

Comparative Study of Anti-Inflammatory Activity of Petroleum Ether Extract of *Myxopyrum smilacifolium* Blume and Ethanolic Leaf Extract of *Pamburus missionis* Swingle.

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Abstract

Petroleum ether and ethanolic extract of *Myxopyrum smilacifolium* Blume and *Pamburus missionis* Swingle were made investigated for anti-inflammatory activity by using Carrageenan induced rat paw oedema and cotton-pellet method. In Carrageenan induced rat paw oedema method, doses with 200mg/kg and 400mg/kg of both *Pamburus missionis* Swingle and *Myxopyrum smilacifolium* Blume had shown maximum inhibition in paw volume, paw thickness and knee diameter. Comparatively dose of 400mg/kg of *Pamburus missionis* Swingle had shown significant anti-inflammatory activity when compared with the same dose of *Myxopyrum smilacifolium* Blume. In cotton pellet method 200mg/kg of *Pamburus missionis* Swingle had shown comparatively effective than *Myxopyrum smilacifolium* Blume and at 400mg/kg *Pamburus missionis* Swingle had shown effective than 400mg/kg *Myxopyrum smilacifolium* Blume and as well as standard drug Diclofenac sodium.

Keywords *Pamburus missionis*, *Myxopyrum smilacifolium*, anti-inflammatory, Diclofenac sodium.

INTRODUCTION

Indian system of medicine has retained its importance from ancient days and it gave the path for discovery of lead medicaments for various diseases to treat. *Myxopyrum smilacifolium* Blume is a large woody twining shrub belonging to the family Oleaceae, grows at an altitude of below 700-1000mm of tropical and subtropical regions of Eastern Asia. It was used traditionally for the treatment of rheumatism, cough etc., *Pamburus missionis* Swingle. is a shrub belonging to the family Rutaceae, grows in southern India which was used traditionally for the treatment of rheumatism and fractures. The present study was to investigate to evaluate the anti-inflammatory activity of both plants viz., *Myxopyrum smilacifolium* Blume and *Pamburus missionis* Swingle.

MATERIAL AND METHODS

Plant material

The leaves of both *Myxopyrum smilacifolium* Blume and *Pamburus missionis* Swingle were procured from botanical garden, Department of Botany, University of Kerala, Kerala and Talakona hills, Tirupati respectively. Both the plants were authenticated by V. Chelladurai, Former Research officer, Central Council of Research in Ayurveda and Siddha, Government Siddha medical College, Tamil Nadu, India and Prof. K. Madhava Shetty, Department of Botany, Sri Venkateswara University, Tirupati. Andhra Pradesh, India.

Preparation of extract

Myxopyrum smilacifolium Blume and *Pamburus missionis* Swingle leaves were shade dried and extracted by Soxhlet apparatus using solvents petroleum ether and ethanol respectively. Further the extract made concentrated by rotary evaporator.

Animal

Healthy albino rats (150–200 g) were used for the study and all the animals were acclimatized under standard husbandry conditions, i.e., room temperature 22 ± 2 °C, relative humidity 45-55% and light dark cycle 12:12 hours. The animals were fed with commercial pellet rat feed and water *ad libitum*. All the animal experiments were strictly compiled with ethical standards of animal handling and approved by Institutional Animal Ethics Committee.

Evaluation of anti-inflammatory activity

Carrageenan induced paw oedema

In this method the animals were divided into six groups of 6 animals each. Group I serves as control which is treated with normal saline, Group II serves as Standard-50 mg/kg Diclofenac sodium, p.o. Group III & Group IV rats were treated with 200mg/kg and 400mg/kg petroleum ether extract of *Myxopyrum smilacifolium* B. (PEMS) Group V and Group VI were treated with 200 & 400 mg/kg of leaf extracts of *Pamburus missionis* S. (EPPM). Initially Carrageenan is administered to all groups of animals through intraplantar route later the test drug and standard drug were given and observed from 0th day to 28th day.

Cotton pellet Granuloma Method:

A sterilized cotton pellet weighing 10mg was implanted subcutaneously into the groin region of rats after which six groups were treated (once daily) with 200mg/kg and 400mg/kg of both extracts of PEMS and EPPM for seven consecutive days. Animals in control and reference groups received normal saline and Dexamethasone Sodium Phosphate injection (0.5 mg/kg) respectively. On the 8th day the animals were sacrificed and thereafter the pellets surrounded by granuloma tissue were dissected out carefully and the weight of wet cotton pellets were noted. The cotton pellets were dried in oven at 60° c for 24 hrs to

obtain a dry cotton pellet weight, the mean weight of granuloma tissue formed around each pellet was obtained and the percentage inhibition was determined.

significance between more than two groups was tested using one way ANOVA followed by the Tukey test using computer based fitting program (Prism graph pad.). Statistical significance was taken as $p < 0.05$.

