

# Pre-Clinical Toxicological Evaluation of *Rostellularia diffusa*: Hematological, Biochemical, and Histopathological Studies

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## ABSTRACT

**Background:** *Rostellularia diffusa* (Willd.) is a traditional herb belongs to acanthaceae family. The whole plants are used as brain tonic in traditional practice. There were no earlier reports on the safety assessment of *Rostellularia diffusa*. **Objective:** the present study was undertaken to assess the safe use of this plant in traditional practice. **Method:** The acute oral toxicity study of hydro alcoholic extract of *Rostellularia diffusa* (AERD) was carried out as per the OECD guidelines 423 and the sub-acute toxicity was carried out at a dose of 150 mg/kg and 300 mg/kg as per OECD 407 guidelines in male and female rats. **Results:** Rats were administered up to 2000 mg/kg as a single dose orally not caused any signs of toxicity or mortality in rats. In sub-acute toxicity study in rats, AERD at two different daily doses of 150 and 300 mg/kg for 14 days did not cause any significant change including the hematological and biochemical parameters. Histopathological examinations showed normal architecture suggesting no morphological disturbances.

**Conclusion:** No deaths or any signs of toxicity was observed after oral administration in acute toxicity study up to a dose of 2000 mg/kg of AERD in rats and up to a dose of 300 mg/kg of AERD in sub-acute toxicity study in rats.

**Key word:** Brain tonic, Toxicity study, *Rostellularia diffusa*, Herbal medicine.

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## INTRODUCTION

*Rostellularia diffusa* (Willd.) (*Justicia diffusa*) is a traditional herb belongs to acanthaceae family. Traditionally the plant recommended as brain tonic to relieve stress related disorders.<sup>1</sup> Recently, in our laboratory anti-stress activity of *Rostellularia diffusa* was evaluated and documented.<sup>1</sup> Ethyl acetate extract of *Rostellularia diffusa* was reported to have 16-Hentriacontanone (22.59%), Hexadecanoic acid (11.23%), Stigmast-5-en-3-ol (6.78%), 9-Octadecenoic acid (6.30%) and many other compounds were identified as low level.<sup>2</sup> As per the literature survey there is no other scientific reports are available regarding pharmacological and phytochemical analysis of any part of this plant.

Plant based medicine play a key role in human health care system of globe. In many developing countries plant based treatment was used in traditional practice.<sup>3,4</sup> Such traditional system of medicine still practice in countries like India, China, Africa etc., and the popularity of herbal medicine in developed countries is due to severe adverse drug effect caused by available synthetic drugs.<sup>4</sup> Most of the medicinal plants were used in common practice because of diverse biological effect of their crude extracts.<sup>5,6</sup> However, recent scientific evidences were documented that some of the plants also possess adverse effect in human and animals.<sup>7,8,9</sup> So, study of toxicological effect of any plant extracts before preclinical and clinical pharmacological evaluation is more important to assess its safe use.<sup>4,10</sup>

Hence, the present study was designed to evaluate the safety profile of *Rostellularia diffusa* to provide the scientific information to develop potential phytomedicine.

## MATERIALS AND METHODS

### Plant material and preparation of extract

The whole plant of *Rostellularia diffusa* was collected (August, 2012) from Tirumala forest, Tirupati, A.P., India and was authenticated by Dr. K. Madhava Chetty, Professor S. V. University, Tirupati. The shade dried plant materials were coarsely powdered and defatted with petroleum

ether (60-80°C) using a soxhlet extractor and further extracted with 70% ethanol for 72 h to obtain hydro-alcoholic extract (AERD).

### Experimental animals

Albino Wistar rats of either sex with the body weight 180-200 g were selected for the study. The animals were housed in clean polypropylene cages under hygienic and standard environmental conditions at 22±2°C, 12:12 h light: dark cycle and 60±5 % RH with free access to standard laboratory food and water *ad libitum* (SaiDurga Feeds and Foods, Bangalore). The experimental protocol was approved by the institutional animal ethics committee (1220/PO/Re/S/08/CPCSEA).

### Acute toxicity study

Acute toxicity test was performed as per the protocol described in the OECD guidelines 423.<sup>11</sup> Briefly, female Wistar albino rats with the body weight of 180-200 g were administered with various doses of (5, 50, 300 and 2000 mg/kg) AERD in distilled water through oral gavage. All the rats were observed for first 30 min individually, then continuous observation was made for next 4 h to observe any toxicity signs like change in respiration, mucous membrane, skin, eyes, fur and behavioral pattern. After 24 h of extract administration the animal were observed for mortality.

### Sub-acute toxicity study

Sub-acute toxicity test was performed as per the protocol described in the OECD guidelines 407.<sup>12</sup> 18 Wistar albino rats were divided in to three groups each containing three male and three female rats. Group I received distilled water and served as control, group II and III rats were received AERD (150 and 300 mg/kg respectively) in distilled water through oral gavage for 14 days. Food and water consumption of the rats was observed during the treatment period and the body weight of the rats were monitored to observe any changes during the treatment period.

