HEPATOPROTECTIVE EFFECT OF ADANSONIA DIGITATA L. (BAOBAB) FRUITS PULP EXTRACT ON CCL₄-INDUCED HEPATOTOXICITY IN RATS

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ABSTRACT

Background: Medicinal plants, for thousands of years, have been used as traditional treatment for numerous diseases. In recent years, focus on plant research has been increased to show the potential of some medicinal plants in discovery of novel compounds which could be used in treatment of some diseases. Objectives: The present study was conducted to investigate the hepatoprotective effect of Adansonia digitata fruits pulp methanolic extract on CCl₄-induced hepatotoxicity in rats. Methods: A. digitata fruit pulp was extracted by maceration using methanol. Silymarin (25mg/kg) and two doses of the methanolic extract (100mg/kg and 200mg/kg) were used to investigate their hepatoprotective effects on CCl₄-induced hepatotoxicity in rats. Results: The two doses of the plant extract showed dose-dependent hepatoprotective effect on CCl₄-induced hepatotoxicity, as evident by the significant reduction (P < 0.05) in serum levels of AST, ALT, ALP and bilirubin along with the improved histopathological liver sections compared to CCl₄-treated animals. Conclusion: The plant material could provide a suitable source for new drug development, and its possible role in treatment of some liver disorders should be further evaluated by well-designed controlled studies.

KEYWORDS: Adansonia digitata; Baobab fruit pulp; hepatoprotection; hepatotoxicity; Silymarin; CCL₄; Liver enzymes.
INTRODUCTION
Liver diseases are one of the major threats to public health and remains as worldwide problem. They are mainly caused by hepatotoxic chemicals (acetaminophen, carbon tetrachloride and alcohol), infections and autoimmune disorders. The hepatotoxic chemicals caused damage to liver cells mainly by inducing lipid peroxidation and other oxidative damages.[1] In recent years, interest has refocused on traditional medicine due to modern drugs high cost, time and expenditure that is necessary to bring a drug to market after appropriate clinical trials, serious side-effects of some modern drugs, and drug-resistance developed by many microorganisms.[2] According to the World Health Organization (WHO) 2003 report, about 80% of the population of developing countries being unable to afford pharmaceutical drugs relies on the use of traditional medicine, which is predominantly based on plant material.[3] Many plants have been shown to possess antioxidant activity (or inhibit the generation of free radicals) which is important in providing hepatoprotective property against hepatic damage.[4] and nearly about 160 phytoconstituents from 101 plant have been claimed to possess liver protecting activity.[5]

The Baobab tree (A. digitata L.) makes an important contribution to people’s livelihoods for food, fiber and medicine throughout its geographical range. Also the Baobab products, such as seed oil and fruit pulp, form an important source of income, especially in the dry season or at times of drought.[6] A variety of chemicals such as terpenoids, flavonoids, steroids, vitamins, amino acids, carbohydrates and lipids have been isolated and characterized from A. digitata.[7] The plant commonly considered rich in antioxidants and the fruit pulp represents the most important natural sources of ascorbic acid, while the leaves were characterized by the content of provitamin A.[8] Ethnomedicinally the fruit pulp has been traditionally used as an immunostimulant, anti-inflammatory, analgesic, pesticide, antipyretic, febrifuge, and astringent in the treatment of diarrhea.[9] Seeds were used in folk medicine to treat diarrhea, and hiccough while their oil extract mainly used to treat skin complaints and for cosmetic applications. The leaves were used to treat a wide variety of conditions including fatigue, as an anti-asthmatic, as a tonic and for insect bites, Guinea worm and internal pains, diseases of the urinary tract, ophthalmia and otitis.[10] In east Africa, the baobab bark, fruit pulp and seeds were used as an antidote to poisoning by a number of Strophanthus species which has been used as an arrow poison. The bark widely used in tradition medicine as a substitute for quinine in case of fever or as a prophylactic.[11]
The present study was conducted to investigate the hepatoprotective effect of *A. digitata* fruits pulp methanolic extract on CCl₄-induced hepatotoxicity in rats which may aid and enhanced the medicinal uses of this plant.

