Study on Activity of Um Galagil (Aristolochia bracteolata Lam) Ethanolic Extracts against the Scorpion Venom of (Leiurus quinquestriatus Hemp rich and Ehrenberg)

Tahany Malik¹, Mutaman Kehail¹, Nour Osman¹, Reem Ahmed², GadAllah Modawe³, Salwa ME Khogali⁴*

¹Center of Biosciences and Biotechnology, Faculty of Engineering and Technology, University of Gezira, Sudan
²National Research Center, Institute of Medicinal and Aromatic Plants, Department of toxicology Vardhman Mahavir Medical College, New Delhi, India
³Department of Biochemistry, Faculty of Medicine and Health Sciences, Omdurman Islamic University, Omdurman, Sudan
⁴Department of Biochemistry, Nutrition, Toxicology and Pharmacology, Veterinary Research Institute, Animal Resources Research Corporation, Alamarat Khartoum, Sudan

*Corresponding author: Salwa ME Khogali, Veterinary Research Institute, Animal Resources Research Corporation, Alamarat Khartoum, Sudan. Tel: +916742300134; Email: salwamuhamed@hotmail.com


Received Date: 26 July, 2017; Accepted Date: 31 August, 2017; Published Date: 7 September, 2017

Abstract

Accidental venoming by scorpion stings may happen anytime and anywhere. Some hypothesis pointed out that, some natural products (e.g. Um Galagil; Aristolochia bracteolata Lam) are beneficial to cure scorpion venom. The objective of this study was to investigate the activity of A. bracteolata against scorpion (Leiurus quinquestriatus Hemp rich and Ehrenberg) venom. The scorpion venom was brought from University of Khartoum, and the polyvalent anti-venom was brought from Khartoum Teaching Hospital, whereas the experimental rats were brought from The Animal House of National Center for Research. Um galagil was collected from Rufaa’s farms. The rats were divided into five groups; the first was the control, the 2nd was injected with ethanolic extract of A. bracteolata, while the 3rd, 4th and 5th groups were injected with the venom. In addition; the 3rd group also injected by ethanolic extract, the 4th was injected by polyvalent anti-venom. The phytochemical screening of A. bracteolata revealed that, the plant contains different compounds. The sero-biochemical tests for blood parameters of the experimental rats showed considerable changes. Um galagil plant succeeds in suppressing scorpion venom, although there was some increase in the parameters of AST, ALT, ALP, cholesterol and urea compared to control. The recommendation of this study was to start the formulation procedures for um galagil as scorpion antivenom.

Keywords: Aristolochia bracteolata; Biochemical Tests; Scorpion Antivenom

Introduction

The three most important orders of Arachnida are Araneae (spiders), scorpions (scorpions) and Acari (ticks and mites) [1-7]. Scorpions are terrestrial arachnids that are easily recognized by their characteristic elongated body and segmented tail ending in a bulbous sac and a stinger (telson). There are approximately 1,500 scorpion species worldwide [8-12]. Scorpion venom, which has lethal and paralytic effects, is a secretion composed of water, salts and simple, low-molecular-weight proteins [9]. It is a unique defense and feeding weapon. Scorpion envenomation still remains a major health problem in many tropical and subtropical countries [13-16]. Antivenom is still widely used in treatment of envenomation as there are no vaccines or other effective agents available against animal venoms [17-22]. Venom pooling is extremely important specifically, a larger number of cases were reported in effectively treat scorpion’s cases [23-30]. Aristolochia bracteolata lam is a perennial herb. This plant belongs to the
family Aristolochiaceae. This species which had been shown nephrotoxic, mutagenic and carcinogenic due to the cytotoxicity of the Aristolochic acid constituents. The leaves of the plant are used by native tribal and the villagers, in traditional medicines as a gastric stimulant and in the treatment of cancer, lung inflammation, dysentery, snakebites and scorpion stings it is commonly called as worm killer in English and aadutheneedapalaai in Tamil [31-35]. The objective of this work was to Study the activity of Um Galagil (Aristolochia bracteolata Lam) ethanolic extracts against the venom of (Leirus quinquestriatus Hemp rich and Ehrenberg), Run a phytochemical screening for A. bracteolata components and assessment of a sero-biochemical test.

Materials and Methods

Venom Sample

The lyophilized L. quinquestriatus venom was obtained from University of Khartoum, Faculty of Science, and was preserved at room temperature. Before use, the venom was dissolved in normal saline, centrifuged at 2000 rpm for 10 minutes, and the supernatant was used for antivenom studies. Venom concentration was expressed in terms of dry weight/ volume solvent.