After 24 h of last treatment (15<sup>th</sup> day) all the rats were anesthetized with ether anesthesia. Blood was withdrawn from retro-orbital sinus route for the analysis of hematological parameters like red blood cell (RBCs), white blood cell (WBCs), hemoglobin (Hb) platelet count, mean corpuscular volume (MCV), packed cell volume (PCV), Mean Corpuscular Hemoglobin Concentration (MCHC) etc., Serum was separated from blood for the analysis of biochemical parameters like total bilirubin, liver transaminases (AST and ALT), alkaline phosphatase (ALP), total protein, urea, and creatinine by using enzyme kits (Transasia Bio-Medicals Limited, Solan). Heart, liver and kidney were isolated and stored in 10% buffered formalin were embedded in paraffin; sections were cut at 5 µm and stained with hematoxylin and eosin. These sections were then examined under a light microscope for histological changes.

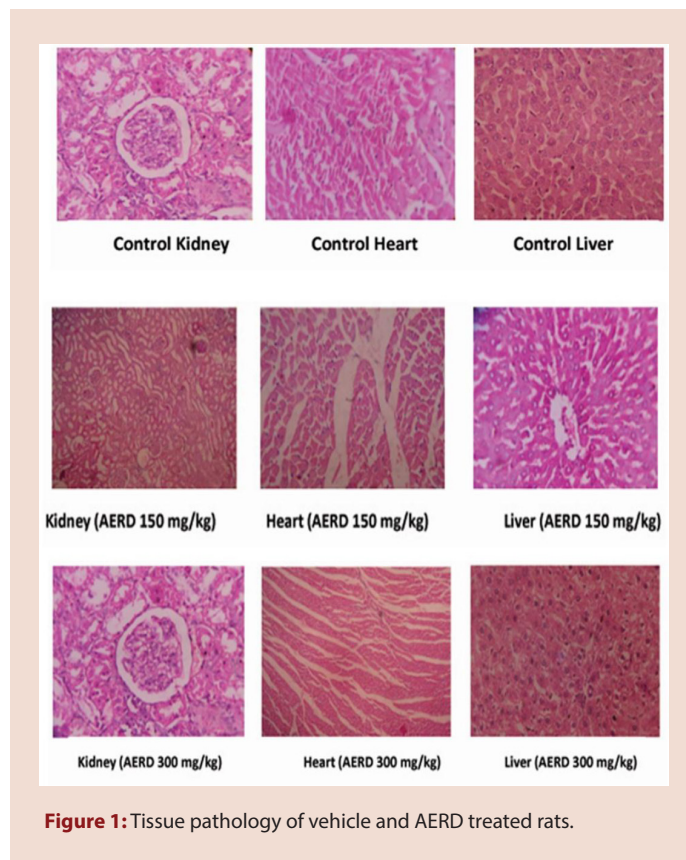
**Statistical analysis**

All values are expressed as mean ± SEM. Statistical analysis carried out by using One-way ANOVA with Dunnett’s post-test was performed using GraphPad Prism version 5.00 for Windows, GraphPad Software, San Diego California USA, www.graphpad.com. Significance is set at p < 0.05.

**RESULTS**

AERD did not produce any sign of toxicity and also not produced any mortality up to the dose of 2000 mg/kg BW after 24 h single dose toxicity study. In 14 days repeated dose studies, AERD was administered to the rats in two different dose (150 and 300 mg/kg). The AERD did not produced any sign of toxicity like change in respiration, mucous membrane, skin, eyes, fur and behavioral pattern. There is no sign of seizures, tremors, salivation, depression and diarrhea also observed in AERD treated group rats. The body weight of the rats also not altered during 14 day of extract treatment.

There is no significant changes in the hematological parameters produced by the AERD in both the dose when compared to control group. The results were presented in Table 1. There is no significant changes observed in the serum level of total bilirubin, AST, ALT, ALP, total protein, urea and creatinine in AERD treated groups rats compared to control group rats. The results were present in Table 2. There is no significant changes in the histological features of heart, liver and kidney. The AERD treated rat heart, liver and kidney shows normal myocardium, normal hepatocytes



**Figure 1:** Tissue pathology of vehicle and AERD treated rats.

and normal cortex and medulla respectively in both the dose. There is no significant inflammation, fatty change and fibrosis observed in the AERD treated group rats. The results were present in Figure 1.

**DISCUSSION**

In this present research we have reported the safety profile of hydro alcoholic extract of plant *Rostellularia diffusa* (AERD) as there were no earlier reports on the safety assessment of *Rostellularia diffusa*.

