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Anti-inflammatory and analgesic property of methanolic extract of *Spinifex littoreus* (Burm.f.) Merr

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Abstract: *The methanol extract of the coastal sand-dwelling grass, Spinifex littoreus was tested for its anti-inflammatory and analgesic property using mouse assay. To ascertain the pharmacological property of each plant part (aerial and root), extracts were prepared and tested.. The results were compared with the standard analgesic and anti-inflammatory drugs viz. paracetamol and dichlofenac sodium respectively. It was observed that the root extract of Spinifex littoreus was more potential in term of analgesic and anti inflammatory properties.*

Key words: *Spinifex littoreus, Methanol extract, Roots, Analgesic, Anti-inflammatory.*

Introduction

Marine plants and animals are reported to possess a wide spectrum of bioactive substances which are structurally novel and biologically active substances. Research in the areas of marine natural products has grown geometrically in the recent past (Aswal *et al.,* 1984a,b and Rawiwon *et al.*, 1990). However, reports on the bioactive substances possessed by coastal plants are very meager. Few reports on the coastal plant *Ipomoea pes-caprae* containing pharmacologically active substances, were indicated by de Souza *et al.* (2000); Krogh *et al.*(1999) and Rogers *et al.*(2000). In the present study, *Spinifex littoreus,* one of the coastal plants growing in the sand-dunes located at the extreme highest high tide mark in the intertidal region of pondicherry coastal zone was selected to ascertain its bioactive property with particular reference to anti inflammatory and analgesic properties.

Materials and Methods

To assess the pharmacological property of the aerial and the root of the sand dune grass, *Spinifex littoreus*, extracts were prepared separately using methanol as solvent. The grasses were collected from the sand dunes along the pondicherry coast during its flowering stage. The grasses were uprooted and washed well in freshwater and dried under shadow. The aerial part and roots separated and cut into small pieces. Each set of samples was refluxed in methanol for 6 hr. After extraction, the crude extract was evaporated under reduced pressure and used for analgesic and anti inflammatory property evaluation studies. For each pharmacological study, two groups of animals were maintained and each group consisted of 6 male abino mice having an average bodyweight of 160 g. Using the method of Winter *et al*. (1962), oedema was produced by injecting 0.1 ml of carrageenan solution (1% w/v) in normal saline under the subplanar region of the right hind foot of the rats. Paw volume was measured using volume differential meter at the scheduled time interval (Table 1a and1b). The percentage inhibition of oedema was assessed comparing with the control group. For the analgesic evaluation, tail clip method was adopted and analgesiometer was used to evaluate the analgesic index of the extracts using mouse assay. Test compound was administered and the reaction time of mice (n=10) to pain due to application of the tail clip, was recorded. All these methods were followed as described by Winter *et al.* (1962). Mean values of all data were tabulated and one way ANOVA was done for anti-inflammatory studies.

Results

The overall inhibitory activity of the root extract was higher than stem extract. The root extract enhanced the inhibitory activity resulting in 60% reduction in oedema. The mean paw volume and its percentage oedema inhibition shown by stem and root extracts as presented in the Tables 1a and 1b. The inhibition was higher during the first 60 min after injection of the extract. But, in the following hours, the inhibitory activity declined. The statistical analysis of the data also revealed significant analgesic property of the root extract. When tested by tail flick method using analgesiometer the analgesic index was very low in the case of stem extract (Table 2) which was recorded as only 33.3% of pain relax. The percentage of analgesic property was high after 15 min (60%) but it decreased to 33.3% in the next 15 min without any sustained activity.

Discussion

There are several tissue-factors that are known to be involved in the inflammatory action such as release of histamines and prostaglandins. However, development of non-steroidal anti-inflammatory agents in recent years have contributed a lot not only in human sufferings but also helped in understanding the tissue mechanisms of inflammation. The inflammatory reaction is readily produced in rats in the form of paw oedema with the help of irritants or inflamogens. Carrageenan induced paw oedema is the most commonly used method in experimental pharmacology causing inflammation by producing histamine, 5-HT and prostaglandins. Therefore, in

Table – 1a: Percent oedema inhibition by stem extract of *Spinifex littoreus**.

