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RESEARCH ARTICLE

PRELIMINARY PHYTOCHEMICAL SCREENING & EVALUATION OF ANTI INFLAMMATORY ACTIVITY OF *MIMOSA PRAINIANA* GAMBLE (MIMOSACEAE)

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ABSTRACT

Crude methanolic extract of the bark of *Mimosa prainiana* Gamble (Mimosaceae) was subjected to the preliminary phyto chemical screening and evaluation of its anti inflammatory activity. Preliminary phyto chemical screening of the methanolic extract of the bark of *Mimosa prainiana* Gamble was followed by HR-LCMS analysis to develop a finger print chromatogram. The studies on the phytochemical screening of the bark extract revealed the presence of flavonoids, phytosterols, carbohydrates and glycosides. Different doses of crude methanolic extract of bark of *Mimosa prainiana* Gamble was tested for its anti inflammatory activity and found to provide the marked anti inflammatory effect induced by *Naja naja* venom.

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INTRODUCTION

Mimosa prainiana (Gamble 1935) (Mimosaceae) is small strangling thorny shrub about 7 to 8 meters tall with lateral dense shoots & pink flowers with rounded leaflets, the pinnae in regular subequal pairs. Specifically found in east coast, deccan & Hyderabad region. Abundantly available in Khandesh region of north Maharashtra. Leaves are over 2 to 3 inch in length while its leaflets are semicordate at base; sutures of the pods are with strong recurved prickles. Pods obtuse at tip, 6 to 8seeded, 4 to5 inch broad, sometimes slightly pubescent, long, pinnae 5-7 pairs about 5 inch apart, leaflets oblong, the end ones subobovate, up to 3 inch long, the 7-S pairs touching, 'l-'2 in. apart; ovary minutely pubescent. Locally known as 'Arkathi' & it is found throughout this region.

MATERIALS AND METHODS

Plant Material & Extracts: Ethno botanical information was collected by interviewing local medicine men, tribal chiefs & bhagats, which prescribe their own herbal medicine. Fresh samples of the bark of the plant *Mimosa prainiana* Gamble (Mimosaceae) were collected from the wild sources in the month of October. The plant was identified & authenticated by Botanical Survey of India, Pune vide Herbarium sheet no. HM01. A voucher specimen has been deposited at the Pharmacology department, R.G.Sapkal College of Pharmacy, Anjaneri, Nashik. Collected bark sample was made free from dirt and thoroughly washed with running water to remove the adherent soil & dried. Dried bark was reduced to coarse powder & extracted in a soxhlet apparatus. Total of 1 kg of drug powder (individual batch of 200 gm) was extracted successively with solvents of increasing polarity as petroleum ether (60-80) and methanol (95%v/v). The petroleum ether extraction was carried in soxhlet assembly for 72 hrs till its completion and it was confirmed by placing a drop of extract from siphon tube on filter paper gives no oily spot (Spot filter test). Then the marc was removed from thimble and dried in air and further subjected to extraction with methanol (95%) in soxhlet assembly. The extracts were concentrated by distillation and dried by heating on water bath at 37^0C. Extract was then concentrated to a semisolid, thick, sticky brown red coloured mass which was kept in refrigerator till further use. All extracts were subjected to the preliminary phyto chemical screening for identification of active constituents (Shah *et al.,* 1995) (Khandelwal, 2001).

Snake Venom: Lyophilized venom of Indian spectacled cobra (*najanaja*) was commercially purchased from Irula Snake catcher's Co Operative Society Chennai and preserved at 4^0C till further use. The venom was dissolved in normal saline solution immediately before the administration. Venom

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concentration was expressed in terms of dry weight. (mg/ml stock venom).

