ACUTE TOXICITY EVALUATION OF ETHANOLIC EXTRACT OF ARISTOLOCHIA ALBIDA DUCH. LEAVES ON WISTAR RATS LIVER AND KIDNEY FUNCTIONS

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INTRODUCTION

Hepatitis is a real public health problem affecting both developed and developing countries. They are most often caused by viruses, but also by toxic substances, alcohol and certain drugs [1]. *A. albida* Duch is a recurrent plant used in the treatment of viral hepatitis B and C in Benin [2]. It is of the branch of the Magnoliophyta and the great family of the Aristolochiaceae, [3]. Commonly known as white Aristolochia (French), Dutchman’s pipe (English), fonwle (Ijo), akogun (yoruba, nagot), kaba têê (bariba), leki reedu (Peuhl) [4] it has a synonym known scientifically under Aristolochia Ledermannii Engl [3]. *A. albida* is a perennial herbaceous climbing shrub. The leaves are alternate, cordate-ovate, acuminate. The flowers are solitary with a dark brown perianth at the top and greenish pink at the base. The fruit is a capsule about 4 cm long [5, 4]. Its capsules are longitudinally dehiscent and often quadrivaluated and the seeds released are black [4]. Ecologically, it is a species that blooms in July, August and October and then grows in October. It is found in the dense dry forest [5]. Recent studies have focused on the evaluation of the antioxidant activity of different extracts of *A. albida* after carrying out the phytochemical screening, the determination of the polyphenolic compounds and showed that the ethanolic extract of *A. albida* provides the best antioxidant activity [6]. Despite the use of this plant to improve or cure pathological processes, very little scientific work has been devoted to its biological properties even less the toxicity tests. This study attempts to evaluate the acute toxicity of ethanolic extract of *A. albida* leaves on Wistar rat’s liver and kidney functions.

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 150 g of the powder of the leaves is soaked and macerated in 750 ml 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 rotavapour pressure. The various clinical signs observed after administration and for 14 d were recorded and no mortality was observed. With the exception of white blood cells, mean cell volume and platelets with statistically significant difference in control (p<0.05), all the haematological parameters showed an insignificant statistical difference to the control ratio (p>0.05). For biochemical parameters, except blood glucose and total protein of control batches, which show a significant statistical difference after 14 d (p<0.05), all the biochemical parameters show the statistically insignificant difference for test and controls batches (p>0.05) as well as the weight variation of the animals.

Conclusion:
The ethanolic extract of the leaves of *A. albida* Duch (EEAr) had not toxic effect on the biochemical and hematological parameters studied at a dose of 2000 mg/kg. The lethal dose is therefore over 2000 mg/kg.

Keywords: *Aristolochia albida* Duch, Acute toxicity, Lethal dose

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ABSTRACT

Objective: *Aristolochia albida* Duch is frequently used by alternative medicine to treat some pathologies like hepatitis. Our main objective was to evaluate the acute oral toxicity of the ethanolic extract of this plant.

Methods: Exploratory tests for acute oral toxicity are performed in vivo on Wistar albino rats in a limit test of 2000 mg/kg for 14 d in accordance with the OECD Guidelines 423. The clinical signs were observed every day, followed by measurement of body weight change, the haematological and biochemical examinations were executed and statistical analysis was performed.

Results: The various clinical signs observed after administration and for 14 d were recorded and no mortality was observed. With the exception of white blood cells, mean cell volume and platelets with statistically significant difference in control (p<0.05), all the haematological parameters showed an insignificant statistical difference to the control ratio (p>0.05). For biochemical parameters, except blood glucose and total protein of control batches, which show a significant statistical difference after 14 d (p<0.05), all the biochemical parameters show the statistically insignificant difference for test and controls batches (p>0.05) as well as the weight variation of the animals.

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marked for individual identification. The limit test at a dose of 2000 mg/kg was chosen because information indicating that *A. Alida* Duch. Are probably not toxic, that is, the toxicity is likely to be above the regulatory dose limit. The rats were divided into two batches of three rats after blood tests to ensure homogeneity of batches. Control batch (I) had not received extract but distilled water while batch (II) received 2000 mg/kg of an ethanolic extract of *A. Alida* Duch. The animals were observed individually at least once during the first 30 min and at least twice during the first 24 h after treatment. Special attention was given to them daily for 14 d after the administration of the extract. All observations were systematically recorded. Particular attention has been paid to observing the various manifestations of tremors, convulsions, salivation, diarrhea, lethargy, sleep and coma. The change of body weight, the hematological and biochemical parameters are searched.

