Evaluation of Hepatoprotective and Nephroprotective Activities of Ethanolic Extract Leaves of Aristolochia Albida Duch. Against CCl₄-Induced Hepatic and Renal Dysfunction

ABSTRACT
Objective Vegetable drugs are taken recurrently to improve or cure pathological processes, without any scientific knowledge of their pharmacodynamic activities. The aim of this study was to evaluate the effects of Aristolochia albida used in virus hepatitis treatment, on the liver and kidneys.

Material and Methods Carbon tetrachloride (CCLₓ) is used to induce toxicity whose main target organs are liver and kidney (hepatotoxicity and nephrotoxicity). After poisoning (CCLₓ), the animals are treated curatively with the extracts, according to the model of Fleurentin and Joyeux. All data is processed using Microsoft Excel 2010 and was analyzed by One-Way Analysis of the variance (ANOVA) followed by Tukey’s post-test for the comparison of the averages. The threshold of significance is 5%.

Experimental The hepatic and renal parameters investigated are transaminases (ASAT, ALAT), alkaline phosphatase (PAL), bilirubin (free and conjugated), urea, total protein, creatinine. Several doses (250 mg/kg, 500 mg/kg, 750 mg/kg) of the ethanolic extract of A. albida were used to evaluate effective dose for liver and kidneys.

Results Biochemical analysis show a significant decrease in transaminases (ASAT, ALAT), alkaline phosphatase (PAL), bilirubin (free and conjugated) at 750 mg/kg. Concerning renal parameters, we notice that A. albida don’t reduce significantly urea level.

Conclusion The Ethanolic extract of P. amarus protect liver against the oxidative stress of CCLₓ at 750 mg/kg and has no beneficial effect on the kidneys at this dose.

KEYWORDS hepatotoxicity, nephrotoxicity, Aristolochia albida, carbon tetrachloride

INTRODUCTION
Benin has a great biodiversity floristic, to which is added a secular traditional medicine with many plants used in the treatment of various pathologies. Vegetable drugs are taken recurrently, without any scientific knowledge of their potential toxicity and biological activities. Experience has shown that the richness of plant biodiversity and the knowledge of our therapists are likely to help improve the management of diseases by opening up new scientific channels for their treatments. A great deal of effort combines to discover new actively therapeutic molecules of natural origin and medicinal plants therefore present themselves as an alternative for the research of its new therapeutic molecules. A. albida is a plant of the branch of Magnoliophyta and the great family of Aristolochiaceae. Previous work on this plant concerns the evaluation of antiplasmodial, anti-malarial and prophylactic activities. Recent studies have focused on evaluating the antioxidant activity of different extracts of A. albida after performing phytochemical screening, polyphenolic compounds, and showing that the ethanolic extract has the best antioxidant activity. The objective of our study is to evaluate the hepatoprotective and nephroprotective properties of A. albida against hepatotoxicity and nephrotoxicity induced by carbon tetrachloride on an in vivo model of Wistar strain rats.

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MATERIALS AND METHODS

Plant material

The leaves of A. albida Duch. were harvested in Covè (Latitude 7° 13’ 8” N, Longitude 2° 20’ 22” E, Altitude 102 m), department of Zou (Benin), in July 2015 and identified under the number AA 6551/HNB in the national herbarium of Benin.

Preparation of ethanolic extract of A. albida (EEAr)

The collected leaves were shade-dried and powdered in a mixer-grinder to get a coarse powder. A quantity of 650 g of the powder of the leaves is soaked and macerated in 3 L of ethanol, under gentle agitation for one night at room temperature forming a maceration. Ethanol extract is recovered after filtration using a paper filter; ethanol is eliminated from the filtrate by evaporation under reduced in a rota-evapour pressure.

Treatment of animals

Wistar albino rats (142–200 g), aged 10 to 15 weeks were obtained and acclimatized in the Laboratory of Animal Physiology and Experimental Pharmacology of the Faculty of Science and Technology of the University of Abomey-Calavi two weeks before the beginning of the experiment at a constant temperature of 22 ± 1°C with a 12-h cycle of light and 12 h of darkness. They are fed with granular feed and ad libitum water without discontinuity in feeding bottles. Carbon tetrachloride, supplied by UBC.HR. Leuven 6172 Belgium, is used for the induction of hepatic and renal poisoning. The Belle France extra virgin olive oil (Francap, BP 30403-75564 Paris Cedex 12) is used to prepare the poisoning solution. The CCL₄ solution. The CCL₄ was prepared by the chemical method of using the semi-automated brand Legalon® (lot B 0902953, MADAUS GmbH 51101 Cologne-Germany) used as liver reference product contains 70 mg of silymarin (SIL). The animals were taken care as per OCDE guidelines, and the experimental protocol was approved by Animal Ethics Committee of Animal Physiology Department of Abomey-Calavi University (Benin).

