Safety evaluation of the ethanol extract of *Ammannia baccifera* (Lythraceae): assessment of Acute and Subacute toxicity

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ABSTRACT

Ethanolic extract of *Ammannia baccifera* was screened for its acute and subacute toxicological effects on rats, because the traditional healers of India use this plant to treat various ailments. Acute oral toxicological study of ethanolic extract of *A. baccifera* revealed that all the animals tolerated the maximum test doses (2000 mg Kg−1 body weight) of the ethanolic extract of *A. baccifera* and suggested that the extract does not cause any apparent acute toxicity. Thirty days subacute toxicity study of *Ammannia baccifera* in Wistar rats at the doses of 50, 100, 250 and 500 mg Kg−1 body weight /day respectively, did not produce any significant dose-related changes in hematological, biochemical parameters and histopathology of vital internal organs. Therefore, it is concluded that the ethanolic extract of *A. baccifera* at the given doses does not produce any significant toxic effect in rats and considered to be safe.

Key words: *Ammannia baccifera*, medicinal herb, natural remedy, toxicity, marker enzymes, histopathology.

INTRODUCTION

The contemporary search for novel molecules has taken a new direction in which ethnobotany and ethnopharmacognosy are being used as guides to lead the chemist to different sources and classes of compounds (1). In the last two decades, numerous publications have described the great importance that the natural products have in the development of new pharmaceuticals or phytopharmaceuticals (2, 3). Consequently, various works have been published signaling the potential toxicological effects that these products could also possess (4, 5). Hence for developing safe phytotherapeutics, it is necessary to characterize its effects on bio-systems, including its toxicological activities.

*Ammannia baccifera* Linn. commonly known as blistering ammannia is erect, branched, smooth, slender, annual, more or less purplish herb (Family: Lythraceae) which is widely distributed throughout India and also naturally occurs in Pakistan and China. It is known to possess a broad spectrum of medicinal, pharmacological and therapeutic properties. It is reported that, this bitter herb is an appetite, stomachic and is useful in treating biliousness; the leaves are beneficial for removing phlegm from the lungs and trachea. According to Ayurvedic pandits, the herbal extract is a good remedy for tuberculosis and typhoid fever. The plant juice mixed with ginger extract is helpful for curing fevers. Tribals believe that the herb is an effective remedy for all blood diseases. In India, the leaves are used to reduce the sexual libido in men. The roots are used for antiurolithiasis, antibacterial and central nervous system depressant activities (7, 8). Recent studies have demonstrated that the ethanolic extract from *Ammannia baccifera* possess antisteroidogenic activity (6).

Despite the varied uses of this plant in treating various ailments, less is known about its toxicological profile. Hence the present study is aimed at investigating the acute and sub-acute (repeat dose) oral toxicity of the plant in rats using the recommended Organization for Economic Cooperation and Development (OECD) guidelines for testing of chemicals for safety evaluation.

MATERIALS AND METHODS

Plant Material and Extraction

*Ammannia baccifera* whole herb was collected during the month of September-2006 from the surroundings of Tirumala Tirupati hills, Chittoor dist, Andhra Pradesh, India. A 100 g of dried powder of whole herb of *A. baccifera* was soxhlet extracted with 500 mL of ethanol for more than six hours. The extract was concentrated in a rotary evaporator and was stored at −20 °C for further usage.

Animals

Wistar rats (200 ± 50 g) were selected for the study and maintained at controlled temperature of 25−28 °C with a 12 h light/dark cycle and fed a standard diet and water ad libitum. Animal studies were conducted according to the Institute Animal Ethics Committee regulations approved by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). The animals were acclimatized to laboratory condition for 10 days before commencement of experiment.

Acute Oral Toxicity Study

Acute oral (gavage) toxicity studies were conducted with ethanol extract of *Ammannia baccifera* (EEAB) in healthy Wistar albino rats. Rats of either sex weighing 200 ± 50 g were divided into six groups of six animals each. Rats were maintained under standard laboratory conditions. After an overnight fast, rats were dosed orally with EEAB in water at doses of 100, 250, 500 and 2000 mg Kg−1 body weight. The control group received 1.0 mL of saline. The animals were observed for a period of 72 h for any signs of behavioral changes, toxicity and mortality.

