

Acute Systemic Toxicity Of Four Mimosaceous Plants Leaves In Mice

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ABSTRACT

Nature provides drugs in the form of medicinal plants to cure all ailments of mankind with fewer side effects. These medicinal plants can cause some serious damaging effects on the vital organs of the body too; there is a need of toxicity studies to provide their safe use both in human and animals. The present study reports the acute systemic toxicity of methanolic extracts of leaves of *Adenanthera pavonina* L., *Peltophorum roxburghii* Degener., *Prosopis cineraria* Druce and *Prosopis juliflora* DC., (Mimosaceae) in swiss albino mice to explore their suitable doses for pharmacological screening. Observations were made immediately and after 1/2, 1, 4, 24 and 48hours of intraperitoneal drug administration. *Adenanthera pavonina* extract at the dose of 150 mg/kg body weight is considered to be safe. Whereas remaining extracts of investigated plants are relatively safe at the dose of 100 mg/kg body weight.

Keywords - Acute systematic toxicity, *Adenanthera pavonina*, Methanol extract, Mimosaceae, *Peltophorum roxburghii*, *Prosopis cineraria*, *Prosopis juliflora*, Swiss albino mice.

1. INTRODUCTION

Herbal drugs are playing an important role in health care all over the world, especially in developing countries because they are being cheap, locally available and consider being safe. Medicinal plants behave as authentic medicines because of bioactive chemical compounds. The natural origin may not signify that they are safe. Limited evidence suggests that herbal drugs associated adverse effects are less. Determination of efficacy and safety of herbal remedies are necessary because of their self medication[1]. Although there is a limited data available about the pharmacology and toxicology for the most commonly used herbal remedies[2]. Therefore, efforts to elucidate health benefits and risks of herbal medicines should be intensified.

Adenanthera pavonina L., *Peltophorum roxburghii* Degener., *Prosopis cineraria* Druce and *Prosopis juliflora* DC are medicinal plants of Pakistan and India belong to the family Mimosaceae[3]. *Adenanthera pavonina* L., leaves decoction is used in blood pressure, cholera, general paralysis[4], gastric complaints (diarrhoea and dysentery)[5], gout and rheumatism[6]. Chemical literature survey of *Adenanthera pavonina* revealed the presence of triterpenes[7], flavonoids[8] and fatty acids[9]. Acute oral toxicity study of the ethanolic extract of leaves revealed 5g/kg as nontoxic dose[10]. The bark of *Peltophorum roxburghii* Degener is used in dysentery, toothache, pains and sores[11]. The hexane and methanol extracts of plant showed anti microbial activity[12]. *Prosopis cineraria* Druce leaves are used in cataract, dyspepsia, earache and toothache[13]. Literature survey of *P. cineraria* revealed the presence of alkaloids[14], fatty acids[15], glycosides and sterols[16]. The acute and sub acute oral toxicity of the methanol extract of leaves suggest 2g/kg as safe dose[17]. Leaf preparations of *Prosopis juliflora* DC are used in dyspepsia, earache, mouth infections, scorpion stings, snake bites[18], bronchitis, dermatitis, parasitic infections and urinary disorders[19]. The leaves of *Prosopis juliflora* contained amino acids, flavonoids[20], alkaloids and diketones[21]. The flavonol glycosides and hydroxycinnamic acid from *Prosopis juliflora* pollen are reported to possess antioxidant with high free radical scavenging activity [22].

The purpose of the present study is to investigate acute systemic toxicity of the methanol extracts (leaves) of four Mimosaceous plants (*Adenanthera pavonina* L., *Peltophorum roxburghii* Degener., *Prosopis cineraria* Druce and *Prosopis juliflora* DC) in swiss albino mice in order to establish their safe doses for systemic use.

2. MATERIALS AND METHODS

- 2.1. Plant material: Leaves of *Adenanthera pavonina* L., *Peltophorum roxburghii* Degener., *Prosopis cineraria* Druce , and *Prosopis juliflora* DC were collected in summer 2011 from University of Karachi and deposited in the herbarium of department for reference.
- 2.2. Extraction: Leaves of *Adenanthera pavonina* L., *Peltophorum roxburghii* Degener., *Prosopis cineraria* Druce , and *Prosopis juliflora* DC., were dried under shade and soaked in methanol for a week. The extracts were filtered separately then concentrated using rotary evaporator at 40°C.