Hepatoprotective activity

Ten batches of six Wistar rats were randomized. They are individually marked and then kept in their cages for acclimation to laboratory conditions for 2 weeks before the experiment. All rats are weighed before the experiment. They received carbon tetrachloride (CCL₄, 1 ml/kg diluted 1/5 in olive oil) to induce toxicity in which the liver and kidney are the primary target organs (hepatotoxicity and nephrotoxicity). Belle France olive oil (HO) is used to prepare CCL₄ solution. The CCL₄ was administered intraperitoneally, and the animals were treated curatively with the ethanolic extract of A. albida (EEAr) according to the model previously described.

- Batch A (negative control): Rats received distilled water
- Batch B (negative control): Rats received olive oil
- Batch C (positive control): Rats received 1 ml/kg CCL₄ (1/5) without treatment
- Batch D (reference): Rats received 1 ml/kg CCL₄ (1/5) and treated with (SIL) at 300 mg/kg PV

Test Batch 1: Rats were given 1 ml/kg CCL₄ (1/5) and treated with EEA rat 250 mg/kg PV
Test Batch 2: Rats were given 1 ml/kg CCL₄ (1/5) and treated with EEA rat 500 mg/kg PV
Test Batch 3: Rats were given 1 ml/kg CCL₄ (1/5) and treated with EEA rat 750 mg/kg PV

The batch C doesn’t receive corrective therapy (Positive Control) while batch D received silymarin (300 mg/kg). The batches 1, 2 and 3 received, respectively, 250 mg/kg, 500 and 750 mg/kg of A. albida extracts orally once daily for 7 days. On day 8, the blood was taken by retroorbital puncture in dry tubes using a micropipette with unheparinized hematocrit. The blood samples were centrifuged at 3000 tr/min for 15 minutes. The serum collected was used for the determination of the various biochemical parameters.

Body weight

The individual weight of each rat is determined 1 hour before the administration of the test substance and then at least once a week. The weight changes are calculated and recorded.

Biochemical examinations

 Portions of the blood are taken from all rats by retroorbital puncture 24 h after the last extract administration. Biochemical examinations were performed at the Laboratory of Applied Biology Research of the Abomey-Calavi Polytechnic School. The biochemical tests are carried out by the kinetic method according to the methodology of using the semi-automated brand RAYTO™. These include determination of transaminases (ASAT, ALAT), alkaline phosphatase (PAL), bilirubin (free and conjugated), urea, total protein, creatinine.

Statistical analysis

All data were processed using Microsoft Excel 2010 and were analyzed by one-way analysis of the variance (ANOVA) followed by Tukey’s post-test for the comparison of the averages. All analyses were performed using the statistical program Minitab version 16.FR. The threshold of significance is 5%.
RESULTS AND DISCUSSION

Morphometric parameters

The animals of the different lots are weighed before and after the treatments. The weights at the beginning and at the end and their variations are summarized in Table 1.

In the same column, the averages that do not share a letter are different. Values are expressed as mean ± SEM (Standard Error Mean); *Significant statistical difference. (P < 0.05), one-way ANOVA followed by Tukey’s test as compared to control; n = Number of Wistar rats.

The lot receiving CCL₄ alone (positive control) showed a significant weight loss whereas all other lots showed an insignificant weight reduction. The fall in weight at the level of the CCL₄ lot is likely to be related to the toxic effects of CCL₄. These data confirm the safety of A. albida in accordance with previous in vivo toxicity work on this plant. These results are similar to those of previous research⁸,⁹, who found a loss in weight during the evaluation of the hepatoprotective activity of Gomphrena celosioides and of the ethanolic extract of Cinnamomum zeylanicum L.

Biochemical Examinations

The various biochemical parameters explored have informed us about the probable effects of EEPh leaves in the liver and kidney. The transaminases (ALAT and ASAT), alkaline phosphatase (PAL), bilirubin (free and conjugated), blood glucose are parameters of the liver while uric acid, creatinine and total proteins are kidney parameters. The results of the various assays are shown in the following tables and figures.