Statistical analysis

All the data was expressed as Mean \pm S.E.M. Statistical

Table 1 Percentage inhibition of paw volume in Carrageenan induced inflammatory rat

S.No	Group	Treatment	Paw volume (ml) (Mean \pm SEM) on				
			0 th day	7 th day	14 th day	21 st day	28 th day
1	I	Control, CFA (0.1ml/rat, s.p)	0.22 \pm 0.02	1.95 \pm 0.09 ^{###}	2.27 \pm 0.08 ^{###}	2.35 \pm 0.10 ^{###}	2.32 \pm 0.06 ^{###}
2	II	Standard, CFA (0.1 ml/rat, s.p) + Diclofenac sodium (15 mg/kg, p.o.)	0.27 \pm 0.02	2.12 \pm 0.07	1.82 \pm 0.06 ^{**}	1.22 \pm 0.06 ^{***}	0.52 \pm 0.04 ^{***}
3	III	Low dose, CFA (0.1 ml/rat, s.p) + PEMS (200 mg/kg, p.o.)	0.25 \pm 0.02	2.22 \pm 0.06	2.15 \pm 0.06	1.87 \pm 0.04 ^{**}	1.85 \pm 0.11 ^{**}
4	IV	High dose, CFA (0.1 ml/rat, s.p) + PEMS (400 mg/kg, p.o.)	0.27 \pm 0.02	1.97 \pm 0.14	1.97 \pm 0.08 [*]	1.32 \pm 0.04 ^{***}	0.6 \pm 0.07 ^{***}
5	V	Low dose, CFA (0.1 ml/rat, s.p) + EEPM (200 mg/kg, p.o.)	0.26 \pm 0.02	2.24 \pm 0.05	1.30 \pm 0.07	1.93 \pm 0.03 ^{**}	1.94 \pm 0.11 ^{**}
6	VI	High dose, CFA (0.1 ml/rat, s.p) + EEPM (400 mg/kg, p.o.)	0.28 \pm 0.02	2.35 \pm 0.14	1.87 \pm 0.08 [*]	1.34 \pm 0.04 ^{***}	0.5 \pm 0.07 ^{***}

All values are shown as mean \pm SEM and n=6.

indicate $p < 0.001$ when compared to normal group.

* indicate $p < 0.05$, ** indicate $p < 0.01$, *** indicate $p < 0.001$ when compared to control group

Table 2 Percentage inhibition of paw thickness in Carrageenan induced inflammatory rat

Group	Treatment	Paw thickness (cm) (Mean \pm SEM) on				
		0 th day	7 th day	14 th day	21 st day	28 th day
I	Control, CFA (0.1ml/rat, s.p)	0.3 \pm 0.04	1.125 \pm 0.04 ^{###}	1.225 \pm 0.08 ^{###}	1.3 \pm 0.04 ^{###}	1.275 \pm 0.04 ^{###}
II	Standard, CFA (0.1 ml/rat, s.p) + Diclofenac sodium (15 mg/kg, p.o.)	0.3 \pm 0.05	0.8 \pm 0.04	0.775 \pm 0.04 ^{**}	0.7 \pm 0.04 ^{***}	0.45 \pm 0.08 ^{***}
III	Low dose, CFA (0.1 ml/rat, s.p) + MS1 (200 mg/kg, p.o.)	0.325 \pm 0.02	1.2 \pm 0.07	1.2 \pm 0.07	1.1 \pm 0.07	0.925 \pm 0.04 ^{**}
IV	High dose, CFA (0.1 ml/rat, s.p) + MS2 (400 mg/kg, p.o.)	0.275 \pm 0.04	1.125 \pm 0.04	0.975 \pm 0.04	0.9 \pm 0.07 ^{**}	0.55 \pm 0.06 ^{***}
V	Low dose, CFA (0.1 ml/rat, s.p) + PM1 (200 mg/kg, p.o.)	0.315 \pm 0.02	1.3 \pm 0.07	1.2 \pm 0.09	0.7 \pm 0.03 ^{***}	0.925 \pm 0.04 ^{**}
VI	High dose, CFA (0.1 ml/rat, s.p) + PM2 (400 mg/kg, p.o.)	0.275 \pm 0.04	1.125 \pm 0.04	0.77 \pm 0.04 ^{**}	0.7 \pm 0.06 ^{***}	0.44 \pm 0.06 ^{***}

All values are shown as mean \pm SEM and n=6.

indicate $p < 0.001$ when compared to normal group.

* indicate $p < 0.05$, ** indicate $p < 0.01$, *** indicate $p < 0.001$ when compared to control group

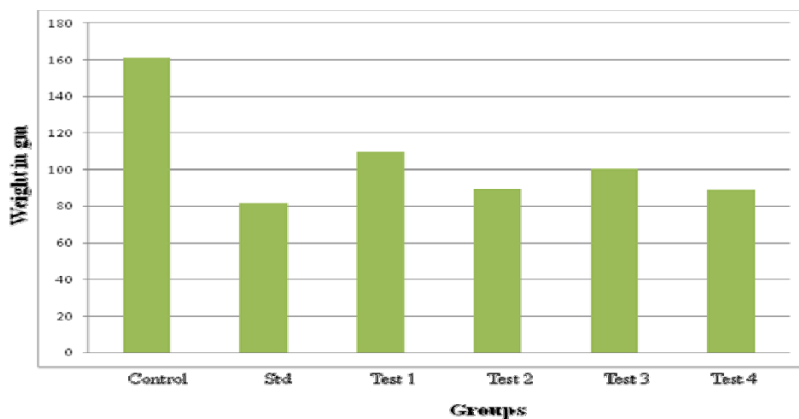
Table 3 Percentage inhibition of knee diameter in Carrageenan induced inflammatory rat