**Body weight**

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

**Hematological and biochemical examinations**

Portions of the blood are taken from all rats by retro-orbital puncture 24 h and 14 d after the extract administration, for hematological and biochemical examinations at the Laboratory of Applied Biology Research of the Abomey-Calavi Polytechnic School. The hematological examinations are carried out using a SYSMEX KX-N21 automaton according to the method used by [8]. These include the enumeration of red blood cells, white blood cells, platelets and determination of hemoglobin, hematocrit, mean globular volume, average corpuscular content in hemoglobin, average corpuscular concentration in hemoglobin. The biochemical tests are carried out by the kinetic method according to the methodology of [8] using the Semi-automate brand RAYTO. These include the determination of transaminases (ASAT, ALAT), alkaline phosphatase (PAL), bilirubin (free and conjugated), blood glucose, urea, total protein, creatinine.

**Statistical analysis**

All data is processed using Microsoft Excel 2010. Minitab version 16. FR. was used for the analysis of the variance (ANOVA One-Way Analysis) for the comparison of the averages. The threshold of significance is 5%.

**RESULTS AND DISCUSSION**

**Clinical signs observed**

After administration of the extract, the animals were observed individually at least once during the first 30 min and at least twice during the first 24 h after treatment. Particular attention was paid to them daily for 14 d after administration of the substance. Few minutes after the administration of EEAr, we recorded a short agitation period of about 2 to 3 min, mostly in lot (II) and then the animals resumed normal habit. All observations were systematically recorded and summarized in table 1.

**Table 1: Clinical signs observed 24 h and during the 14 d after injection of EEAr**

<table>
<thead>
<tr>
<th>Lots clinical signs</th>
<th>Lot(I)</th>
<th>Lot(II)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salivation</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Accelerated breathing</td>
<td>-</td>
<td>+++</td>
</tr>
<tr>
<td>Tremors</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Sleep</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Lethargy</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Paralysis</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Abdominal constrictions</td>
<td>-</td>
<td>++</td>
</tr>
<tr>
<td>Coma</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

- : absence of signs +: Presence of signs

The *in vivo* oral toxicity tests of EEAr carried out at a limit test of 2000 mg/kg did not cause any mortality and the various clinical signs appeared reversible and disappeared after two weeks. Similar results were reported by [9], who by studying the acute and subacute oral toxicity of *Aristolochia longa* L., noted a mortality at 4000 mg/kg which was accentuated at 5000 mg/kg for acute oral toxicity while the mortality was observed at 2500 mg/kg for subacute oral toxicity.

**Fig. 1: Effect of EEAr (2000 mg/kg) on the average weight of wistar rats, n=3, results represent the means±SEM (standard error of mean)**

The averages obtained are shown on fig. 1 and fig. 2. There is a variation of average weight over time. (p>0.05).
Effect of EEAr on the weight of Wistar rats and their organs

Analysis of the variation in body weight at the level of the batch having received (EEAr) and the control group showed a slight weight drop 7 d after treatment (fig. 1). This variation is not significant (p>0.05) so it is certainly caused by other factors such as stress and nervousness of animals during and after swab. The EEAr, therefore, has no effect on the weight change of the animals. On the other hand, previous work has shown that the presence of polyphenols such as tannins can be responsible for poor assimilation of food and can promote a reduction in weight. These results are comparable to the work [10] in the study of the sub-chronic toxicity of Argemone mexicana. In addition, variation in body weight is used as an indicator of the adverse effects of chemical compounds [11].

This weight reduction can be explained by a consumption of food reduction, but also by the possibility of dose/absorption interactions and by the reduction of the quantity of food absorbed. Other studies have also shown the weight reduction of rats after oral administration of the Chicococca alba extract [12] and Stryphnodendron adstringens [13].