In the same column, the averages that do not share a letter are different. Values are expressed as mean ± SEM (Standard Error Mean); *Significant statistical difference. (P < 0.05), one-way ANOVA followed by Tukey’s test as compared to control; n = Number of Wistar rats.

In the same column, the averages that do not share a letter are different. Values are expressed as mean ± SEM (Standard Error Mean); *Significant statistical difference. (P < 0.05), one-way ANOVA followed by Tukey’s test as compared to control; n = Number of Wistar rats.

Figure 1 shows the effects (expressed as a percentage) of EEAr on transaminases and bilirubins.

Figure 2 shows the effects (expressed as a percentage) of EEAr on alkaline phosphatases, urea and creatinine.

Figure 3 shows the effects (expressed as a percentage) of EEAr on total proteins.

These results express that there is no significant difference between the liver and renal parameters of the two negative controls (water, olive oil). The olive oil (HO) used as vehicle for the dilution of CCL₄ has no effect on the physiology of the rats and may have a protective effect by causing an increase in the activity of the antioxidant enzymes and decreased signs of damage to the liver¹⁰,¹¹. CCL₄ starts its biotransformation by a reductive dehalogenation reaction catalyzed by P₄₅₀ to give the trichloromethyl free radical (CCL₃). The highly reacted CCL₄ formed readily interacts with the molecular oxygen

Table 1 Average weights of Wistar rats at the beginning and at the end of treatments (n = 6).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Initial average weight (g)</th>
<th>Final average weight (g)</th>
<th>Change average weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control HO</td>
<td>147.00 ± 11.08⁶</td>
<td>143.67 ± 14.49⁶</td>
<td>4.00 ± 3.03⁶</td>
</tr>
<tr>
<td>Control H₂O</td>
<td>164.50 ± 12.11⁵</td>
<td>158.67 ± 11.69⁵</td>
<td>6.13 ± 4.83⁵</td>
</tr>
<tr>
<td>CCL₄</td>
<td>173.50 ± 33.16</td>
<td>128.20 ± 15.01</td>
<td>41.80 ± 24.41</td>
</tr>
<tr>
<td>CCL₄-SIL</td>
<td>185.00 ± 7.21</td>
<td>177.60 ± 14.22</td>
<td>10.20 ± 1.10</td>
</tr>
<tr>
<td>CCL₄-EEAr (250 mg)</td>
<td>152.33 ± 16.84&lt;sup&gt;b&lt;/sup&gt;</td>
<td>144.80 ± 12.15&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.80 ± 2.28&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>CCL₄-EEAr (500 mg)</td>
<td>162.17 ± 21.45&lt;sup&gt;b&lt;/sup&gt;</td>
<td>159.83 ± 17.20&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.20 ± 2.96&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>CCL₄-EEAr (750 mg)</td>
<td>152.80 ± 9.15&lt;sup&gt;b&lt;/sup&gt;</td>
<td>151.00 ± 7.97&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.60 ± 0.89&lt;sup&gt;b&lt;/sup&gt;</td>
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</table>

Table 2 Effects of A. albida on the hepatic parameters of the control batches (negative and positive) and of the test batches (intoxicated and treated batches). (n = 6).