Group	Treatment	Knee diameter (cm) (Mean \pm SEM) on				
		0 th day	7 th day	14 th day	21 st day	28 th day
I	Control, CFA (0.1ml/rat, s.p)	1.275 \pm 0.04	2.3 \pm 0.07 ^{###}	2.85 \pm 0.104 ^{###}	2.8 \pm 0.08 ^{###}	2.75 \pm 0.13 ^{###}
II	Standard, CFA (0.1 ml/rat, s.p) + Diclofenac sodium (15 mg/kg, p.o.)	1.3 \pm 0.07	2.02 \pm 0.07	2.22 \pm 0.07 ^{***}	1.77 \pm 0.04 ^{***}	1.45 \pm 0.02 ^{***}
III	Low dose, CFA (0.1 ml/rat, s.p) + MS1 (200 mg/kg, p.o.)	1.325 \pm 0.04	2.22 \pm 0.06	2.5 \pm 0.04 [*]	2.35 \pm 0.08 ^{**}	2.175 \pm 0.07 ^{**}
IV	High dose, CFA (0.1 ml/rat, s.p) + MS2 (400 mg/kg, p.o.)	1.3 \pm 0.04	2.2 \pm 0.07	2.3 \pm 0.04 ^{**}	1.8 \pm 0.04 ^{***}	1.525 \pm 0.04 ^{***}
V	Low dose, CFA (0.1 ml/rat, s.p) + PM1 (200 mg/kg, p.o.)	1.325 \pm 0.04	2.01 \pm 0.06	2.5 \pm 0.04 [*]	2.45 \pm 0.08 ^{**}	2.17 \pm 0.07 ^{**}
VI	High dose, CFA (0.1 ml/rat, s.p) + PM2 (400 mg/kg, p.o.)	1.3 \pm 0.04	2.2 \pm 0.07	2.2 \pm 0.04 ^{***}	1.74 \pm 0.04 ^{***}	1.44 \pm 0.04 ^{***}

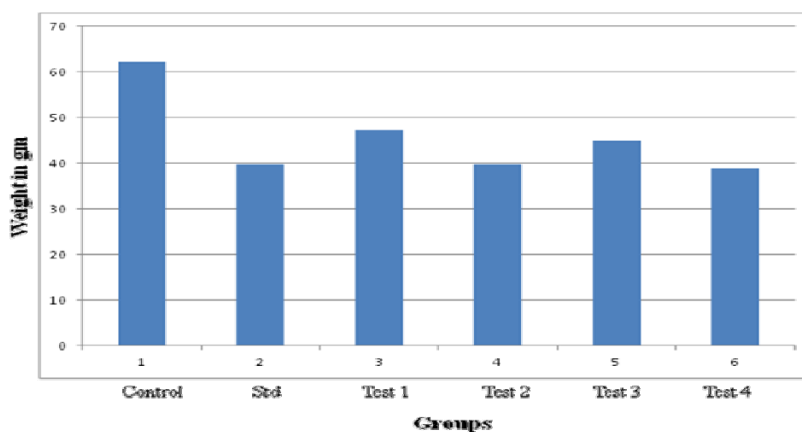
All values are shown as mean \pm SEM and n=6.

indicate $p < 0.001$ when compared to normal group.

* indicate $p < 0.05$, ** indicate $p < 0.01$, *** indicate $p < 0.001$ when compared to control group



Graph 1 Effect of PEMS & EEPM on weight of wet cotton pellets



Graph 2 Effect of PEMS & EEPM on weight of Dry cotton pellets

RESULTS AND DISCUSSION

The leaves of PEMS and EEPM were extracted by soxhlet apparatus with petroleum ether and ethanol respectively. The extracts were subjected for evaluation of anti-inflammatory activity. In Carrageenan induced paw oedema, EEPM had shown % inhibition of paw volume of about 0.5% where as Standard drug (Diclofenac sodium) and PEMS had shown about 0.52 and 0.6%. Percentage inhibition of paw thickness is about 0.45% by standard drug where as 0.55% and 0.44% by both extracts of PEMS and EEPM. Percentage inhibition of knee diameter by Standard drug was about 1.45% where as by PEMS and EEPM was about 1.52% and 1.44%. Both PEMS and EEPM had shown similar mean wet weight of cotton pellets where as in case of dry weight of cotton pellets EEPM has shown good inhibition activity when compared with PEMS.

CONCLUSION

The above study revealed that both leaf extracts of *Myxopyrum smilacifolium* Blume and *Pamburus missionis* Swingle were persisting anti-inflammatory activity when compared with that of standard drug Diclofenac sodium. Leaf extract of *Pamburus missionis* were shown a significant activity than leaf extract of *Myxopyrum smilacifolium* Blume.

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