- 2.3. **Animals:** Swiss albino mice of both sexes (17-23g) were purchased from Aga Khan University and Hospital animal house. All animals were kept under laboratory conditions of room temperature with 12/12h light and dark cycles. They were allowed to free access to food and water *ad libitum*. The animals were divided into drug treated as test group and DMSO treated as control groups of six mice ($n = 6$) per cage for a minimum of three days prior to toxicity assessment. The groups of animals were transferred in different cages and marked with their identification.
- 2.4. **Materials:** Disposable syringes 1 ml, 0.2 μ membrane filters, filtration assembly, methanol, DMSO.
- 2.5. **Preparation of Test Sample and Blank / Control Solution:** The methanol extracts (100,150 and 200mg/kg body weight) of all investigated plants were solubilized in 0.5ml DMSO. Before injection the solution was passed through 0.2 μ membrane filter. Each extract was agitated vigorously prior to withdrawal of injection doses to ensure even distribution of the extract. These extracts were administered intraperitoneally to the test group whereas control receiving only vehicle (DMSO).
- 2.6. **Acute systemic toxicity studies:** The acute systemic toxicity test of the methanol extract of leaves were carried out by the method described by Lorke [23] and modified by Vongtau *et al* [24]. Mice were treated intraperitoneally (i.p.) at the doses of 100,150 and 200mg/Kg and were observed for biological reactivity continuously for the first 4 hour, then the number of survivors was noted after 24 and 48 hours of dosing. The protocol of the study is given in Table 1. The LD₅₀ was calculated by using following formula [25].

$$LD_{50} = \sqrt{\text{Highest non-lethal dose} \times \text{Least lethal dose}}$$

Table 1: Study Protocol of Acute Systemic Toxicity of Mimosaceous plants.

Name of study	Acute systematic toxicity of Mimosaceous plants
Test materials	Methanolic extract of <i>Adenanthera pavonina</i> , <i>Peltophorum roxburghii</i> , <i>Prosopis cineraria</i> , <i>Prosopis juliflora</i> , leaves.
Details of animals used	Healthy swiss albino mice (03 Female and 03 Male)
Route of test drug administration	Intraperitoneal administration with the help of disposable syringes
Dose of drug administration	0.5ml / 20gm of the mice body weight intraperitoneally
Study duration	Acclimatization for 3 days and 48 hours study period
Parameters observed	Mortality, sign of illness, injury, pain distress, allergic reactions, changes of outer appearance, difficulty in breathing, paralytic effect on hind limbs, behavioral alterations (i.e., ataxia, hypoactivity, hyperactivity) and sedation

3. RESULTS AND DISCUSSION

After the intraperitoneal administration of 100 mg/kg dose of *Adenanthera pavonina* L., *Peltophorum roxburghii* Degener., *Prosopis cineraria* Druce, and *Prosopis juliflora* DC., no adverse reactions were seen for 48 hours so, all studied extracts at the same dose considered to be safe as they give normal activity. Where as 150 mg/kg i.p. considered to be safe only for the extract of *Adenanthera pavonina* and remaining three plant extracts are being considered toxic as they cause 100% mortality of mice till 48 hours. The 200 mg/kg i.p. of all studied extracts considers being toxic (Table 2). The final median lethal dose (LD₅₀) value was calculated by recorded 0 and 100% survival as the square root of the product of the lowest lethal dose and highest non-lethal dose[25]. The calculated LD₅₀ for *Adenanthera pavonina* was to be 141.42 mg/kg body weight where as for *Peltophorum roxburghii* Degener., *Prosopis cineraria* Druce, and *Prosopis juliflora* DC., was. 122.47 mg/kg body weight.

Table 2: Acute Systemic Toxicity of the leaves extracts of Mimosaceous plants.

