89</td>
<td>196.00 ± 1.73</td>
<td>198.20 ± 2.50</td>
<td>190.50 ± 1.10</td>
<td>191.70 ± 2.07</td>
<td>192.60 ± 1.23</td>
</tr>
<tr>
<td>Body weight gain (g)</td>
<td>53.22 ± 1.38</td>
<td>56.66 ± 3.15</td>
<td>60.66 ± 3.50</td>
<td>53.50 ± 1.14</td>
<td>54.07 ± 2.91</td>
<td>55.33 ± 1.51</td>
</tr>
<tr>
<td>Liver relative weight (g/100 g b.w)</td>
<td>2.74 ± 0.10</td>
<td>2.66 ± 0.07</td>
<td>2.71 ± 0.04</td>
<td>3.04 ± 0.08</td>
<td>2.97 ± 0.14</td>
<td>2.87 ± 0.12</td>
</tr>
<tr>
<td>Kidney relative weight (g/100 g b.w)</td>
<td>0.242 ± 0.007</td>
<td>0.235 ± 0.009</td>
<td>0.238 ± 0.007</td>
<td>0.310 ± 0.033</td>
<td>0.276 ± 0.013</td>
<td>0.263 ± 0.008</td>
</tr>
</tbody>
</table>

b.w, body weight; HgCl₂, mercuric chloride; H. thebaica 500, H. thebaica extract (500 mg/kg body weight); H. thebaica 1000, H. thebaica extract (1000 mg/kg body weight). Values are means, with their standard errors. (One-Way ANOVA with Tukey’s multiple comparison test).
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ly decreased (*P* < 0.05 to *P* < 0.001) by -6.9%, -8.5% and -38.6%, while hepatic MDA concentration significantly increased (*P* < 0.001) by 67.5% in HgCl₂ intoxicated rats compared with the healthy control animals. The changes in serum total protein, albumin levels, hepatic GSH and MDA concentrations shown in intoxicated rats were reverted to normal levels upon treatment with *H. thebaica* 1000 only (*P* > 0.05 compared with the healthy control group). The percentages of changes of these parameters measured, compared with the healthy control group, in *H. thebaica* 1000 plus HgCl₂ groups were -5.4%, -4.1%, -6.4% and 9%. Healthy rats consumed high dose of *H. thebaica* showed a significant increase in the liver GSH concentration and a significant decrease in the liver MDA concentration (*P* < 0.05) by 2.1% and -2.4%, respectively compared with the healthy control animals (Table 2).

Figure 1 and 2 revealed that the significant increase (*P* < 0.001) in the liver and kidney markers for cellular toxicity (serum ASAT, ALAT activities, urea and creatinine levels) in HgCl₂ intoxicated rats by 51.8%, 103.4%, 89.4% and 103.8%, respectively compared with the healthy control animals. All of these changes were completely modulated in intoxicated rats that received high dose of *H. thebaica* only (*P* > 0.05) compared with the control animals. The percentages of changes of serum ASAT, ALAT activities, urea and creatinine levels, compared with the healthy control group, in *H. thebaica* 1000 plus HgCl₂ groups were 5.2%, 10.4%, 13.8% and 28.4%, respectively.

As shown in Figure 3 revealed that liver anti-oxidants enzymes (GSH-Px, GST and CAT activities) significantly decreased (*P* < 0.01) in HgCl₂ intoxicated rats by -57.4%, -78.1% and -46.1% compared with the healthy control animals. Both different doses of *H. thebaica* extract significantly alleviated (*P* < 0.05–0.001)

Figure 1. Liver biomarker enzymes (serum ASAT and ALAT) of different experimental groups.

**Table 2.** Effect of *H. thebaica* on serum protein profiles, hepatic GSH and hepatic MDA concentrations on different experimental groups of rats.