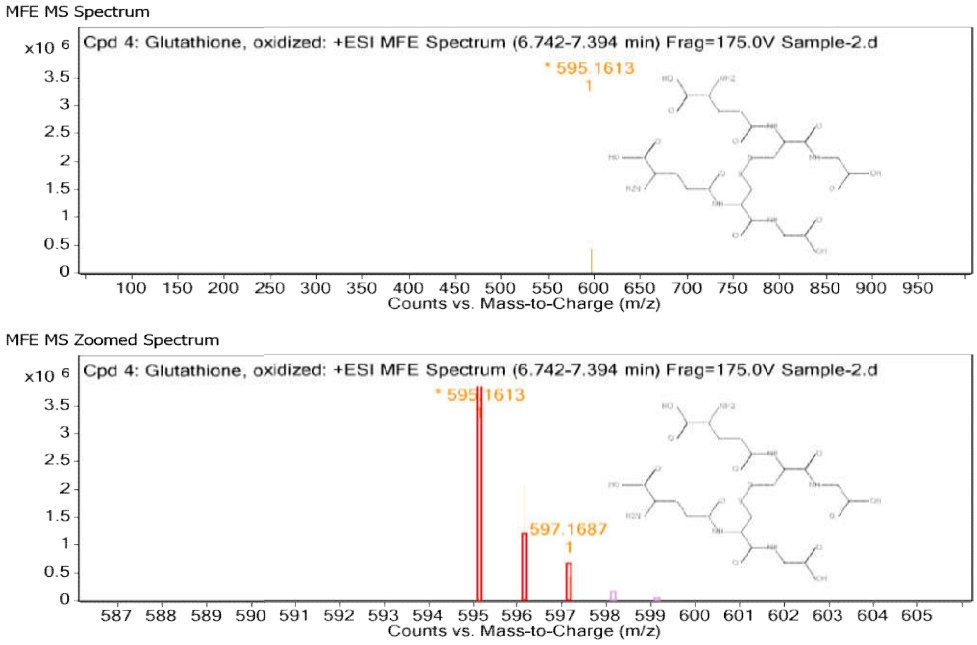
Animals: Swiss albino mice (18-20 gm) of both sexes were used for this study. They were housed in plastic cages at room temperature with a12 hr light/dark cycle. They had a free access to drinking water and standard diet. Experimental protocol was submitted to the Institutional Animal Ethics Committee and was cleared by the same vide No. RGSCOP/Ph.D/2013-2014/051405/11.

Phyto chemical screening: Ether as well as methanol extract was subjected to the qualitative phyto chemical analysis using standard methods. (Table 1)

HR-LCMS Analysis of methanolic extract of the bark of *Mimosa prainiana* Gamble

Methanolic extract of the bark of *Mimosa prainiana* Gamble was analyzed by HR-LCMS to develop a finger print chromatogram. The extract was tested against the available flavonoid reference compounds (markers) and the results were further used for the standardization of the extract. (Figure 1a, b, c)

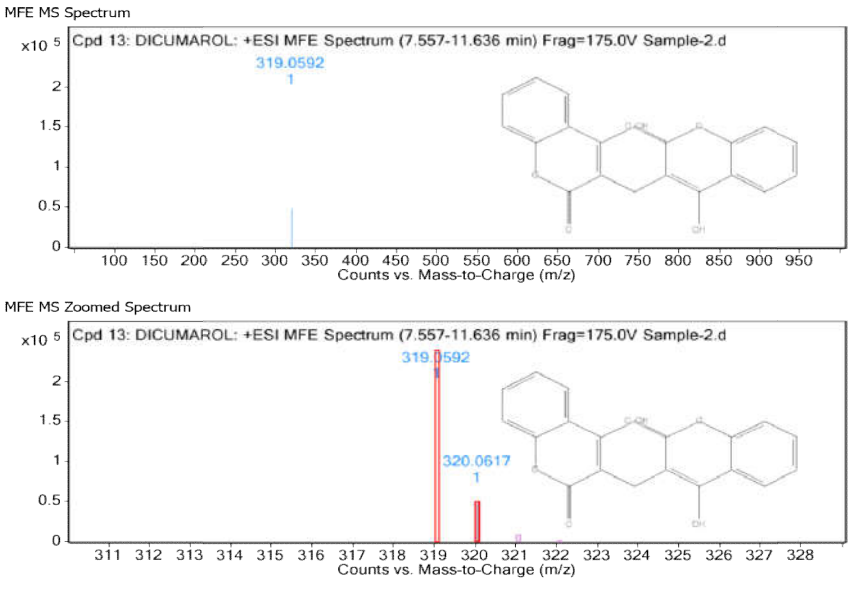
Fig. 1a. HR-LCMS fingerprint chromatogram of Methanolic extract of *Mimosa Prainiana* (Glutathione)



MS Spectrum Peak List

m/z	z	Abund	Formula	Ion
595.1613	1	3832035.75	$C_{20}H_{32}N_6O_{12}S_2$	(M+H)+
596.1668	1	2094510.85	$C_{20}H_{32}N_6O_{12}S_2$	(M+H)+
597.1687	1	426088.93	$C_{20}H_{32}N_6O_{12}S_2$	(M+H)+

Fig.1b. HR-LCMS fingerprint chromatogram of Methanolic extract of *Mimosa prainiana* (Dicumarol)



