ACUTE AND SUB – ACUTE TOXICITY STUDIES OF
Hemidesmus indicus var. pubescens R.Br.

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ABSTRACT: Toxicity studies of crude aqueous ethanolic extract of the roots of Hemidesmus indicus var. pubescens R. Br. Was carried out in two growth phases. The drug was administered orally and intra-peritonealy. It was found to possess nonspecific changes in liver. The results are discussed in detail.

INTRODUCTION

Hemidesmus indicus, a member of the Asclepiadaceae family grows wild in India. It is widely used for biliousness, blood diseases, diarrhea, respiratory disorders, skin diseases, syphilis, fever, bronchitis, asthma, eye diseases, epileptic fits in children, kidney and urinary disorders, loss of appetite, burning sensation and rheumatism in traditional systems of medicine. β-sitosterol, flavonoid glycoside, tannins and saponins have been detected in the root of this plant. Though, var. indicus is commonly used, var. pubescence is also used in some instances. Ethanolic (50%) extract exhibited cytoprotective effect on gastric mucosa. The toxicity of the root extract of this plant has not been investigated on animals. Results of the study on the root extract are reported here. To have a better insight plants were collected in two growth phases and studied.

MATERIALS AND METHODS

Root were collected from Vallanad, Tirunelveli district, Tamilnadu, India and identified by the coauthor Dr. Jegadeesan. A voucher specimen is deposited in the department herbarium for future reference (TUH No. 187). Two seasonal samples were collected i.e., flowering (November) [FP] and vegetative [VP] season (May) with a gap of six months between sample collections. The roots were cleaned, shade dried and disintegrated into coarse powder. Cold extract (aqueous ethanolic) was extracted by soxlet apparatus and subjected to toxicity studies. The extract (Yield: 18.34%) was dark brown in colour, sticky in nature and gave positive results for alkaloids, tannins and phenols, saponins, and negative for gums and mucilage.

Wistar strain albino rats of either sex weighting between 180 to 220g and Swiss albino mice of either sex weighting between 20 to 25g were used. These animals were taken form the in – bread group, which is being maintained Tamil University animals house. The animals were fed with standard pelleted diet supplied by Lipton & Co. Ltd., Bangalore. Feed and water were made available to animals ad libitum. Studies were carried according to the recommendations of animals ethical committee.
Gross behavioural and acute oral toxicity studies \(^6\) (LD\(_{50}\)) were carried out in Swiss albino mice. They were grouped into 6 mice per group and administered at different dose levels (1000, 2000, 3000 & 4000 mg/kg b.w) dissolved in distilled water and administered once orally for overnight fasted animals. The group receiving water (1ml/kg) was kept as control. The animals were subjected to primary screening studies and observed at \(\frac{1}{2}\), 1, 2 and 4 hours and finally overnight mortality was noted. Behaviour as well as any other toxic symptoms was observed for 24, 48 and 72 hours and animals were kept under observation up to 14 days after drug administration to find out delayed mortality if any.

Acute toxicity studies \(^6\) (LD\(_{50}\)) by intra-peritoneal route was carried out in Swiss albino mice. They were grouped into 12 mice per group. Extracts at different dose levels (250, 500, 750, 1000, 1250, 1500, 1750, 2000, 3000 & 4000 mg/kg) was administered intra-peritonealy. They were screened for mortality within 2 hours and kept under observation for 7 days for delayed toxicity, if any.

Sub-acute toxicity studies were carried out in wistar strain of albino rats \(^6\), \(^9\). They were divided into 6 rats per group. Extract dissolved in water was given orally at a dosage of 150, 30, 450 mg/kg b.w. Control group received water. Study was continued up to 28 days. On 29\(^{th}\) day, animals were sacrificed and vital organs were subjected to haematological and biochemical analysis.

Haematological parameters like total R.B.C total W, B.C., differential count, haemoglobin concentration (Hb) and erythrocyte sedimentation rate (ESR) were estimated\(^{10}\). Biochemical parameters were estimated in auto-analyser using Ecoline diagnostic kits for blood glucose\(^{11}\), blood urea\(^{12}\), serum cholesterol\(^{13}\), total proteins\(^{14}\), alkaline phosphatase, aspartate aminotransferase (ASAT), alanine aminotransferase (ASAT)\(^{15}\), serum bilirubin\(^{16}\) and serum creatinin\(^{17}\). Once blood samples were collected, the abdomen was explored and liver, kidney, spleen, stomach, heart and brain were excised quickly and fixed in 10% buffered neutral formalin, the tissues are mounted by peterfi’s double embedding technique Paraffin sections of 5-10 \(\beta\) size were prepared, stained with H & E \(^{18}\). The histopathological interpretations were made.