TREATMENT	DOSES (mg/kg body weight)	OBSERVATIONS						MORTALIY %
		INJECTION IMMEDIATELY	AFTER					
			½ hr	1hr	4hrs	24hrs	48hrs	
DMSO (Control)	-----	Normal activity	Normal activity	Normal activity	Normal activity	Normal activity	Normal activity	-----
<i>Adenanthera pavonina</i>	100	Normal activity	Normal activity	Normal activity	Normal activity	Normal activity	Normal activity	0
	150	Normal activity	Normal activity	Normal activity	Normal activity	Normal activity	Normal activity	0
	200	Breathing problem	Breathing problem	Paralytic effect	No movement	Died	-----	100
<i>Peltophorum roxburghii</i>	100	Normal activity	Normal activity	Normal activity	Normal activity	Normal activity	Normal activity	0
	150	Lose of senses	Paralytic effect on hind limbs	Paralytic effect on hind limbs	03 mice were died while remaining 03 were survived	Died	-----	100
	200	Breathing problem	Breathing problem	Paralytic effect with hair erection	Died	----	----	100
<i>Prosopis cineraria</i>	100	Normal activity	Normal activity	Normal activity	Normal activity	Normal activity	Normal activity	0
	150	Lose of senses	Paralytic effect on hind limbs	Paralytic effect on hind limbs	03 mice were died while remaining 03 were survived	Died	-----	100
	200	Breathing problem	Breathing problem	Paralytic effect with hair erection	Died	----	----	100
<i>Prosopis juliflora</i>	100	Normal activity	Normal activity	Normal activity	Normal activity	Normal activity	Normal activity	0
	150	Normal activity	Paralytic effect on hind limbs	Paralytic effect on hind limbs	03 mice were died while remaining 03 were survived	Died	-----	100
	200	Breathing problem	Breathing problem	Paralytic effect with hair erection	Died	----	----	100

Plants and their derivatives played a key role in world health and have long been known to possess biological activity[26]. The importance of biological, chemical and pharmacological evaluations of plants derived agents used in the treatment of human ailments has been increasingly recognized in the last decades[27]. The data of the acute, subchronic and chronic toxicity studies (oral and systemic) on medicinal plants or preparations derived from them should be obtained in order to increase the confidence in its safety to human, particularly for use in the development of pharmaceuticals. The calculated LD₅₀ values for investigated Mimosaceous plants may suggest the suitable and safe dose for biological studies using systemic route.

4. CONCLUSION

It can be concluded that the methanol extracts of investigated Mimosaceous plants is relatively safe at 100 mg/kg body weight intraperitoneally. Further toxicity studies using different animal species recommended to elucidate the exact mechanism and loci of the fatal action of the extracts are necessary.