Subacute Oral Toxicity Study

Treatment of Animals

Wistar albino rats of either sex weighing 200 ± 50 g were divided into five groups (group I-V) of six animals each and were housed under standard laboratory conditions. The control animals (group I) received 1.0 mL of saline daily for 30 days. Group II to Group V animals were administered daily with EEAB for 30 days at doses of 50, 100, 250 and 500 mg Kg−1 body weight, respectively. Rats were observed for mortality and viability twice daily and observed for any toxic manifestations once before treatment and once daily thereafter. On 28th day of the experiment, 24 h urine samples were collected by placing the animals in the metabolic cage with free access to tap water but no feed was given. The urine was free from fecal contamination. Toluene is used as a preservative while collecting the sample. The sediments present in the urine were removed by centrifugation and the collected urine was used for biochemical estimations.

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On 30th day, the animals were fasted for approximately 18 h, then slightly anesthetized with ether and blood samples were collected from the retro-orbital plexus into two tubes: one with EDTA for immediate analysis of haematological parameters, the other without any anticoagulant and was centrifuged at 4000 rpm at 4 °C for 10 minutes to obtain the serum. Serum was stored at 20 °C until analyzed for biochemical parameters.

Relative Organ Weight

At the end of the experiment, animals were sacrificed and different organs such as liver, kidneys, heart, brain, testis and adrenals were surgically dissected out, washed and weighed in grams (absolute organ weight). The relative weight of each animal was then calculated as follows:

\[
\text{Relative organ weight} = \frac{\text{Absolute organ weight} (g)}{\text{Body weight of rats on sacrifice day} (g)} \times 100
\]

Hematological Studies

Blood samples of control and experimental rats were analysed for haemoglobin content, total red blood corpuscles (RBC), white blood corpuscles (WBC) count, Mean corpuscular volume (MCV) and packed cell volume (PCV) following the methods of Sahli (9); Davidson and Henry, (10) and Schalm et al. (11) respectively. From the estimated values of RBC count (millions/mm³) and PCV (volumes percent), mean corpuscular volume (MCV) was calculated as follows:

\[
\text{MCV (µm/red cell)} = \frac{\text{PCV}}{\text{RBC count}} \times 10
\]

Biochemical Studies

Freshly removed liver and kidney separated from extraneous material in chilled saline medium were homogenised 50 mM phosphate buffer (pH 7.0), and then centrifuged at 10,000 rpm for 15 min to remove cell debris. Serum, urine and kidney homogenates were used for the estimation of biochemical parameters. The estimation of protein was carried by the method of Lowry et al. (12). Samples of control and experimental rats were analysed for bilirubin, urea, uric acid, creatinine, triglycerides, cholesterol and glucose levels following the methods of Malloy and Evelyn, (13); Natelson et al. (14); Van Handel (15); Owen et al. (16); Sasaki et al. (17); Parekh and Jung (18) and Sasaki et al. (19) respectively. Activities of glutamate oxaloacetate transaminase/alanine aminotransferase (GOT/GPT), aspartate aminotransferase (GOT/AST), glutamate pyruvate transaminase/Alanine amino transferase (GPT/ALT) and alkaline phosphatase were estimated as per the colorimetric procedure of King (20, 21).