Hematological and biochemical examinations

The results of the hematological examinations are summarized in the following table:

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Lot I</th>
<th>Lot II</th>
</tr>
</thead>
<tbody>
<tr>
<td>GB (10^9/l)</td>
<td>5.35±0.07</td>
<td>6.5±0.25 a</td>
</tr>
<tr>
<td>HGB (g/dl)</td>
<td>14.45±0.35</td>
<td>10.45±1.485 b</td>
</tr>
<tr>
<td>GR (10^12/l)</td>
<td>8.380±0.255</td>
<td>7.820±2.942 b</td>
</tr>
<tr>
<td>HCT (%)</td>
<td>42.50±3.536</td>
<td>31.90±1.273 b</td>
</tr>
<tr>
<td>VGM (FL)</td>
<td>51.850±1.061</td>
<td>61.650±1.52 a</td>
</tr>
<tr>
<td>TMH (pg)</td>
<td>16.100±1.414</td>
<td>18.200±1.414 b</td>
</tr>
<tr>
<td>CCMH (g/dL)</td>
<td>31.850±1.061</td>
<td>29.150±1.344 b</td>
</tr>
<tr>
<td>IDR-CV (%)</td>
<td>11.200±2.970</td>
<td>13.650±2.192 a</td>
</tr>
<tr>
<td>IDR-DS (FL)</td>
<td>24.500±1.414</td>
<td>23.000±5.536</td>
</tr>
<tr>
<td>PLT (10^9/l)</td>
<td>534.00±19.80</td>
<td>718.50±40.31 a</td>
</tr>
<tr>
<td>VMP (FL)</td>
<td>7.700±1.697</td>
<td>6.700±2.121 b</td>
</tr>
<tr>
<td>IDP</td>
<td>13.550±1.768</td>
<td>12.800±2.121 b</td>
</tr>
<tr>
<td>PCT (%)</td>
<td>0.690±0.1117</td>
<td>0.3845±0.0912 b</td>
</tr>
</tbody>
</table>

Indeed, it is interesting to note that except the white blood cells and the mean globular volume whose statistical difference compared to the control is significant (p<0.05), all the hematological parameters present an insignificant statistical difference compared to the control (p>0.05), for example, the absence of the hematocrit variation leads us to exclude the hypothesis of hemococoncentration. On the other hand, the increase in the levels of white blood cells and platelets suggests that the EEAr leaves stimulates the functions of the immune system as opposed to the methanolic extract of Alstonia scholaris which reduces the content of white blood cells and platelets, weakens the immune system by causing infections [14].

These results are in part similar and contrary to those of [15] and [16] which showed that the total aqueous extract of Sacoglossis gabonensis does not cause changes in the erythrocyte and leukocyte lines. Indeed [15], studying the influence of Sacoglossis gabonensis on the side effects of 2,4-dinitrophenylhydrazine on blood and cell metabolism, showed that administration of Sacoglossis gabonensis to rats did not alter the levels of red globules, hemoglobin, hematocrit, white blood cells, lymphocytes, neutrophils and monocytes.

The various biochemical parameters explored have informed us about the probable effects of EEAr 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 fig. ranging from 3 to 11.
Fig. 3: Effect of EEAr. (2000 mg/kg) on blood glucose, n=3, results represent the means±SEM (standard error of the mean).

There was no significant difference (p>0.05) in glucose (lot II) treated with EEAr after 14 d, whereas that in the control batch (lot I) showed a significant difference (p<0.05).

Fig. 4: Effect of EEAr (2000 mg/kg) on the ASAT transaminase, n=3, results represent the means±SEM (standard error of mean).

The ASAT transaminase of (lot II) treated with EEAr and of the control lot (lot I) showed no significant difference (p>0.05) after 14 d.

Fig. 5: Effect of EEAr (2000 mg/kg) on the ALAT transaminase, n=3, results represent the means±SEM (standard error of mean).

The ALAT transaminase of (lot II) treated with EEAr and of the control lot (lot I) showed no significant difference (p>0.05) after 14 d.

Fig. 6: Effect of EEAr (2000 mg/kg) on alkaline phosphatase (PAL), n=3, results represent the means±SEM (standard error of mean).

The alkaline phosphatases (PAL) of (lot II) treated with EEAr and of the control lot (lot I) showed no significant difference (p>0.05) after 14 d.

Fig. 7: Effect of EEAr (2000 mg/kg) on total bilirubin, n=3, results represent the means±SEM (standard error of mean).

Total bilirubin (lot II) treated with EEAr and control batch (lot I) showed no significant difference (p>0.05) after 14 d.

Fig. 8: Effect of EEAr (2000 mg/kg) on direct bilirubin, n=3, results represent the means±SEM (standard error of mean).