<table>
<thead>
<tr>
<th>Groups Parameters</th>
<th>Control</th>
<th><em>H. thebaica</em> 500</th>
<th><em>H. thebaica</em> 1000</th>
<th>HgCl₂</th>
<th><em>H. thebaica</em> 500 + HgCl₂</th>
<th><em>H. thebaica</em> 1000 + HgCl₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum total protein (mg/dl)</td>
<td>9.92 ± 0.06</td>
<td>9.96 ± 0.05</td>
<td>9.98 ± 0.06</td>
<td>9.23 ± 0.13**</td>
<td>9.36 ± 0.25</td>
<td>9.38 ± 0.13</td>
</tr>
<tr>
<td>Serum albumin (mg/dl)</td>
<td>6.57 ± 0.05</td>
<td>6.70 ± 0.03</td>
<td>6.75 ± 0.06</td>
<td>6.01 ± 0.04***</td>
<td>6.21 ± 0.16</td>
<td>6.30 ± 0.15</td>
</tr>
<tr>
<td>Serum globulin (mg/dl)</td>
<td>3.35 ± 0.05</td>
<td>3.27 ± 0.05</td>
<td>3.31 ± 0.07</td>
<td>3.22 ± 0.13</td>
<td>3.15 ± 0.35</td>
<td>3.08 ± 0.16</td>
</tr>
<tr>
<td>A/G ratio</td>
<td>1.97 ± 0.04</td>
<td>2.05 ± 0.04</td>
<td>2.09 ± 0.04</td>
<td>1.88 ± 0.08</td>
<td>2.12 ± 0.34</td>
<td>2.08 ± 0.15</td>
</tr>
<tr>
<td>Hepatic GSH (mmol/g tissue)</td>
<td>34.56 ± 0.41</td>
<td>35.28 ± 0.29</td>
<td>37.55 ± 1.19***</td>
<td>21.23 ± 0.51***</td>
<td>30.85 ± 0.49***</td>
<td>32.36 ± 0.34***</td>
</tr>
<tr>
<td>Hepatic MDA (nmol/g tissue)</td>
<td>127.10 ± 3.73</td>
<td>124.00 ± 2.79</td>
<td>111.30 ± 3.70*</td>
<td>212.90 ± 3.59***</td>
<td>145.40 ± 3.94***</td>
<td>138.50 ± 1.31***</td>
</tr>
</tbody>
</table>

HgCl₂, mercuric chloride; *H. thebaica* 500, *H. thebaica* extract (500 mg/kg body weight); *H. thebaica* 1000, *H. thebaica* extract (1000 mg/kg body weight); GSH, reduced glutathione; MDA, malondialdehyde. Values are means, with their standard errors. Mean value was significantly different from that of the control group: * P < 0.05, **P < 0.01, ***P < 0.001. Mean value was significantly different from that of the lead acetate only treated group: ††† P < 0.001 (One-Way ANOVA with Tukey’s multiple comparison test).
the severe decrease in liver anti-oxidants induced by 
HgCl2. The utmost modulation on this changes was 
shown in high dose of *H. thebaica* by -9.6%, -37% and 
-4.6% compared with the healthy control animals. 
These modulatory effects of *H. thebaica* extract was 
dose dependent.

Figure 4 revealed that pro-inflammatory cytokines 
(TNF-α and IL-1β) significantly increased (P<0.001) 
in HgCl2 intoxicated rats by 118.7%, and 125.5% 
compared with the healthy control animals. Both dif-
ferent doses of *H. thebaica* extract significantly allevi-
ated (P<0.001) the severe increase in pro-inflamma-
tory cytokines induced by HgCl2. The percentages 
of changes of pro-inflammatory cytokines (TNF-α 
and IL-1β) levels, compared with the healthy control 
group, in low vs high dose of *H. thebaica* plus HgCl2 
groups were 74.5 vs 55.0%, and 73.7 vs 36.6%, respec-
tively.

**Discussion**

In this current work, elevation hepatic MDA 
concentration, due to toxic effects of HgCl2 were 
accompanied by significant reductions in hepatic non-
enzyme antioxidant (GSH concentration) and he-
patic enzymic antioxidant (GSH-Px, GST and CAT 
activities), implicating of oxidative tissue damage in 
the hepatocytes. HgCl2 can inactivate a number of en-
zymes by blocking the functional sites through bind-
ing to sulf-hydryl groups, which are part of catalytic or 
binding domains of enzymes (27). As a common fac-
tor in HgCl2 exposure, an imbalance in the antioxidant 
defense system leading to oxidative stress in the cells
is being identified in HgCl₂. Hepatic marker enzymes activities were used as very important biomarkers for detection of hepatotoxicity. In the present study, serum ASAT and ALAT activities were found to be significantly elevated in the HgCl₂ treated group. This elevation of hepatic serum enzymes may be due to increase lipid peroxidation which led to cellular necrosis of liver cells, which causes increases in the permeability of cell resulting release of ASAT and ALAT enzymes into the blood circulation (27).