Statistical Analysis

All the grouped data has been statistically evaluated with SPSS/10.0 software. The results were expressed as mean ± SE for six animals each group. Differences between control and experimental groups were assessed by hypothesis testing methods included one-way analysis of variance. Dunnett’s test has been employed for multiple comparisons because here the mean value in each group is to be compared with that of control group.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I (Control)</th>
<th>Group II (EEAB treated- 50 mg/kg)</th>
<th>Group III (EEAB treated- 100 mg/kg)</th>
<th>Group IV (EEAB treated- 250 mg/kg)</th>
<th>Group V (EEAB treated- 500 mg/kg)</th>
<th>ANOVA F-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST</td>
<td>34.12 ± 0.016</td>
<td>35.14 ± 0.018</td>
<td>34.62 ± 0.016</td>
<td>34.73 ± 0.019</td>
<td>34.68 ± 0.016</td>
<td>0.639</td>
</tr>
<tr>
<td>ALT</td>
<td>20.31 ± 0.017</td>
<td>20.42 ± 0.014</td>
<td>20.56 ± 0.015</td>
<td>20.61 ± 0.014</td>
<td>20.60 ± 0.015</td>
<td>0.628</td>
</tr>
<tr>
<td>ALP</td>
<td>18.89 ± 0.012</td>
<td>18.92 ± 0.013</td>
<td>18.95 ± 0.014</td>
<td>19.01 ± 0.015</td>
<td>19.02 ± 0.015</td>
<td>0.748</td>
</tr>
<tr>
<td>Glucose(mg/dl)</td>
<td>165.90 ± 0.016</td>
<td>167.00 ± 0.017</td>
<td>166.00 ± 0.018</td>
<td>166.00 ± 0.019</td>
<td>166.00 ± 0.020</td>
<td>1.034</td>
</tr>
<tr>
<td>Protein(g/dl)</td>
<td>6.98 ± 0.015</td>
<td>7.02 ± 0.016</td>
<td>7.05 ± 0.017</td>
<td>7.07 ± 0.018</td>
<td>7.08 ± 0.019</td>
<td>0.657</td>
</tr>
<tr>
<td>Total cholesterol(mg/dl)</td>
<td>185.00 ± 0.016</td>
<td>186.00 ± 0.017</td>
<td>187.00 ± 0.018</td>
<td>188.00 ± 0.019</td>
<td>189.00 ± 0.020</td>
<td>0.988</td>
</tr>
<tr>
<td>Urea(mg/dl)</td>
<td>14.31 ± 0.014</td>
<td>14.35 ± 0.015</td>
<td>14.37 ± 0.016</td>
<td>14.40 ± 0.017</td>
<td>14.42 ± 0.018</td>
<td>1.132</td>
</tr>
<tr>
<td>Total triglycerides(mg/dl)</td>
<td>109.00 ± 0.016</td>
<td>110.00 ± 0.017</td>
<td>111.00 ± 0.018</td>
<td>112.00 ± 0.019</td>
<td>113.00 ± 0.020</td>
<td>0.657</td>
</tr>
<tr>
<td>Total cholesterol(mg/dl)</td>
<td>151.00 ± 0.016</td>
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<td>1.132</td>
</tr>
</tbody>
</table>

Values are expressed as Mean ± SE of six rats in each group. * indicates significantly (P<0.05) different from control at 5% level. Data was analyzed by one way ANOVA followed by Dunnet’s test. Hb = hemoglobin; RBC = Red Blood Corpuscles; WBC = White Blood Corpuscles; MCV = Mean Cell Volume; PCV = Packed Cell Volume.

Table 3: Effect of EEAB on hematological parameters of control and treated animals during subacute toxicity studies.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I (Control)</th>
<th>Group II (EEAB treated- 50 mg/kg)</th>
<th>Group III (EEAB treated- 100 mg/kg)</th>
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<th>Group V (EEAB treated- 500 mg/kg)</th>
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<tr>
<td>PCV (%)</td>
<td>39.51 ± 0.016</td>
<td>39.56 ± 0.017</td>
<td>39.61 ± 0.018</td>
<td>39.66 ± 0.019</td>
<td>39.71 ± 0.020</td>
<td>0.756</td>
</tr>
<tr>
<td>MCV(µm/mm)</td>
<td>5.59 ± 0.016</td>
<td>5.64 ± 0.017</td>
<td>5.69 ± 0.018</td>
<td>5.74 ± 0.019</td>
<td>5.79 ± 0.020</td>
<td>0.748</td>
</tr>
<tr>
<td>RBC count</td>
<td>6.92 ± 0.016</td>
<td>6.98 ± 0.017</td>
<td>7.05 ± 0.018</td>
<td>7.12 ± 0.019</td>
<td>7.20 ± 0.020</td>
<td>0.657</td>
</tr>
</tbody>
</table>

Values are expressed as Mean ± SE of six rats in each group. * indicates significantly (P<0.05) different from control at 5% level. Data was analyzed by one way ANOVA followed by Dunnet’s test.