Data were expressed as means ± standard error of the means (S.E.M) and statistical analysis was carried out using student’s t-test\(^{8}\). P-values less than 0.05 were considered significant.

**RESULTS AND DISCUSSION**

H. Indicus var. pubescens administered by gastric intubation, at 1000 and 2000 mg/kg dose levels, showed stimulative effect. But depressive effect like sedation and analgesia was noticed above 3000 mg/kg dose level. At 400 mg/kg dose level there were abnormal secretion from the mouth and the animals showed hypnosis. There was no marked difference in gross behavioural and acute toxicity study between seasonal samples. Since all animals were alive up to 4000 mg, LD\(_{50}\) could not be ascertained for oral route.

Acute toxicity for VP by intra-peritoneal routes, 1, 3, 5, 7 and 9 animals died at 500, 750, 1000, 1250, and 1500 mg respectively and all were dead at 1750 mg dosage LD\(_{50}\) was found to be 848.3 ± 8.93 mg/kg. FP at 500, 750, 1000, 1250 and 1500 mg levels, 4, 6, 7 and 8 animals died respectively and at 1750 mg all were dead. LD\(_{50}\) was calculated as 813.7 ± 8.57 mg/kg.
Extracts were administered at three dose levels for sub-acute toxicity studies in albino rats. 150, 300 and 450 mg dose levels were selected for sub-acute toxicity studies, which was pharmacologically active. The dose was calculated form the gross behavioural and acute toxicity studies where in at 3000 mg dose level produced drowsiness. Change in body weight of the animals after drug administration at different dose is given in figure 1. The weight of the animals monitored weekly was increasing steadily. There was no abnormal increases or decrease in body weight at different dose levels, food and water intake when compared to control.

Haematological parameters are given in table 1. Significant increase was noted in total R.B.C., treated with VP at 450 mg. Except ASAT and ALAT, other biochemical parameters didn’t show any significant change compared to control (Table 2).

Histopathology of liver showed dilated and congested central vein (5/6) at 450 mg. Hepatocytes are arranged in cords with irregular sinusoidal dilation (4/6) above 300mg. portal tract appears normal. The changes are nonspecific hepatomegaly.

Splenic section showed irregular dilatation of sinusoids with congested red pulp (4/6) from 3000 mg onwards. The white pulp appears prominent at 450 mg/kg dosage and appears prominent in focal areas with thick pencillary arterioles (2/6) suggestive of nonspecific congestive spleen. Kidney showed normal glomeruli with potent capillary loops and widened bowman’s space. Tubules are dilated with focal areas of cloudy swelling at 450 mg Interstitium shows mononuclear cell infiltration (4/6) in all doses suggestive of ischemic changes of the tubules. Stomach section shows normal foveolar mucosa. Gastric glands appear normal and lined by cuboidal cells, the lamina propria is compact in all doses. Heart section shows myocardial component showing focal intra-myofibril haematoma and degeneration (5/6) in all doses suggesting organized haematoma. Cerebral tissue shows edema with widely separated neurons. Cerebrum and cerebellum are evident with normal neuronal and glial cells. Cerebellum shows normal columnar architecture.

Even though gross observation reveals nonspecific hepatomegaly, other parameters are not pronounced. The changes are nonspecific which can produce reversible changes. 150 and 300 mg dose was found to be safe, when compared to higher dose in sub-acute toxicity. Earlier works on an allied species var. indicus hepatotoxicity has been reported 19, 20 with hydrophic degeneration and focal hepatocellular necrosis, where in the dose was high. However, in the present study no such degenerative changes were observed. So, care should be taken for patients with liver diseases.

Difference between two different periods might be due to difference in inherent variation of biological active compounds present in the plant the difference in quantitative and qualitative change in biological active compounds is due to genetic, climatic and developmental phase of the medicinal plants. Further isolation and characterization studies of toxic principles would throw more light on the nature and mechanism of action of toxicity in var pubescens, which is in progress.

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REFERENCES

1. Nadkarni, A.N., Indian Materia Medica, vol I. Popular Book Depot, Bombay, 619, (1989).

2. Anoop Austin, Jegadeesan, M. and Shanthi, G., Pharmacognostical studies on roots of varieties of Hemidesmus indicus R.Br., J Swamy Bot. Cl., 19,37-43 (2002).