Treatment	Mean body weight (g)	Control	Mean paw volume (ml) as measured by mercury displacement (min)			
		0	15	30	60	120
Control	160	0.4050±0.02	0.6750±0.002	0.800±0.020	1.225±0.010	1.225±0.025
Treated (stem)	164	0.350±0.015	0.425±0.020	0.575±0.020	0.625±0.015	0.675±0.020
% of oedema inhibition		5%	25%	22.5%	60%	55%

Dose: Carageenan 0.1 ml of 1% (w/v) [left paw only]
Extract of stem: 0.1ml of 1 % (w/v)

- % inhibition of inflammation = vehicle values–treated group mean values of paw volume multiplied by 100 (Alexandre-Moreira *et al.,* 1999*).*

Table – 1b: Percent oedema inhibition by root extract of *Spinifex littoreus*.*

Treatment	Mean body weight (g)	Control	Mean paw volume (ml) as measured by mercury displacement (min)			
		0	15	30	60	120
Control	160	0.4050±0.02	0.6750±0.002	0.800±0.020	1.225±0.010	1.225±0.025
Treated (root)	160	0.375±0.020	0.450±0.030	0.600±0.015	0.650±0.010	0.725±0.025
% of oedema inhibition		2.5%	22.5%	20%	57%	49.5%

Dose: Carrageenan 0.1 ml of 1% (w/v) [left paw only]
Extract of root: 0.1ml of 1 % (w/v)

- % inhibition of inflammation = vehicle values – treated group mean values of paw volume multiplied by 100 (Alexandre-Moreira *et al.,* 1999*).*

Table – 2: Percentage inhibition of analgesic effect of extract of *Spinifex littoreus* analgesic index *.

Treatment	Dose (mg)	Mean values of reaction after morphine challenge	
		15 min	30 min
Control	0.1 ml	8.545 ± 0. 02	12.0 ± 0. 02
Stem extract	0.1 ml	4.025 ± 0.02 (33.3%)**	4.025 ± 0. 02 (33.3%)**
Root extract	0.1 ml	5.523 ± 0.02 (60%)**	6.750 ± 0. 02 (33.3%)**

* Analgesic Index: vehicle values – treated group mean values multiplied by 100 (Alexandre-Moreira *et al.,* 1999)
** percentage inhibition of analgesic effect.

the present study, the anti-inflammatory property of the methanol extract of *Spinifex littoreus* was evaluated by challenging the animal with carageenan. The percentage decrease in the inflammation (paw volume measured by mercury displacement) slightly increased from 25% to 57% upto one hour in the case of aerial part extract. Similarly in the case of root extract, there was a sustained decrease in the inflammmation to the level of 57% upto one hour. Further, the rate of decrease in the inflammation was very slow and finally after 2 hr there was no effect. These observations indicated that the extract of both aerial part and root are capable of reducing the inflammation only upto one hour and beyond that the potency of the extract reduced very fast and was nil within next one hr. The factor responsible for such anti inflammatory property of the extract might be due to the presence of organic substances/compounds like tannin which is capable of inhibiting the flamogen like histamine and prostaglandin system (Muruganandham *et al*., 1999). Moreover, the non-steroid compound like phenylbutazone, is also capable of inhibiting the migration of leucocytes in carrageenan induced pleuracy (Meacock and Kitchen, 1979; Blackman and Oven, 1975). Therefore, it is presumed that the extract of *Spinifex littoreus* might possess a compound capable of inhibiting the internal flamogens. Secondly, the short term effect of the extract on anti-inflammation and analgesic property might be due to the presence of less dose of the anti inflamogen, in the extract. Therefore, a meticulously designed and dose-based experimental evaluation on this encouraging subject would bring out a new input in the realm of naturally present anti-inflamogens to substitute the existing synthetic agents. Further, it is also equally important to understand whether such anti inflamogen is a steroid or non-steroid in nature as plants are reported to possess both types of compounds. This would be clear if the extract is subjected to biochemical characterization in future.

Attempts to ascertain the analgesic property of the two extracts viz., aerial parts and the roots of *Spinifex littoreus* indicated that extracts of the aerial parts of the plant showed appreciable analgesic effect. The tail flick method using analgesiometer revealed that the analgesic index or the percentage increase in reaction time was relatively higher in the case of root extract (upto 15 min of injection of the extract) but it

was lowered towards next 15 min. Therefore, from the present observation it could be presumed that out of two extracts tested, root seemed to be more potent than the aerial parts in terms of analgesic property; however, a dose based study to increase the sustainability of the pharmacological activity of the extract, is very much required in future to consider the extract for clinical trials.

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