MS Spectrum Peak List

m/z	z	Abund	Formula	Ion
319.0592	1	239418.38	$C_{19}H_{12}O_6$	(M+H)+
320.0617	1	47530.83	$C_{19}H_{12}O_6$	(M+H)+

Table 1. Results of the Phytochemical screening of Methanolic Extract of *M. Prainiana*

S. No.	Test	Petroleum ether extract	Methanol extract
1	Test forPhytosterols		
	Salkowski test	-	+
	Lieberman-Burchard test	-	+
	Sulphur test	-	+
2	Test for Alkaloids		
	Mayer's reagent test	-	+
	Dragendorff's reagent test	-	+
3	Test for Steroids and Terpenoids	-	+
4	Test for Flavonoids	-	+
5	Test for Phenols	+	+
6	Test for Tannins	+	+
7	Test for Saponins		
	Foam Test	-	-
8	Test for Carbohydrates		
	Molisch's Test	-	+
	Fehling's Test	-	+
9	Test for Proteins		
	Biuret test	-	-
	Millon's test	-	-
10	Test for Amino acids		
	Ninhydrin test	-	-
11	Tests for Glycosides		
	Test for cardiac glycosides		
	Legal's Test	-	+
	Test for deoxysugars(Keller-Killiani test)	-	+
	Test for Anthraquinone glycosides	+	+
	Borntrager's test		

(-) = absent; (+) = present; *-bioactive crude extract

Table 2. Effect of Methanolic Extract of *Mimosa prainiana* Gamble bark on the cobra venom induced edema in mice

Treatment With Extract	Venom µg/0.1 ml	Paw volume ml ± SEM				Inhibition of edema %
Mg/kg		0.25 hr	0.5hr	1hr	2hr	
-	4	0.36±0.020	0.52±0.022	0.58±0.023	0.85±0.020	-
20	4	0.31±0.018	0.47±0.021	0.54±0.020	0.68±0.014*	36.6
40	4	0.36±0.020	0.46±0.020	0.55±0.020	0.64±0.013*	28
60	4	0.34±0.018	0.41±0.028	0.49±0.027	0.71±0.017	36.9
80	4	0.33±0.017	0.39±0.019	0.48±0.026	0.72±0.016	38.9
Control	-	--	--	--	--	--

N=4, P*<0.001, Values are expressed as ± SEM

Anti-inflammatory activity of *M. prainiana*: The anti inflammatory activity of the crude methanolic extract of *Mimosa prainiana* Gamble bark was assessed by inducing paw edema in albino mice by cobra venom. All the experiments were carried out as per the methods described by Al-Asmari (Al-Asmari 2005) and Alam *et al*. (Alam *et al.,* 1998). The Indian cobra (*najanaja*) venom (5µg/paw) was injected in the sub plantar region of the right hind paw followed by different doses of the extract intra peritoneally. 0.1 ml solution of cobra venom was administered each time to produce time and dose dependent swelling leading to paw edema. The paw volume was measured after 0.25, 0.5, 1 and 2 hours respectively, by using an arm plethysmograph (make DOLPHIN Mercurial Treatment). Appropriate controls were performed by injecting the normal saline solution into the sub plantar region of the left foot of mice. (Table 2)

Statistical Analysis: All values are expressed as mean ± SEM. Statistical significance was calculated using Student's t test with $p < 0.05$being considered significant.

RESULTS

Phyto sterols, glycosides, flavonoids proteins carbohydrates, steroids were observed to be present in the crude extract of the bark of *M. prainiana* by qualitative tests. Dicumarol, Glutathione were identified in the HR-LCMS analysis used for the standardization of the extract. Administration of different doses of methanolic extract of *Mimosa prainiana* bark with cobra venom (*Najanaja)* has shown a marked protection and anti inflammatory effect in albino mice.

DISCUSSION

Present investigation was conducted to explore the anti inflammatory effect of the methanolic extract of *Mimosa prainiana* Gamble in experimental animals against cobra venom. Effectiveness of M. *prainiana* Gamble against inflammation produced by Indian spectacled cobra (*Najanaja*) venom indicates its antivenom property. Cobra bite is often associated with severe necrosis of the local tissues & selective neuro muscular block leading to respiratory failure (Reid *et al.,* 1983) in human beings. Reports are also available about sudden respiratory failure in the patients bitten by the cobra, because of pulmonary hemorrhage (Bonta, 1970) induced by the venom. Venom of the Indian cobra (Parikh, 1990) mainly contains three distinct substances neurotoxin, haemolycin & cardiotoxin along with two basic, heat stable proteins (Larsen, 1968) namely cobramine A & B. Enzymes (Kini 2006) like ATPase,