<table>
<thead>
<tr>
<th>Hepatic parameters</th>
<th>ASAT/GOT</th>
<th>ALAT/GPT</th>
<th>BT</th>
<th>BC</th>
<th>PAL</th>
</tr>
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<tbody>
<tr>
<td><strong>Batches</strong></td>
<td></td>
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<tr>
<td>Control HO</td>
<td>48.12 ± 15.97&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>38.03 ± 13.39&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>4.08 ± 0.62&lt;sup&gt;e&lt;/sup&gt;</td>
<td>3.27 ± 0.83&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>32.07 ± 4.52&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>Control H₂O</td>
<td>37.00 ± 8.1&lt;sup&gt;d&lt;/sup&gt;</td>
<td>30.00 ± 5.9&lt;sup&gt;d&lt;/sup&gt;</td>
<td>3.89 ± 0.67&lt;sup&gt;d&lt;/sup&gt;</td>
<td>2.55 ± 0.75&lt;sup&gt;d&lt;/sup&gt;</td>
<td>25.67 ± 7.94&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>CCL₄</td>
<td>140.50 ± 14.89&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>150.63 ± 17.20&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>14.07 ± 1.407&lt;sup&gt;d&lt;/sup&gt;</td>
<td>8.10 ± 1.96&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>69.82 ± 23.59&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>CCL₄-SIL</td>
<td>33.17 ± 8.01&lt;sup&gt;d&lt;/sup&gt;</td>
<td>32.82 ± 9.91&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>3.38 ± 1.02&lt;sup&gt;e&lt;/sup&gt;</td>
<td>3.28 ± 1.52&lt;sup&gt;d&lt;/sup&gt;</td>
<td>63.27 ± 23.33&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>CCL₄-EEAr (250 mg)</td>
<td>73.23 ± 23.16&lt;sup&gt;d&lt;/sup&gt;</td>
<td>61.21 ± 21.51&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>9.96 ± 16.15&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>8.72 ± 3.21&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>67.70 ± 11.99&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>CCL₄-EEAr (500 mg)</td>
<td>70.12 ± 11.34&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>68.27 ± 7.61&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>9.87 ± 0.47&lt;sup&gt;e&lt;/sup&gt;</td>
<td>8.35 ± 0.54&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>69.12 ± 1.13&lt;sup&gt;cd&lt;/sup&gt;</td>
</tr>
<tr>
<td>CCL₄-EEAr (750 mg)</td>
<td>46.26 ± 7.85&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>42.55 ± 19.66&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>3.97 ± 1.69&lt;sup&gt;e&lt;/sup&gt;</td>
<td>3.45 ± 1.09&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>52.54 ± 15.62&lt;sup&gt;bc&lt;/sup&gt;</td>
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</table>
Table 3 Effects of A. albida on renal parameters. Control batches (negative and positive) and test batches (intoxicated and treated batches). (n = 6).

<table>
<thead>
<tr>
<th></th>
<th>Urea</th>
<th>Creatinine</th>
<th>Total proteins</th>
</tr>
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<tbody>
<tr>
<td>Control HO</td>
<td>0.37 ± 0.06&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>18.82 ± 2.99&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>57.10 ± 4.33&lt;sup&gt;cd&lt;/sup&gt;</td>
</tr>
<tr>
<td>Control H&lt;sub&gt;2&lt;/sub&gt;O</td>
<td>0.35 ± 0.03&lt;sup&gt;d&lt;/sup&gt;</td>
<td>17.39 ± 1.47&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>58.62 ± 17.36&lt;sup&gt;d&lt;/sup&gt;</td>
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<tr>
<td>CCL&lt;sub&gt;4&lt;/sub&gt;</td>
<td>0.50 ± 0.06&lt;sup&gt;abc&lt;/sup&gt;*</td>
<td>27.08 ± 4.97&lt;sup&gt;a&lt;/sup&gt;*</td>
<td>47.74 ± 4.48&lt;sup&gt;c&lt;/sup&gt;*</td>
</tr>
<tr>
<td>CCL&lt;sub&gt;4&lt;/sub&gt;-SIL</td>
<td>0.38 ± 0.03&lt;sup&gt;bcd&lt;/sup&gt;</td>
<td>16.53 ± 3.00&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>53.49 ± 3.08&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>CCL&lt;sub&gt;4&lt;/sub&gt;-EEAr (250 mg)</td>
<td>0.55 ± 0.09&lt;sup&gt;a&lt;/sup&gt;*</td>
<td>20.79 ± 4.03&lt;sup&gt;b&lt;/sup&gt;</td>
<td>71.84 ± 9.00&lt;sup&gt;bc&lt;/sup&gt;*</td>
</tr>
<tr>
<td>CCL&lt;sub&gt;4&lt;/sub&gt;-EEAr (500 mg)</td>
<td>0.51 ± 0.11&lt;sup&gt;abc&lt;/sup&gt;*</td>
<td>17.57 ± 1.40&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>74.68 ± 4.56&lt;sup&gt;c&lt;/sup&gt;*</td>
</tr>
<tr>
<td>CCL&lt;sub&gt;4&lt;/sub&gt;-EEAr (750 mg)</td>
<td>0.49 ± 0.05&lt;sup&gt;ab&lt;/sup&gt;*</td>
<td>16.15 ± 1.35&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>57.73 ± 9.58&lt;sup&gt;abcd&lt;/sup&gt;</td>
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Fig. 1 Effects of different doses of EEA<sub>r</sub> on ASAT, ALAT, total and conjugated bilirubin.