Table 4: Effect of ethanolic extract of Ammannia baccifera on marker enzymes in serum, liver and kidney of control and treated rats during subacute toxicity studies.

<table>
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<tr>
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<th>Group I (Control)</th>
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RESULTS

Acute Toxicity Study

The results of acute toxicity study of EEAB revealed no mortality, abnormal signs and behavioral changes in rats up to the maximum dose (2000 mg Kg⁻¹ body weight) of EEAB administered orally, which is the single high dose recommended by Organization for Economic Cooperation and Development (OECD 2000) guidelines.

Subacute Toxicity Study

Effect of EEAB on Mortality and Body and Organ Weights

Table 1 illustrates that there were no significant differences found in body weights and organ weights of control and EEAB treated rats. Moreover, no mortality was recorded at any dose up to the maximum dose of 500 mg Kg⁻¹ body weight during the experimental period.

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Urinary, uric acid, and creatinine levels of control and treated rats were presented in Table 3. Creatinine level in urine was found to be significantly (p<0.01) increased at the highest dose of EEAB treated rats. All other parameters were found to be non-significantly varying when compared with that of control rats.

The results of the activity of glutamate oxaloacetate transaminase, Glutamate pyruvate transaminase and alkaline phosphatase of control and treated rats are shown in Table 4. Significant changes in the levels of AST, ALT and ALP were not found in serum, liver and kidney of treated groups when compared to the control groups.

Effect of EEAB on Histopathology of Internal Organs
Histopathological examinations of some selected vital organs (liver, kidney, brain and heart) of rats treated with 500 mg Kg⁻¹ body weight of EEAB, the high dosage of the present subacute toxicity study revealed normal architecture of the liver (Figures 1A), kidney (Figures 2A), brain (Figures 3A) and heart (Figures 4A) when compared to control groups (Figures 1B, 2B, 3B and 4B). No abnormal signs of internal organs were observed by gross examination.

DISCUSSION
Acute oral toxicological study of EEAB revealed that all the animals tolerated the maximum test doses of the EEAB and suggested that EEAB does not cause any apparent acute toxicity. Prasad et al. (23) reported a similar acute toxicity results and found that ethanol extract of A. baccifera is safe at a dosage level of above 2000 mg Kg⁻¹ body weight.

The changes in body and organ weights have been used as an indicator of adverse effects of drugs and chemicals (24). During subacute toxicological evaluation of EEAB, no changes were observed in animal behaviour, mortality, body weights and organ weights at all doses of EEAB treated rats when compared to the control rats. Blood, the fluid connective tissue is most essential for existence of life. Any change in haematological leads to impairment of physiological functions. All haematological parameters in all EEAB treated groups remained within the normal limits. Decrease in the glucose level of rats receiving EEAB at the dose of 500 mg Kg⁻¹ body weight was not a dose-dependent change and should not be due to the plant extract. Raised urea and non-protein nitrogen level in blood have been observed with impaired renal function (or) in acute renal failure (25). In this study, a significant increase in serum and urine creatinine level at 500 mg Kg⁻¹ body weight of the extract treated group indirectly manifests the non-hazardous nature of EEAB in maintaining the homeostasis of protein and non-protein nitrogen in the body fluids of treated and control groups. Transaminases (AST and ALT) and ALP are good indices of liver and kidney damage, respectively. From the results it is evident that EEAB did not provoke any deleterious effects on the activities of these enzymes in serum, liver and kidney tissues in control and experimental groups of rats.

Thirty days subacute toxicity study of A. baccifera in Wistar rats indicated that the ethanol extract of A. baccifera at the doses of 50, 100, 250 and 500 mg/kg/day respectively, did not produce any significant dose-related changes in hematological, biochemical parameters and histopathology of any internal organs. Therefore, it is concluded that the ethanol extract of A. baccifera at the given doses does not produce any significant toxic effect in rats and considered to be safe when administered orally.

REFERENCES
Lavanya Goodla et al. / Journal of Pharmacy Research 2010, 3(11), 2634-2637


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