Plant Material

Whole plants of A. bracteolata were collected freshly from Gezira, around the Blue Nile River, Rufaa area, in September, 2011. The collected plants were dried under shade at room temperature. The dried plants were crushed into small particles by using the ordinary mortar and pestle and were then kept in plastic bags for further tests.

Animals, Housing and Management

Forty, male and female healthy adult Wister albino rats, weighing about 40-65 g, were brought from the Animal House of NCR, Soba Khartoum for this study. Housed individually in polypropylene cages, maintained under standard conditions (12 hr light and 12 hr dark cycle; 25-30°C; 35-60% humidity), the animals were fed with standard rat pellet diet (ready-made) and water, as was described by Ranjana et al. 2010[36-40].

Polyvalent Anti-scorpion Serum

Lyophilized polyvalent anti-scorpion serum (as reference serum) was obtained from Khartoum Teaching Hospital Pharmacy.

Preparation of the Plant Extract

An amount of 80g of the dried plant sample was soaked in 1000 ml of 96% ethanol for about 72 hr. Extraction continued till the color of the solvent turn colourless. Solvent were evaporated under reduced pressure using rotary evaporator apparatus. The air was allowed to pass through extract till complete dryness and the yield percentage was calculated. Extract was carried out according to method descried by (Harborne, 1984) [41]. The ethanolic extract of A. bracteolata at the dose of 0.5 mg/g (dissolved in 10 ml saline, (each was 40g in weight) and the Polyvalent anti-scorpion serum at the dose of 0.03 µg/g, were injected to rats five minutes after they were injected with 1.82 µg/g of L. quinquestriatus venom.

Photochemistry

Preliminary phytochemical screening carried out according to the procedure given by Harborne (1984) [41] for the presence of compounds such as steroids, carbohydrates, flavonoids and alkaloids. General phytochemical screening for the active constituents was carried out using the methods described by Sofowora (1993) [42], Martinez (1999) [43-44], with some modifications.

Alkaloids

An amount of 0.2 g of the extract was dissolved in 2 ml of 2N HCl, and heated on water bath with continuous stirring for 10 minutes, cooled, filtered and divided into two test tubes. To one test tube, a few drops of Mayer’s reagent were added, while to the other tube, a few drops of Valser’s reagent were added. A slight turbidity or heavy precipitate in either of the two test tubes was taken as presumptive evidence for the presence of alkaloids. Mayer’s and Valser’s reagents were brought by NCR. Aromatic and medical plant center.

Sterols and Triterpenes

0.2 g of the extract was washed three times with 10 ml of petroleum ether and dissolved in 10 ml of chloroform. To 5 ml of the solution, 0.5 ml acetic anhydride was added and then 3 drops of conc. sulphuric acid. At the contact zone of the two liquids, the gradual appearance of green to blue or pink to purple color was taken as an evidence of the presence of sterols (green to blue) and or triterpenes (pink to purple) in the sample.

Flavonoids

An amount of 0.2 g of the extract was washed three times with 10 ml of petroleum ether and dissolved in 30 ml of 80% ethanol and filtered. The filtrate was used for following tests: A/ to 3 ml of the filtrate, in a test tube, 1.0 ml of 1% aluminum chloride solution was added. Formation of a yellow color indicated the presence of flavonoids, flavones or/and chalcone.

B/ to 3 ml of the filtrate, in a test tube, 1.0 ml of 1% potassium hydroxide solution was added. A dark yellow color indicated the presence of flavonoids compounds (flavones or flavonenes) chalcone and or flavonols. C/ to 2 ml of the filtrate 0.5 ml of magnesium turnings was added. Producing of defiant color to pink or red was taken as presumptive evidence that flavonenes were present in the plant sample.
Saponins

An amount of 0.2 g of the extract was placed in a clean test tube. 10 ml of distilled water was added, the tube stoppered and vigorously shaken for about 30 seconds. The tube was then allowed to stand and observed for the formation of foam, which persisted for at least an hour, was taken as an evidence for presence of saponins.

Cumarin

0.2 g of the extract dissolved in 10 ml distilled water in test tube. A filter paper was attached to the test tube to be saturated with the vapor after a spot of 0.5N KOH was put on it. The filter paper was inspected under UV light; the presence of cumarins was indicated, if the spot found to be adsorbed the UV light.