**MATERIALS AND METHODS**

**Plants materials**

Fruits of *A. digitata* were obtained from the local market, Wad Medani, Sudan. The plants materials were identified and authenticated at the Herbarium of the Phytochemistry and Taxonomy Department, Medicinal and Aromatic Plants Institute, National Center for Research, Khartoum, Sudan.

**Extraction of plants materials**

The fruits of *A. digitata* were crushed and powdered pulp was collected. Three hundred grams of the plant dry powder were extracted by maceration for 72 hours using 1.5 Litters of methanol (99%) as solvent system in conical flasks, with intermittent shaking, and then filtered under vacuum using Buchner funnel. The filtrate was allowed to evaporate at room temperature for 7 days, then collected and stored in an amber glass container (in refrigerator) until used for biological testing. The test material (brownish gummy texture extract) was prepared as a water suspension to be administered to the experimental animals by intragastric feeding tubes.

**Hepatotoxicity study**

Hepatoprotective activity of *A. digitata* fruit pulp methanolic extract against carbon tetrachloride (CCl₄)-induced hepatotoxicity in rats were carried out based on the methods described by Sapakal *et al.*, (2008),[12] and Abdel Azeem *et al.*, (2009).[13]

Twenty five mature Albino rats weighing about 130 - 200g (12 - 15 weeks old) males and females were selected, and randomly divided into five groups of five rats each. They were obtained from the animal house, Faculty of Pharmacy, University of Gezira and housed in polycrylic cages and maintained under standard laboratory conditions (temperature 25 ± 2°C) with dark and light cycle (14/10 hours). They were allowed free access to diet and water. The animals were acclimatized to laboratory condition for 14 days before commencement of the experiment. Hepatotoxicity has been induced in all rats (except the rats of group I) with intrapretonial administration of 2ml/kg CCl₄ (Sana and Hela, 2003).[14] The groups have been divided as following:
• Group I (normal control) received water.
• Group II (negative control) received CCl₄ only.
• Group III (standard control) received, in addition to CCl₄, an oral daily dose of Silymarin (25 mg/kg).
• Group IV and V (test control) administered orally with A. digitata extract 100mg/kg/day and 200mg/kg/day, respectively along with CCl₄.
• Treatment duration was carried out for 21 days and CCl₄ (2ml/kg) was administered every 72 hours.
• The experiment was ethically approved by Faculty of Pharmacy, University of Gezira ethical committee.

Blood biomarkers
Blood samples were obtained from all animals by puncturing the retro-orbital plexus under diethyl ether anaesthesia at day 7 and 14 of the treatment and after scarification at day 21, centrifuged at 3500 rpm for 15 minutes at 4 °C. Serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total bilirubin and total proteins were determined using an auto-analyzer (Cobas Integra 400, Roche Diagnostics, Switzerland).

Histopathological examination
Liver samples were subjected for histopathological examination at the Histopathology Department - Medical Laboratory – University of Gezira, using hematoxylin-eosin staining.

Data analysis
Data were statistically analyzed using paired t-test, and expressed as mean ± standard mean error. For comparisons with the normal control group, differences were considered significant if $P$-value < 0.01, and for comparisons with the CCl₄-only treated group, differences were considered significant if $P$-value < 0.05. The percentage of the hepatoprotection (H) was calculated as described by Singh et al., (2001).¹¹⁵ using the following equation:

$$H = \left( 1 - \frac{T - N}{C - N} \right) \times 100$$

Where (T) is mean value of group treated with test drugs, (C) is mean value of group treated with CCl₄ alone, and (N) is the mean value of the normal control group.
RESULTS

The serum levels of ALT and AST obtained at day 7, 14, and 21 of the experimental course were significantly elevated ($P < 0.01$) in CCl$_4$ group. Silymarin treated group showed significant reduction ($P < 0.05$) in ALT and AST at day 7 and day 14 of the experimental course. However, at day 21, all treatment groups (group III, IV and V) showed significant reduction ($P < 0.05$) in ALT and AST values compared to group II with greatest reduction recorded in group V (200mg/kg of A. digitata fruit pulp extract). Results were displayed in Table 1.

All biochemical parameters recorded at day 21 of the treatment, along with the percentage of hepatoprotection for each biochemical parameter (Table 2) showed that, the plant extract and the standard drug (Silymarin) decreased the levels of liver enzymes that elevated by CCl$_4$ administration when compared to CCl$_4$-only treated group. The two different concentrations that used in this study for the plant extract showed a concentration dependent effect.