REFERENCES

1. L. Rodriguez-Fragoso, J. Reyes-Esparza, S.W. Burchiel, D. Herrera-Ruiz and E. Torres, Risks and benefits of commonly used herbal medicines in Mexico, *Toxicology and Applied Pharmacology*, 227(1), 2008, 125-135.
2. A. Gurib-Fakim, Medicinal plants: traditions of yesterday and drugs of tomorrow, *Molecular Aspects of Medicine*, 27, 2006, 1-93.
3. S.I. Ali. *Flora of West Pakistan*, No.36 (Eds., E. Nasir and S.I. Ali), (Department of Botany: University of Karachi: Karachi, 1972).
4. C. Asima and C.P. Satyesh, editors. *The treatise on Indian medicinal plants. Vol.2*, (New Delhi: Publications and Information Directorate, 1992).
5. D.K. Holdsworth. *Medicinal Plants of Papua New Guinea*, South Pacific Commission Technical paper No.175, (Noumea: New Caledonia, 1977).
6. H.M. Burkill. *The useful plants of West Tropical Africa. Vol. 2*. (Royal Botanical Gardens: Kew, 1994).
7. N. Yadav, G. Misra and S.K. Nigam. Triterpenoids of *Adenathera pavonina* bark. *Planta Medica*, 29(2), 1976, 176-178.
8. A.M. Gennaro and G. Nasini. Flavonoids from *Adenathera pavonina*. *Phytochemistry*, 11(4), 1972, 1515.
9. S. Sotheeswaran, M.R. Sharif, R.A. Mureau and G.J. Piazza. Lipids from the seven Fijian plant species, *Food Chemistry*, 49, 1994, 11-13.
10. C. Mayuren and R. Ilavarasan, Anti-inflammatory activity of ethanolic leaf extracts from *Adenathera pavonina* (L) in Rats, *Journal of Young Pharmacists*, 1(2), 2009, 125-128.
11. R.C. Jagessar, A. Mohamed and G. Gomes, Antibacterial and antifungal activity of leaf extracts of *Luffa operculata*, vs *Peltophorum pterocarpum*, against *Candida albicans*, *Staphylococcus aureus* and *Escherichia coli*. *Nature and Science* 5(4), 2007, 81-93.
12. D. Veeramuthu, A. Muniappan and I. Savarimuthu, Antimicrobial activity of some ethnomedicinal plants used by Paliyar tribe from Tamil Nadu, India. Entomology Research Institute, Loyola College, India. *BMC Complementary and Alternative Medicine*, 6, 2006, 35.
13. A. Shahina, Ghazanfar and A.M.A. Al-Sabahi, Medicinal plants of Northern and central Oman (Arabia), *Economic Botany* 47(1), 1993, 89-98.
14. R.P. Rastogi and B.N. Mehrotra. *Compendium of Indian Medicinal Plants: A CDRI Series. (Vol. IV)* (Lucknow: Publication and Information Directorate, New Delhi, 1995).
15. S. Gangal, S. Sharma and A. Rauf, Fatty Acid Composition of *Prosopis cineraria* Seeds, *Chemistry of Natural Compounds* 45(5), 2009, 705 – 707.
16. H. Akhtar and O.P. Virmani, Dictionary of Indian Medicinal Plants. (1st Ed) (Central Institute of Medicinal and Aromatic Plants: Lucknow, 1992).
17. M.P.M. Naina, V. Ravichandiran and M. Gobinath. Acute and Subacute Toxicity of Methanol Extract of *Prosopis cineraria* in Rats. *Middle East Journal of Scientific Research*, 7(6), 2011, 931-936.
18. N.M. Pasiecznik, P. Felker, P.J.C. Harris, L.N. Harsh, G. Cruz, J.C. Tewari, K. Cadoret and L.J. Maldonado. The *Prosopis juliflora* - *Prosopis pallida* Complex: A Monograph HDRA, Coventry, UK. 2001, p.172.
19. ICFRE (Indian Council Of Forestry Research And Education). Khejri (*Prosopis cineraria*), (ICFRE: Dehra Dun, India, 1993).
20. N.J. Carman. Systematic and ecological investigations in the genus *Prosopis* emphasising the natural products chemistry. *Chemical Abstracts*, 34, 1974, 4240.
21. R.G. Cates and D.F. Rhoades, *Prosopis* leaves as a source for insects. In: Mesquite, Its Biology in Two Desert Scrub Ecosystems. (Ed.) Simpson BB, Dowden, Hutchinson, Ross. (Stroudsburg: Pennsylvania, US, 1977) 61-83.
22. M.G. Campos, K.R. Markham and A. Proença da Cunha, Quality assessment of Bee- pollens using flavonoid/ phenolic profiles. *Polyphenol Communications*, 96, 1997, 53-54.
23. D. Lorke, A New Approach to Practical Acute Toxicity Testing, *Archives of Toxicology*, 54, 1983, 275-287.

24. H.O. Vongtau, S. Amos, L. Binda, D. Saidu, K.S. Gamaniel, O.F. Kunle, C. Wambebe, Pharmacological effects of the aqueous extract of *Neorautanenia mitis* in rodents, *Journal of Ethnopharmacology*, 72, 2000, 207-214.
25. F.N. Kalu, V.N. Ogugua, C.O. Ujowundu and C.R.K. Chinekeokwu, Chemical Composition and Acute Toxicity Studies on the Aqueous Extract of *Combretum dolichopentalum* Leaf in Swiss Albino Mice, *Research Journal of Chemical Sciences*, 1(8), 2011, 72-75.
26. M.M. Burns. Alternative medicine: Herbal preparation. *Clinical Pediatric Emergency Medicine*, 1, 2000, 186-190.
27. A.N. Dasture, Herbal Therapy. *Ancient Science of Life*, 21(4), 2002, 281-286.