Tannins

An amount of 0.2 g of the extract was washed with 10 ml of n-hexane three times, dissolved in 10 ml of hot saline solution and divided in two test tubes. To one tube, 2-3 drops of ferric chloride reagent was added, and to the other one 2-3 drops of gelatin salts reagent was added. The occurrence of a blackish blue colour in the first test tube and turbidity in the second one denotes the presence of tannins.

Anthraquinone Glycoside

An amount of 0.2 g of the extract was boiled with 10 ml of 0.5N KOH containing 1.0 ml of 3% hydrogen peroxide solution. The mixture was extracted by shaking with 10 ml of benzene; 5 ml of the benzene solution was shacked with 3 ml of 10% ammonium hydroxide solution, and the two layers were allowed to separate. The presence of anthraquinones was indicated, if the alkaline layer was found to assumed pink or red color.

Sero-Biochemical Tests

Blood samples from experimental rats were collected and allowed to clot and sera were separated by centrifugation at 3000 rpm for 5 minutes and stored at -20°C until analyzed. The blood samples which were collected from injected rats were submitted to sero-biochemical tests so as to compare the variation in some blood parameters. All values were taken in term of mg/dl. All sero biochemical parameters were estimated by colorimeric methods using commercial kits.

Statistical analysis

The data was subjected to data presentation (histogram), descriptive statistics (mean), regression analysis (regression equation and R-square) and ANOVA analysis using Microsoft Excel Program (2007).

Results and Discussion

Phytochemistry

(Table 1) showed the phytochemical screening result. The tests revealed the presence of alkaloids in high concentration (depending on the darkness of the turbulent solution), sterols (in moderate cons.) and triterpenes (in moderate cons.), flavonoids (in high cons.), saponins (in high cons.), cumarins (in moderate cons.), and tannins (in moderate cons.), while that, anthraquinones was not detected. The quantities remarked (high, moderate, trace and negative) in these tests were determined according to the observed end product of each test (appendix 1, 2, 3, 4, 5 and 6), as was suggested by [45-49]. Alkaloids, flavonoids, saponins, and tannins, were the most abundante ingredients in this natural product ($A. bracteolata$), in comparison to sterols, triterpenes and cumarins. In similar tests of $A. bracteolata$ [45], revealed the presence of alkaloids, saponins, glycosides, steroids, tannins, phenolic compounds and flavonoid glycosides as was tested by Shirwaikar et al. (2003) [50-53].

<table>
<thead>
<tr>
<th>S/No</th>
<th>Phytochemicals</th>
<th>Observation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0</td>
<td>Alkaloids</td>
<td>+++</td>
</tr>
<tr>
<td>2.0</td>
<td>Sterols</td>
<td>++</td>
</tr>
<tr>
<td>3.0</td>
<td>Triterpenes</td>
<td>++</td>
</tr>
<tr>
<td>4.0</td>
<td>Flavonoids</td>
<td>+++</td>
</tr>
<tr>
<td>5.0</td>
<td>Saponins</td>
<td>+++</td>
</tr>
<tr>
<td>6.0</td>
<td>Cumarins</td>
<td>++</td>
</tr>
<tr>
<td>7.0</td>
<td>Tannins</td>
<td>+++</td>
</tr>
<tr>
<td>8.0</td>
<td>Anthraquenones</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 1: Phytochemical screening of $A. bracteolata$.

Acute Toxicity of $L. quinquestriatus$ Venom against Experimental Rats

The $L. quinquestriatus$ venom was used in this test. The test period was in all cases 24 hour. A series of doses of 0.9, 1.2, 1.7 and 2.0µg/g were injected to 4 groups of experimental rats (each group consist of 5 rats). Respectively, the corresponding mortalities were (20%), (60%), (100%) and (100%). It was also noticed that, all rats in the control group (that did not injected with scorpion venom) was survived. The $R^2$ was 0.957, which reflect the correlation between doses and the corresponding mortalities. The regression equation for this case was $Y= 36.52 + 238.71X$, and this equation can be used to determine the corresponding mortality for any given dose or vice versa (e.g. LD$_{99}$). The LD$_{99}$ of $L. quinquestriatus$ venom against experimental rats was 1.83µg/g (Table 2) [54-57]. Found that, the LD$_{99}$ of methanol extract of $Indigofera conferta$ against
Naja nigricollis venom was 12.5µg/g after 45 minutes on rats. Certain scorpion venom is able to induce severe hematological changes that appear after envenoming [58-60].