Table 1: Effects of methanolic extract of A. digitata fruit pulp and Silymarin on serum levels of ALT and AST in CCl$_4$-induced hepatotoxicity.

<table>
<thead>
<tr>
<th>GROUP</th>
<th>Day (7)</th>
<th>Day (14)</th>
<th>Day (21)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ALT (U/L)</td>
<td>AST (U/L)</td>
<td>ALT (U/L)</td>
</tr>
<tr>
<td>Group I (Normal control)</td>
<td>90 ± 12</td>
<td>134 ± 14</td>
<td>63 ± 3</td>
</tr>
<tr>
<td></td>
<td>94 ± 5</td>
<td>119 ± 16</td>
<td>76 ± 9</td>
</tr>
<tr>
<td>Group II (CCl$_4$ only)</td>
<td>3165 ± 190</td>
<td>4483 ± 284</td>
<td>1826 ± 367</td>
</tr>
<tr>
<td></td>
<td>1344 ± 121</td>
<td>2259 ± 117</td>
<td>2259 ± 117</td>
</tr>
<tr>
<td>Group III (CCl$_4$ + Silymarin 25mg/kg)</td>
<td>791 ± 61</td>
<td>1220 ± 115</td>
<td>1985 ± 112</td>
</tr>
<tr>
<td></td>
<td>543 ± 27</td>
<td>629 ± 99</td>
<td>629 ± 99</td>
</tr>
<tr>
<td>Group IV (CCl$_4$ + A. digitata 100mg/kg)</td>
<td>2560 ± 295</td>
<td>3020 ± 174</td>
<td>2300 ± 101</td>
</tr>
<tr>
<td></td>
<td>543 ± 27</td>
<td>1030 ± 68</td>
<td>500 ± 37</td>
</tr>
<tr>
<td>Group V (CCl$_4$ + A. digitata 200mg/kg)</td>
<td>2550 ± 166</td>
<td>3040 ± 209</td>
<td>2480 ± 136</td>
</tr>
<tr>
<td></td>
<td>199 ± 55</td>
<td>500 ± 37</td>
<td>500 ± 37</td>
</tr>
</tbody>
</table>

Table 2: Hepatoprotective percentage of A. digitata fruit pulp methanolic extract and Silymarin on CCl$_4$-induced hepatotoxicity at day 21.

<table>
<thead>
<tr>
<th>Group</th>
<th>ALT (U/L)</th>
<th>AST (U/L)</th>
<th>ALP (U/L)</th>
<th>Bilirubin (mg/dL)</th>
<th>Total proteins (g/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I (Normal control)</td>
<td>94 ± 5</td>
<td>119 ± 16</td>
<td>38 ± 5</td>
<td>0.0</td>
<td>6.8 ± 0.12</td>
</tr>
<tr>
<td>Group II (CCl$_4$ only)</td>
<td>1344 ± 121</td>
<td>2259 ± 117</td>
<td>383 ± 23</td>
<td>1.4 ± 0.08</td>
<td>5.4 ± 0.2</td>
</tr>
<tr>
<td>Group III (CCl$_4$ + Silymarin 25mg/kg)</td>
<td>214 ± 26 (90 %)</td>
<td>629 ± 99 (76 %)</td>
<td>255 ± 15 (42 %)</td>
<td>0.3 ± 0.06 (79 %)</td>
<td>5.7 ± 0.2 (21 %)</td>
</tr>
<tr>
<td>Group IV (CCl$_4$ + A. digitata)</td>
<td>543 ± 27</td>
<td>1030 ± 68</td>
<td>355 ± 16</td>
<td>0.4 ± 0.08</td>
<td>5.6 ± 0.14</td>
</tr>
</tbody>
</table>
Histopathological liver section of normal control group (group I) showed normal cellular architecture with distinct hepatic cells, sinusoidal spaces, and central vein. In the CCl₄-only intoxicated group (group II), necrosis of hepatic architecture were observed in the liver section. The liver sections of the rats treated with Silymarin and the methanolic extract of *Adansonia digitata* fruit pulp, showed minimal necrosis and regeneration of hepatocytes compared to CCl₄-only intoxicated group, supplementing the protective effect of the tested extract and the standard hepatoprotective drug. Results were showed in Figure 1.