3. Anoop Austin and Jegadeesan, M., Anti-ulcer potential of Hemidesmus indicus var pubescens R.Br., Hamdard Medicus, 46,3,(2003). (Accepted)

4. Gamble, J.S. Flora of the presidency of Madres, Vol LL Adlard & Son Ltd., Hard street, W.C London, 824, (1985).

5. Anonymous, The Indian Pharmacopoeia, Government of India, Ministry of Health and Family Welfare, Published by the Controller of publications, New Delhi, 57,(1966).

6. Turner, R.A., Screening methods on pharmacology, Vol I, 2nd Academic press Inc Ltd., London, 1187, (1965).

7. Miller, L.C. and Trainter, M.L., Behavioural toxicity studies, Proc. Soc. Exptl. Biol. Med., 57,261, (1944).

8. Ghosh, M.N., Statistical analysis in fundamentals of experimental pharmacology. II edn, Scientific book agency, Calcutta, 78, (1984).

9. Biswas, N.R., Sen, Singh, S., Gopal, N., Pandey, R.M. and Giri, D., Sub-acute toxicity study of a polyherbal drug (Prostina® ) in rats, Indian J. Pharmacol., 30,239, (1998).

10. Ghai, C.L., A textbook of practical physiology, Jaypee brother’s medical publishers (P) Ltd., New Delhi, 87, (1993).

11. Constantino, N.V. and Kabat, H.F., Drug induced modification of laboratory test values, Amer. J. Hosp. Pharm., 30,24, (1973).

12. Kassirer, J.P., Photometric analysis of urea, N. Eng. J. Med., 285, 385, (1971).

13. Richmond, J.P. Photometric analysis of urea, N. Eng, J Med., 285,385, (1971).

14. Josephson, G and Gyllensward, C., Chemical analysis of albumin and total proteins, Scand.J. Clin Lab Invest., 9. 29, (1957)

15. Engelhardi, A. and Norges, A., Biochemical analysis of aspartate aminotransferase, Artl. Lab., 16,42, (1970).
16. Schellong, G. and Wende, U., Analysis of total bilurubin, Arch. Kinderheilk., 162, 126, (1960)

17. Ullmann, R. and Bontiz K., Analytical methods of serum creatinine, Med. Labor., 29,137, (1976).

18. Bancroft, J.D. and Stevens, A., Theory and practice of histological techniques. Churchill Livingston, New York, 78, (1996).

19. Arseculeratne, S.N., Gunatilaka, A.A.L. and Panabokke, R.G., Studied on medicinal plants of srilanka. Part 124: Toxicity of some traditional medicinal herbs, J. Ethnopharmacol., 13, 323, (1985).

20. Atal, C.K., Sharma, M.L., Kaul, A. and Khajuria, A., Immunomodulating agents of plant origin, Part I: preliminary screening. J. Ethnopharmacol., 18, 133, (1986).

21. Trease, G.H. and Evans, W.C., Text book of Pharmacognosy, 12th edn., Bailliere Tindall, London, 125, (1983).
Fig 1: Weight of animals in weekly intervals in sub-acute toxicity study

Ctr - Control; V - vegetative period; F - flowing period; 150, 300 & 450 are respective dosages.
Table 1: Haematological parameters – sub – acute toxicity of H. indicus var. pubescens