The principle toxic effects of inorganic Hg in the Hg²⁺ form involve interactions with large number of cellular processes, including the formation of complexes with free thiols (—SH) and protein SH groups, which may lead to promote formation of reactive species via SH complexation (2). In the present study, a significant decrease in serum protein and albumin levels was recorded. The decrease in the protein level of HgCl₂ treated rats might be due to changes in protein synthesis and/or metabolism by hepatic cell and increase rate of excretion due to renal damage (2). Lipid peroxidation have been proposed for the biological toxicity of HgCl₂, and it occurs in the liver, kidney and other tissues of the experiment animals (27). A significant reduction in GSH levels as a consequence of HgCl₂ due to its antioxidant property exerted by flavonoids in this plant (32). Another study reported that phytochemical studies revealed the presence of flavonoids, coumarins and saponins in H. thebaica (13). Saponin has a protective effect against HgCl₂ toxicity. It inhibited peroxyl radical induced lipid peroxidation in rat liver. Phenolic acids of H. thebaica such as coumaric acid may scavenger free radicals generated by HgCl₂ and reduces the lipid peroxidation. Furthermore, flavonoids are a large group of naturally occurring plant phenolic compounds that inhibit lipid oxidation (33). The antioxidant activity of polyphenolics is principally defined by the presence of ortho-dihydroxy substituents, which stabilize radicals and chelate (27). Explanation of the possible mechanism underlying the hepato-protective properties of the H. thebaica is having five flavone glycosides were isolated and identified from H. thebaica fruits (9). In the current study, the improvement of total protein and albumin results of H. thebaica extract may be attributed to promote liver cell protein synthesis by decreasing the oxidation of GSH.

It was observed from our results that protective effect of H. thebaica on HgCl₂ toxicity resulted mainly from increasing hepatic GSH concentration, GSH-Px, GST and CAT activities as antioxidant potential and reducing the MDA concentration as a marker of lipid peroxidation. H. thebaica possess strong antioxidant activity which provokes free radical scavenging enzyme system (31). GSH, specifically bind with methylmercury, forms a complex that prevents Hg from binding to cellular proteins and causing damage to both enzymes and tissue (34). GSH-Hg complexes also reduce intracellular damage by preventing Hg from entering tissue and cells, and becoming an intracellular toxic. Additionally, the elevated level of GSH-Px, GST and CAT by H. thebaica as compared with the HgCl₂ may have facilities the conjugation reaction of xenobiotics metabolism and may have increased the availability of non-critical nucleophile for inactivation of electrophiles and therefore might be playing a major role in metallo-protection. Antioxidants are compounds that can delay and/or inhibit oxidation of lipids or other molecules by inhibiting the initiation or propagation of oxidative chain reaction (35). In the present study, the pretreatment with H. thebaica increased total protein and albumin levels in serum resulted mainly from elevation concentration of GSH which protects cellular protein against oxidation.
through glutathione redox cycle and directly detoxifies reactive species (36). The protective effect of *H. thebaica* is probably due to its high contents of powerful antioxidants, particularly: saponins, coumarins, hydroxycinnamates, essential oils, flavonoids, alkaloids, reducing sugars, glycosides, and water-soluble phenolic contents, polyphenols and polyunsaturated/unsaturated fatty acid which are known as powerful antioxidants (9, 11, 31). Polyunsaturated and unsaturated fatty acid have protective effects against oxidative stress induced by HgCl₂ in rats, which are explained by their double bounds, that is difficult to oxidize and that involved in the fluidity of lipoproteins (37). Several fatty acids were identified and isolated from the *H. thebaica* as caprylic, capric, lauric, myristic, palmitic, stearic, oleic and linoleic while oleic was found to constitute the major fatty contents in the edible part of *H. thebaica* (11). Also, the presence of some elements such as magnesium, manganese, copper, zinc and calcium in *H. thebaica* plays major role in prevention of heavy metals toxicity by increasing antioxidant enzymes. These compounds make *H. thebaica* fruits an important source of antioxidant, which certainly play an important role in *vivo*. Pro-inflammatory cytokines are potent inducers of free radicals and inflammatory mediators. The uncontrolled production of ROS due to the inflammatory mediators leads to a decrease in enzymatic/non-enzymatic anti-oxidant which resulted from membrane lipid peroxidation (increasing MDA), as shown in this study. Both doses of *H. thebaica* extract (especially high dose) were able to significantly prevent oxidative damage and cellular toxicity through scavenging the free radicals and terminating the membrane lipid peroxidation (MDA) by improving cellular antioxidants and pro-inflammatory cytokines (TNF-α and IL-1β). Finally, the antioxidant property of *H. thebaica* is claimed to be one of the mechanisms of hepatotox/nephro-protective against oxidative damage. Indeed consumption of *H. thebaica* extract did not induce any adverse effects in healthy rats. On the other hand, *H. thebaica* extract (especially high dose) significantly increased and decreased (*P<0.05*) the concentration of hepatic GSH and MDA concentrations, respectively, in healthy rats. All of these findings indicated that *H. thebaica* is considered as a safer anti-oxidant agent that possess stronger free radical scavenging activity.

Conclusions

Hence, it can be concluded from this study that *H. thebaica* extract has potent activity against hepatotoxicity experimentally induced by HgCl₂, due to its antioxidant properties and we can be advised it as a protective plant to delay the progression of heavy metal toxicity and complications.

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References

10. Abdel-Rahim E A, El-Beltagi H, Fayed A. Comparative

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