The direct bilirubin of (lot II) treated with EEAr and the control lot (lot I) showed no significant difference (p>0.05) after 14 d.

Fig. 9: Effect of EEAr (2000 mg/kg) on creatinine, n=3, results represent the means±SEM (standard error of mean).

Creatinine (lot II) treated with EEAr and control (lot I) showed no significant difference (p>0.05) after 14 d.

Fig. 10: Effect of EEAr (2000 mg/kg) on total proteins, n=3, results represent the means±SEM (standard error of mean).

The total proteins of (lot II) treated with EEAr and of the control batch (lot I) showed no significant difference (p>0.05) after 14 d.
The total protein (lot II) treated with EEar showed no significant difference (p>0.05) after 14 d, whereas those of the control (lot I) group showed a significant difference (p<0.05).

It is important to note that except of blood glucose (fig. 3) and total proteins (fig. 10) of the control batch, the contents of which are 24 h after administration and 14 p<0.05), other biochemical parameters such as ALAT/GTP transaminises (fig. 5) and ASAT/GOP (fig. 4), total bilirubin's (fig. 7), and conjugated (fig. 8), alkaline phosphastase (fig. 6), creatinine (fig. 9), and urea (fig. 11) Show statistically insignificant difference (p>0.05) in test (II) and control batch (I).

The variations in glycemia and total proteins in the control batch may be related to the result between inputs (food, synthesis, mobilization of reserves) and outputs (storage, catabolism, elimination). The EEar had no effect on plasma biochemical parameters.

With regard to the results obtained, we can deduce that the EEar leaves proved to be non-toxic for the parameters tested, thus has no influence on the blood tissue and then on the vital organs such as the liver and the kidneys. This shows practically the safety of this plant used in the treatment of hepatitis by some traditional healers and herbalists in Benin. Serum enzymes ASAT, ALAT are enzymes synthesized in the cytoplasm of the cell and discharged into the circulation in the case of damaged cells [17].

These are considered good indicators of hepatic cytolysis. Thus, high levels of liver enzymes, including ALAT and ASAT, are frequently attributed to the metabolic and/or toxic effects of different drugs such as psychotropic drugs [18]. At 2000 mg/kg body weight the extract caused a slight, non-significant increase in ASAT while the ALAT did not increase. The realization of the histological sections will allow us to confirm these observations.

These results are comparable to those obtained by [10] with the Argemone mexicana L., where they did not notice the variation of biochemical parameters during sub-chronic toxicity. This shows the safety of the aqueous extract of this plant.

Our results are partly comparable to those of [19] who are not arrested for acute toxicity but have further investigated their work by the subacute toxicity of the extracts of Aristolochiae fructus (A. fructus) and Honey-Fried Aristolochiae fructus (H. A. fructus) in Rodents. The subacute toxicity results showed a dose-dependent relationship of nephrotoxicity of A. fructus and H. A. fructus. However, the toxicity of A. fructus was higher than that of H. A. fructus. Even when the content of aristolochic acids was equivalent, the toxic effect of A. fructus was more severe than H. A. fructus.

The likely explanation can be attributed to differences in the pharmacokinetics or toxicokinetics of A. fructus and H. A. fructus and require further study. Clinical doses of A. fructus and H. A. fructus are 3.0 g/d for adults. They conclude that Honey and decoction with water may decrease aristolochic acids contents, and mitigate toxic effects.

Like This, some medications carry the indications "slightly toxic", "toxic" and "extremely toxic" an overdose. In order to mitigate or eliminate their toxicity and the secondary effects of these drugs, a plethora of theory and technique are frequently used whose main methods of toxicity elimination are drug treatment and compatibility. Some plants containing aristolochic acids such as A. manusharesis [20,21], A. yangchi [22,23] and A. radix [24] have been reported on the possibility of reducing toxicity by drug therapy [24,25] and compatibility [20,23].

CONCLUSION

The acute toxicity tests in vivo of EEar leaves showed no toxic effect on the biochemical and haematological parameters studied at 2000 mg/kg. The lethal dose is therefore over 2000 mg/kg. These results appear to be in favor of its use in hepatitis treatment in some areas of Benin. However, other studies such as chronic or subchronic toxicity tests in vivo of extracts A. albida Duch and histological examinations of the organs (liver and kidney) deserve to be carried out in order to confirm it's a toxic character.

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interests regarding the publication of this paper.

REFERENCES


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