| Particulars                  | Control | Vegetative period | Flowering period |
|------------------------------|---------|-------------------|------------------|
|                              | (mg.kg) | Dose (mg/kg)      |                  |
|                              | 150     | 300               | 450              |
|                              | 150     | 300               | 450              |
| Total R.B.C. (Millions/mm³)  | 6.85 ± 0.28 | 7.73 ± 0.29 | 7.23 ± 0.37 | 7.48 ± 0.29 | 7.85 ± 0.24 | 7.38 ± 0.36 | 8.15a ± 0.38 |
| Total W.B.C. (Thousands/mm³) | 10.28 ± 0.64 | 9.62 ± 0.23 | 9.55 ± 0.28 | 9.73 ± 0.15 | 9.12 ± 0.38 | 9.52 ± 0.28 | 9.47 ± 0.40 |
| Neutrophils (%)              | 29.50 ± 0.76 | 29.00 ± 0.58 | 27.50 ± 2.11 | 28.33 ± 0.88 | 28.67 ± 0.67 | 27.83 ± 1.66 | 27.33 ± 0.76 |
| Lymphocytes (%)              | 63.00 ± 1.00 | 65.17 ± 1.08 | 66.00 ± 1.81 | 65.33 ± 1.15 | 65.50 ± 0.85 | 55.67 ± 9.80 | 66.00 ± 1.32 |
| Eosinophils (%)              | 3.50 ± 0.43 | 2.50 ± 0.43 | 3.00 ± 0.52 | 3.00 ± 0.45 | 2.33 ± 0.33 | 2.67 ± 0.42 | 3.17 ± 0.60 |
| Monocytes (%)                | 2.83 ± 0.60 | 1.67 ± 0.33 | 2.17 ± 0.48 | 2.00 ± 0.26 | 2.50 ± 0.22 | 2.50 ± 0.49 | 2.67 ± 0.42 |
| Basophils (%)                | 1.17 ± 0.31 | 1.67 ± 0.21 | 1.33 ± 0.21 | 1.33 ± 0.21 | 1.00 ± 0.26 | 0.17 ± 0.17 | 0.83 ± 0.31 |
| Haemoglobin (g%)             | 14.00 ± 0.50 | 13.83 ± 0.44 | 14.08 ± 0.24 | 13.92 ± 0.35 | 14.58 ± 0.27 | 15.03 ± 0.31 | 14.45 ± 0.33 |
| E.S.R. (mm/1/2hr)            | 7.67 ± 0.49 | 8.00 ± 0.73 | 9.00 ± 0.58 | 8.67 ± 0.88 | 9.83 ± 0.60 | 9.67 ± 0.56 | 10.17 ± 0.60 |
| E.S.R. (mm/1hr)              | 16.67 ± 0.60 | 15.67 ± 1.33 | 17.50 ± 1.18 | 16.83 ± 1.62 | 19.17 ± 1.22 | 18.50 ± 0.99 | 20.17 ± 1.22 |

All the values are Mean ± S.E.M. of 6 samples. *P<0.01 compared with control group
Table 2: Biochemical parameters – sub –acute for H. indicus var. pubescens

| Particulars                  | Control | Vegetative period Dose (mg.kg) | Flowering period Dose (mg/kg) |
|-----------------------------|---------|--------------------------------|-----------------------------|
|                             |         | 150   | 300   | 450   | 150   | 300  | 450   |
| Blood sugar (mg/dl)         | 75.67±  | 66.00 | 69.00 | 70.00 | 77.67± | 76.17± | 79.00± |
|                             | 5.04    | ±3.57 | ±4.20 | ±4.88 | 2.50    | 3.22  | 3.46  |
| Blood urea (mg/dl)          | 25.17±  | 22.67 | 26.00 | 23.50 | 26.50± | 27.17± | 27.17± |
|                             | 1.60    | ±1.26 | ±1.32 | ±1.91 | 1.38    | 1.05  | 1.08  |
| Serum cholesterol (mg/dl)   | 75.50±  | 70.17 | 74.60 | 75.17 | 76.83± | 7383± | 75.00± |
|                             | 5.35    | ±5.23 | ±5.34 | ±5.72 | 3.23    | 3.10  | 2.10  |
| Total proteins (g%)         | 5.38±   | 5.22  | 5.52  | 5.47  | 5.60±   | 5.63± | 5.35± |
|                             | 0.26    | ±0.31 | ±0.35 | ±0.35 | 0.31    | 0.31  | 0.32  |
| Alkaline phosphatase (K.A. units/ml) | 15.17± | 13.00 | 16.00 | 15.67 | 13.67± | 14.17± | 13.33± |
|                             | 1.38    | ±0.37 | ±0.37 | ±0.61 | 0.88    | 0.48  | 0.84  |
| ASAT (Units/ml)             | 1.83±   | 10.33 | 11.33 | 11.53a| 11.83± | 12.83a| 13.17a|
|                             | 0.60    | ±0.49 | ±0.49 | ±0.48 | 0.48    | 0.48  | 0.87  |
| ALAT (Units/ml)             | 16.00±  | 15.83 | 17.83a| 18.00a| 17.67a±| 19.00a| 18.83±|
|                             | 0.58    | ±0.48 | ±0.48 | ±0.37 | 0.95    | 0.58  | 0.75  |
| Serum bilirubin (mg/dl)     | 0.62±   | 0.52  | 0.53  | 0.57  | 0.60±   | 0.65  | 0.60± |
|                             | 0.01    | ±0.006| ±0.004| ±0.003| 0.005   | 0.004 | 0.004 |
| Serum creatinine (mg/dl)    | 0.72±   | 0.55  | 0.63  | 0.58  | 0.67±   | 0.75± | 0.73± |
|                             | 0.01    | ±0.004| ±0.003| ±0.004| 0.007   | 0.004 | 0.004 |

All the values are Mean ± S.E.M. *P<0.01 compared with control group