Fig. 2 Effects of different doses of EEA<sub>r</sub> on alkaline phosphatase, urea and creatinine.

Fig. 3 Effects of different doses of EEP<sub>h</sub> on total proteins.

to form the peroxyl trichloromethyl radical (CCl<sub>3</sub>OO<sup>•</sup>).<sup>12</sup> These radicals bind to proteins, lips or abstract a hydrogen atom of an unsaturated lipid to cause lipid peroxidation and lesions, thus contributing significantly to the pathogenesis of diseases.<sup>13</sup> The toxicity of CCL<sub>4</sub> is mainly due to the appearance of free radicals or toxic forms of oxygen, which induce lipid peroxidation leading to the destruction of cell membranes<sup>14</sup>. This is a mandatory and predictable indirect toxicant<sup>15,16</sup>. The increase in serum levels of transaminases and alkaline phosphatases after CCL<sub>4</sub> injection is evidence of significant hepatic involvement. CCL<sub>4</sub>-induced liver lesions are commonly used as a model for liver drug screening and the extent of damage is assessed by the level of cytoplasmic transaminases (ALT and ASAT) and circulating APL<sup>17,18</sup>. The test batch, which has received only CCL<sub>4</sub> exhibited a significant increase in transaminases (ASAT, ALAT), alkaline phosphatase (PAL), as well as bilirubin (total and conjugated) of urea, creatinine and a significant decrease in proteins. Increased serum levels of ALT and AST in CCL<sub>4</sub>-mediated rats is an indication of the damaged structural and functional integrity of liver cell membranes since these cytosolic enzymes are released into the circulation after cellular lesions hepatic function<sup>19</sup>. The carbon tetrachloride, besides exerting its toxic effect on the liver, also reportedly gets distributed at higher concentrations in the kidney than in the liver.<sup>20</sup> The mechanism of CCL<sub>4</sub> renal toxicity is almost the same as that of the liver, but the cytochrome P<sub>450</sub> predominantly shows a high affinity to the kidney cortex<sup>21,22</sup>. The CCL<sub>4</sub> caused hepatorenal injury and the transport function of hepatocytes and nephrotic cells gets disturbed in the leakage of plasma membrane, thereby
causing an increased enzyme level in the serum\textsuperscript{13}. The variation of hepatic and renal parameters recorded the extensive disruption of the structure and function of the liver and kidney. Silymarin has hepatoprotective properties and is used in various liver diseases\textsuperscript{14}. Various studies indicate that Silymarin exhibits strong antioxidant activity\textsuperscript{15} and shows protective effects against hepatic toxicity induced by a wide variety of agents by inhibiting lipid peroxidation\textsuperscript{16–18}, while antioxidant activity has also been linked to the hepatoprotective effect of some extracts.

The batch having received silymarin after intoxication with CCL\textsubscript{4} shows that the liver and renal parameters present insignificant difference compared to the negative control with the exception of alkaline phosphatases. The results obtained in the test batches having received different doses of EEA extracts show that the dose of 750 mg/kg prevents the appearance of lesions in the liver because the levels of ASAT, ALT, BT and BC show insignificant statistical difference versus negative control with respective reductions percentages of 91.05%; 81.30%; 99.21%; 83.78% versus. 103.7%; 97.66%; 106.15%; 86.84% for silymarin. Hepatic lesions induced by free radicals can be prevented or corrected by antioxidants\textsuperscript{19}. EEA has a dose-dependent hepatoprotective effect on the ASAT transaminase and bilirubin tandis that has no dose-dependent effect on the ALAT transaminase and conjugated bilirubin. Concerning renal parameters, this same dose lowers significantly the creatinine level and increases the conductivity of the liver and kidney. Silymarin has hepatoprotective activity has also been linked to the hepatoprotective activity of some extracts.

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CONCLUSION

\textit{A. albida} has significant protective effect against CCL\textsubscript{4}–induced hepatotoxicity. The ethanolic extract of \textit{A. albida} protect the liver against CCL oxidative stress and damage at 750 mg/kg and don’t protect the kidney at the same dose. This hepatoprotection is preserved through amelioration of lipid peroxidation by its scavenging activity of free radicals and enhancement of the antioxidant defense systems.

ACKNOWLEDGEMENTS

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REFERENCES