![Histopathological liver sections](image)

**Figure 1: Histopathological liver sections (10x).**

A: Central vein surrounded by normal hepatocytes, B: Hepatocytes necrosis and inflammatory cell infiltration, C: Central vein, D: Minimal necrosis and inflammatory cell infiltration compared to CCl₄-alon intoxicated group.

**DISCUSSION**

Liver function testing are performed to reveal or confirm hepatocyte necrosis/damage (serum aminotransferases), biliary obstruction (serum bilirubin and alkaline phosphatase) as well as
assessing the synthetic functions of the liver by means of measuring the serum levels of total protein and prothrombin time.\textsuperscript{[16]} The normal values for the main serum parameters that used in liver function tests in humans are 10-55 units/liter for ALT, 10-40 units/liter for AST, 45-115 units/liter for ALP, 0-1 mg/dL for total bilirubin, and 6.0-8.0 g/dL for total protein.\textsuperscript{[17,18]} Acute hepatitis due to viruses, drugs or toxins generally produces markedly elevated levels of aminotransferases, often in the thousands.\textsuperscript{[19]} Induction of liver injury by carbon tetrachloride (CCl\textsubscript{4}) in animal model studies is commonly used to evaluate the of hepatoprotective agents because CCl\textsubscript{4} administration significantly elevate the serum levels of ALT, AST, ALP and bilirubin.\textsuperscript{[20]} Carbon tetrachloride is a xenobiotic that induce oxidative stress by initiating free radical mediated lipid peroxidation leading to the accumulation of lipid-derived oxidation products that cause liver injury and excess collagen deposition in the liver.\textsuperscript{[21]} As described by Sana and Hela, (2003),\textsuperscript{[14]} the intraperitoneal route was found to be the best route for CCl\textsubscript{4}-induced hepatotoxicity in rats and the optimum dose was found to be 2 ml/kg (dissolved in an equal volume of olive oil), and this increased the level of serum enzymes significantly, without causing death of the animals. Generally, when using low doses of CCl\textsubscript{4} natural healing of the liver started at three days of the liver damage inducement while high doses of CCl\textsubscript{4} (90 – 120 mg/Kg) could caused massive liver damage and delay natural healing.\textsuperscript{[22]} Hepatoprotective agents exert their action against CCl\textsubscript{4}-induced liver injury by impairment of CCl\textsubscript{4}-mediated lipid peroxidation, either through decreased production of free radical derivatives or due to the antioxidant activity of the protective agent itself.\textsuperscript{[23]} Administration of different antioxidants together with CCl\textsubscript{4} may help to prevent the development of liver injury due to the free radical scavenging properties possessed by antioxidants that decreased the overload on the endogenous body antioxidants.\textsuperscript{[13]} An ethnobotanical studies have confirmed that the Baobab fruit pulp could be considered a much valuable source containing levels of vitamin C (2.8 - 3 g/kg) and the antioxidant capacity of products derived from A. digitata plant were attributed to the high content of vitamin C of the fruit.\textsuperscript{[8]} Our results indicated that, the plant extract could protect the liver against CCl\textsubscript{4} chemical toxicity as evident by the obtained biochemical data which were further supported by histopathological observations. The obtained results were in accordance with that previously described by Al-Qarawi et al., (2003).\textsuperscript{[9]} who reported that, the protection and restoration against CCl\textsubscript{4}-induced liver damage by the A. digitata fruit pulp extract could result from the
fruit content of triterpenoids, β-sitosterol, β-amyrin palmitate, or/and α-amyrin, and ursolic acid along with the antioxidant, anti-inflammatory, analgesic, immunostimulant, and antimicrobial activities of A. digitata fruit pulp.

CONCLUSION
As experimentally evident, it could be concluded that, the oral administration of A. digitata fruit pulp methanolic extract exhibited hepatoprotective properties on CCl₄-induced hepatotoxicity in rats which could be attributed to the antioxidant capacity of the plant extract. Therefore, efficacy, safety, and the possible role of A. digitata in treatment of some liver disorders should be further evaluated by well-designed controlled studies.

ACKNOWLEDGMENTS
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Conflict of Interest
Authors declared that no conflict of interest.

REFERENCES