cholineasterase, & Phospholipase A2 are also present. All these components of snake venom are responsible for its severe neurotoxic, cardiotoxic & local effects (Gupta, 1999) in the human body. Reid (Reid, 1964) has reported the systemic, cardiovascular & biochemical effects of venom in 47 patients bitten by common cobra in detail. Neuro toxins are the major components of the cobra venom, which are low molecular weight proteins. Neurotoxic action of the cobra venom components is primarily on the postsynaptic Ach receptor in the motor end plate of the muscle fiber, which interferes with impulse transmission leading to the respiratory paralysis (Misra *et al.,* 1999). Cytotoxins constitute more than 50%of total venom proteins in some cobravenoms. These cytotoxins are lytic in nature and act synergistically with venom Phospholipase A_2 to rapidly lyse erythrocytes leading to the massive haemolysis and release of $K^{+,}$ causing cardiac arrest in victims. Blisters (Reid, 1968) around the site of bite are common in cobra bite. Local swelling and necrosis is the characteristic of poisoning from Asian cobra bites. Necrosis is extensive but superficial and involvement of tendons, muscles and bones is exceptional. Most of the times bacterial infection follows the necrosis and spreads to the joints. A constant feature of local swelling from cobra bite is a dusky discolouration around the bite marks. This deepens in colour each day. Sanguineous blisters develop over the middle of the dusky area. They are usually small, rarely extending 2-3 cm in diameter. After four to five days of the bite sloughing occurs and reveals necrosis of the subcutaneous tissue. The extent of local necrosis and inflammatory response (Voronov *et al.,* 1999) after the snake bite is due to the release of cytokines TNFα & IL-1 which are induced by metalloproteinases. Cytokines ultimately amplify the total inflammatory response leading to the cell destruction & necrosis.

Conclusion

So far no systematic study has been reported about the anti-inflammatory & snake venom detoxification mechanism of *M.prainiana* Gamble. From the above results it seems that *M. prainiana* Gamble can be recommended for further studies as an antidote to the snake bite envenomation.

REFERENCES

Alam MI, Gomes A. 1998. An Experimental Study On Evaluation of Chemical Antagonists Induced Snake Venom Neutralization. *Indian Journal of Medical Research*, 107 pp 142-146.

Al-Asmari AK. 2005. Pharmacological Characterization of Rat Paw Edema Induced By *NajahajeArabica* Venom. *J Venom Anim Toxins incl Trop Dis.,* 11 (1) pp 51-67.

Bonta IL, De Vrieskragt Bhargava N. 1970. Preventive effect of Local Heparin Administration on Micro vascular Pulmonary Hemorrhages Induced by Cobra Venom in Mice. *European Journal of Pharmacology,* 13 pp 97-102.

Gamble JS. 1935. *Flora of Presidency of Madras*. National Institute of Science Communication (CSIR): New Delhi; 95

Gupta I 1999. In Snakes in India-A source Book, edited by B. D. Sharma, Asiatic Publishing House, Delhi - 92 pp. 281-283.

Khandelwal K.R. 2001. *Practical Pharmacognosy, Techniques & Experiments*, 8th Ed. NiraliPrakashan Pune, 153-155.

Kini RM. 2006. Anti coagulant Proteins from Snake Venoms: Structure, Function and Mechanism. *Biochemistry Journal,* (397) pp 377-387.

Larsen PR. 1968. The Basic Proteins of Cobra Venom. *The Journal of Biological Chemistry,* 249 (6) pp 1283-1289.

Misra NP, Misra SC. 1999. Snakebite: A Review in Snakes in India edited by B.D. Sharma, Asiatic Publishing House, Delhi 92 pp: 221-256.

Parikh CK. 1990. Parikh's Textbook of Medical Jurisprudence and Toxicology, CBS Publishers New Delhi, pp 800-823.

Reid HA, Theakston RDG. 1983. Development of Simple Standard Assay Procedures for The Characterization of Snake Venoms, *Bull*etin WHO 61(6): 949-956.

Reid HA. 1964. Cobra Bites, *British Medical Journal*, 2 pp. 540-545.

Reid HA. 1968. Snakebite in the Tropics *British Medical Journal* 3, pp 359- 362.

Shah C.S. Quadry J.S. 1995. *A Textbook of Pharmacognosy*, 11th Ed. B.S.ShahPrakashan, Ahmedabad 157-160.

Voronov E, Apte RN, Soffer S. 1999. The Systemic Inflammatory Response Syndrome Related to the release of Cytokines Following Severe Envenomation. *Journal of Venomous Animal Toxins,* 5(1) pp 5 -33.

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