<table>
<thead>
<tr>
<th>Dose (µg/g)</th>
<th>Log dose</th>
<th>n</th>
<th>Dead</th>
<th>% mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.9</td>
<td>-0.05</td>
<td>5</td>
<td>1</td>
<td>20</td>
</tr>
<tr>
<td>1.2</td>
<td>0.079</td>
<td>5</td>
<td>3</td>
<td>60</td>
</tr>
<tr>
<td>1.7</td>
<td>0.231</td>
<td>5</td>
<td>5</td>
<td>100</td>
</tr>
<tr>
<td>2</td>
<td>0.301</td>
<td>5</td>
<td>5</td>
<td>100</td>
</tr>
<tr>
<td>Control</td>
<td>-</td>
<td>5</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

R² = 0.958, Equation: \( Y = 36.52 + 238.71X \), \( LD_{99} = 1.82 \mu g/g \), \( b = \) slope = 238.71

Table 2: Acutetoxicity of L. quinquestriatus venom against experimental rats.

**The Antivenom Effect of A. bracteolata on Scorpion Venom**

The L. quinquestriatus venom at any tested doses (0.9, 1.2, 1.7 and 2.0 µg/g) produced significant rate of mortality (20% and more) in rats (Table, 3). The ethanolic extract of A. bracteolata significantly increase mean survival rate (mortality was 40%, while the survived was 60%) in the experimental rats compared to those which injected with the serum drug (mortality was 60%, while the survived was 40%). Also, some bleeding was noticed in the venom and extract injected rats in comparison to control (Figure 1). According to Hutt and Houghton (1998), some compounds like, flavonoids, cumarins, tannins and alkaloids are known to have antivenin activity by stimulating the immune system of the victims. This might contribute to the reduction in the effects of venoms and improvement in recovery by contributing to a more rapid removal of the venom from the victims when properly administered and in the right manner.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Ethanollic extract +Venom</th>
<th>Polyvalent anti-serum +Venom</th>
<th>Groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST</td>
<td>138.96</td>
<td>↑203</td>
<td>↑161.9</td>
<td>↑187.3</td>
</tr>
<tr>
<td>ALT</td>
<td>18.66</td>
<td>↑25</td>
<td>18.56</td>
<td>↑31.61</td>
</tr>
<tr>
<td>ALP</td>
<td>130.2</td>
<td>192.6†</td>
<td>↑139</td>
<td>↑134.3</td>
</tr>
<tr>
<td>Total protein</td>
<td>7.7</td>
<td>↓6.9</td>
<td>↓7.2</td>
<td>7.7</td>
</tr>
<tr>
<td>Albumin</td>
<td>4.5</td>
<td>4.46</td>
<td>↓4.03</td>
<td>4.36</td>
</tr>
<tr>
<td>Globulin</td>
<td>3.16</td>
<td>↑2.43</td>
<td>3.16</td>
<td>3.3</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>81.3</td>
<td>↑100.6</td>
<td>↑127.3</td>
<td>↑93</td>
</tr>
<tr>
<td>Total bilirubin</td>
<td>4.5</td>
<td>4.4</td>
<td>↓4.03</td>
<td>↓3.8</td>
</tr>
<tr>
<td>Urea</td>
<td>52.9</td>
<td>↑199.6</td>
<td>↑101.6</td>
<td>↑97</td>
</tr>
</tbody>
</table>

ANOVA analysis (column)

| f-stat | 1.371 |
| f-crit | 3.01  |

Table 4: Sero-biochemical Tests for blood of the experimented rat mg/dl.
Figure 1: Sero-biochemical Tests for blood of the experimented rats.

In Figure 1 group 1 (control), group 2 (ethanolic extract of Aristolochia bracteolata+ L. quinquestriatus venom), group 3 (Polyvalent anti-scorpion serum + L. quinquestriatus venom), group 4 (L. quinquestriatus venom), group 5 (ethanolic extract of A. bracteolata).

Conclusions

Phytochemical screening of A. bracteolata revealed the presence of alkaloids, sterols, triterpenes, flavonoids, saponins, cumarins and tannins while that, anthraquinones were not detected. Sero-biochemical tests in the rats that injected with scorpion venom, scorpion venom and plant extract, scorpion venom and serm drug, and plant extract, showed considerable changes in AST, ALT, ALP, cholesterol, total bilirubin and urea, while the total protein, globulin, and albumin did not differ greatly than those of standards.

Acute toxicity of L. quinquestriatus venom against experimental rats was found LD$_{50}$ 1.82 µg/g. The ethanolic extract of A. bracteolata significantly increase a mean the survival rate in the experimental rats compared to serum drug treatment.

References