**Table 1: Hematological studies on rats after administration of AERD.**

Treatment groups	Hb g%	Total WBC x10 <sup>9</sup> /L cells/ Cu mm	Total RBC x10 <sup>12</sup> /L Mil/ Cu mm	PCV %	MCV (fl)	MCH (PG)	MCHC g/dl	Platelet x10 <sup>9</sup> /L cells Cu mm
G1	10.8±0.26	4.9±0.8	6.9±0.3	42.6±2.1	60±3.7	15.5±1.2	25.8±0.4	499±16.2
G2	11.6±0.41	6.2±0.4	8.4±0.5	48.4±1.4	56±4.2	15.8±1.4	24.6±0.6	719±22.4
G3	14.2±0.38	6.9±0.7	9.6±0.1	43.2±0.98	61±1.8	16±1.3	28±0.8	648±14.3

G1-Control; G2-AERD 150 mg/kg; G3-AERD 300 mg/kg

All values are expressed as mean±SEM. ONEWAY ANOVA followed by Dunnet’s tests.

**Table 2: Biochemical studies on rats after administration of AERD.**

Treatment groups	TB mg/dl	AST U/L	ALT U/L	ALP U/L	TP mg/dl	Urea mg/dl	Creatinine mg/dl
G1	0.1±0.01	53±2.8	54±1.7	79±5.4	5.2±0.4	34±0.8	0.7±0.02
G2	0.3±0.02	76±5.1	26±1.8	70±6.9	5.6±1.2	61±2.8	1.3±0.04
G3	0.2±0.01	47±3.4	77±1.5	77±2.8	5.8±3.6	28±1.4	0.8±0.01

G1-Control; G2-AERD 150 mg/kg; G3-AERD 300 mg/kg

All values are expressed as mean±SEM. ONEWAY ANOVA followed by Dunnet’s tests.

Toxicity screening is a preliminary step in the pharmacological evaluation of medicinal plants. The acute toxicity study may provide preliminary information to find out the LD<sub>50</sub> of the plant extracts to fix the dose for further pharmacological evaluation. Subacute toxicity evaluation may provide information on dose dependent toxicity, organ toxicity and other observable adverse effect that may affect the normal life of experimental animals.<sup>4</sup>

In the present study rats were treated with 150 and 300 mg/kg dose of AERD for 14 days did not produce any mortality, change in water and food intake. Change in body weight and internal organ weight is first sign of toxicity that indicate the general health status of the animals exposed by any toxic substances.<sup>13,14</sup> In the present study there is no significant changes in body weight and organ weight of AERD treated rats indicate there is no toxicity sign caused by this extract.

The preliminary target of many toxic compounds were hematopoietic system/bone marrow that provide essential index of physiological and pathological status of animal and human. Evaluation of hematological parameters during animal toxicity study may provide higher predictive value for human toxicity.<sup>6,15,16</sup> Rats exposed with AERD did not shows any significant changes in hematological parameters.

Serum biochemical evaluation is an important factors to predict the effect of toxic substance in the specific organ system like liver and kidney. Analysis of serum level of liver function and kidney function parameters are good indicators of organ functions.<sup>17</sup> In addition, AST found in the serum is of both mitochondrial and cytoplasmic origin and any increase can be taken as a principal sign of cell injury that leads to the leakage of the enzymes into the serum.<sup>18</sup> Changes in serum level of urea, uric acid and creatinine is an indicators of kidney damage also.<sup>19</sup> In the present study, we have observed that there is no significant changes in the serum level of AST, ALT, ALP, total bilirubin, total protein, urea and creatinine shows AERD treatment did not produce any damage to the liver and kidney. These observations were further confirmed by the histological assessment of the liver, kidney and heart showed in Figure 1.

## CONCLUSION

Based on the above findings, it has been concluded that the hydroalcoholic extract of plant *Rostellularia diffusa* seems to be nontoxic in animal model. Further, chronic toxicity studies want to be conduct to find out the teratogenic and carcinogenic safety profile of this plant.

## CONFLICT OF INTEREST

The authors have no conflict of interest regarding this study.

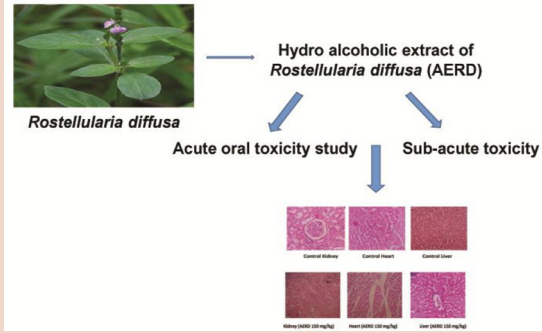
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### PICTORIAL ABSTRACT



### SUMMARY

- Safety evaluation of plant extracts is more important before using these extracts for treatment. In the present study *Rostellularia diffusa* was evaluated for acute and sub acute toxicity study by using experimental animal model. The aqueous extract of *Rostellularia diffusa* does not shows any signs of toxicity in experimental rats after 14 days treatments, which revealed that *Rostellularia diffusa* will be used for further pre-clinical and clinical evaluation for animals and human.

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