



Evaluation of antiarthritic activity of ethanolic leaf extract of *Myxopyrum smilacifolium Blume.*

Short running title: Antiarthritic activity of *Myxopyrum smilacifolium Blume.*

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Abstract

Herbal medicines play an important role in primary health care which ensures safety, efficacy and cost effective. The current study is to evaluate anti-arthritis activity of leaf extract of *Myxopyrum smilacifolium Blume*. The plant leaves were collected, dried and extracted by using the solvent ethanol. Further the extract was subjected for anti-arthritis activity by Complete Freund's Adjuvant Induced Arthritis (CFA) model using Wistar albino rats weighing about 150-200g of either sex. The rats were divided into five groups containing six rats each. Primly CFA was injected for induction of arthritis followed by the treatment with 200mg/kg and 400mg/kg of ethanolic extract of *Myxopyrum smilacifolium Blume* where Diclofenac sodium 10mg/kg was taken as standard. The animals were examined for inflammatory, haematological parameters and the joints were radiographically analysed. Further the ethanolic extract was subjected for phytochemical investigations by Infra red (IR), Nuclear Magnetic Resonance (NMR) and Mass spectroscopy. The experimental findings revealed that the rats treated with 400mg/kg ($P < 0.001$) of ethanolic extract of *Myxopyrum smilacifolium Blume* elicited decrease in paw edema, knee diameter and paw thickness. In haematological parameters it had shown increase in RBC and haemoglobin content whereas decrease in white blood cells count (WBC) and rheumatoid antibody factor (RA). From the radiographical images it was keenly observed the recovery of joints from inflammation. The phytochemical investigations revealed that the *plant* consists of phytoconstituent myxopyroside-an irridoid glycoside. Thus the ethanolic leaf extract of *Myxopyrum smilacifolium Blume*. at the dose of 400mg/kg ($P < 0.001$) had shown significant antiarthritic activity due to the presence of phytoconstituent myxopyroside.

Keywords:

Myxopyrum smilacifolium Blume, antiarthritic, Myxopyroside, Freund's adjuvant, Radiography.

INTRODUCTION

Plants have high importance for the presence of secondary metabolites which are used to treat various diseases. The mortality and morbidity rate was high in musculo skeletal diseases like rheumatoid arthritis (Arora et al., 2013). The preliminary phytochemical examination of leaves of *Myxopyrum smilacifolium Blume* shown presence of secondary metabolites which exerts therapeutic activity (Raveesha Peeriga et al., 2016). *Myxopyrum smilacifolium Blume* is a large woody twining shrub commonly called as chaturdharalata in telugu. The leaf of this plant is used traditionally for the treatment of rheumatism, cough, cephalalgia, notalgia, anodyne, febrifuge and otopathy. Vegetatively, the genus *Myxopyrum* is very distinct plant belonging to the family Oleaceae. The leaves were used in the treatment of rheumatism in Indian traditional system of medicine. Even though they have a long history in Indian traditional system of medicine but there is a lack of systemic scientific evidence that it exerts an anti-arthritic activity. Hence the current study demonstrates the evaluation of antiarthritic activity of ethanolic extract of *Myxopyrum smilacifolium Blume* (Kiew R, 1984).

MATERIALS AND METHODS

Collection and authentication of *Pamburus missionis*

Myxopyrum smilacifolium Blume leaves were procured from botanical garden, Department of Botany, University of Kerala, Kerala. It was identified and authenticated by V. Chelladurai, Former Research officer. Central Council of Research in Ayurveda and Siddha, Government Siddha medical College, Tamil Nadu. India. The leaves of *Myxopyrum smilacifolium Blume* were dried in shade, coarsely powdered and stored in air tight container.

Preparation of extract:

The dried leaves of *Myxopyrum smilacifolium B* were extracted with ethanol by soxhlet apparatus for 72hr and resulted extract was made concentrated by rotary evaporator, under reduced pressure.

Animal

Experimental work was carried out by using healthy male albino rats (150–200 g). 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.

Anti-arthritic activity

Male albino rats of Wistar strain were divided into four groups. The first group act out as control group. The second group acquired the standard drug Ibuprofen, a dose of 15mg/kg. The 3rd and 4th groups received the ethanolic extract of *Myxopyrum smilacifolium* Blume at a dose of 200mg/kg and 400mg/kg. After 30 min. 0.1mL 6mg of complete Freund's adjuvant intercalated into the subplanatar region of left hind paw on prime day. Saline and extracts were presided from day 0 and continued until 28th day. Anti-arthritic effects of EEMS and standard drug Ibuprofen were evaluated by measuring inflammatory parameters viz., Paw volume, Paw thickness and Knee diameter. On 28th day blood was introverted from each and every animal through retro-orbital venous plexus of rats and it is collected into vial containing EDTA which is subjected for haematological parameters viz., RBC count, WBC count, Hb, ESR and RA factor.

Radiological analysis

The animals x-rays were taken at the joints of the hind paw of the animals for evaluating the bone damage. Radiographs were taken using x-ray apparatus.

Statistical analysis

The data précised in Mean±SEM and the results were statistically by one way analysis of difference (ANOVA) method. Differences in mean were considered to be significant at (P<0.01).

Isolation and Interpretation

The column chromatography for the leaf extracts of *Myxopyrum smilacifolium* were carried out and Silica gel G of mesh size 60-120 µm were used for isolation of phytoconstituents. The elution was done by using the solvents of increasing polarity viz., chloroform and ethanol in different ratios. The fraction of 100 ml each were collected and made concentrated, the residue was examined by TLC. The Chloroform and methanol in 60:40 ratio was subjected for TLC studies. The fractions were pooled and evaporated yielded pale yellow residue. The fractions were evaporated and the isolated compound I were subjected for spectral analysis by using Infra red (Perkin-Elmer RX1 FTIR), Nuclear Magnetic Resonance and Mass spectroscopic (Schimadzu QP 5000) methods for elucidation of structure.

RESULTS

The percentage of inhibition of paw volume in complete Freund's adjuvant induced arthritis rats was observed that decrease in paw volume was gradually observed from 0th day to 28th day in II, III, groups when compared with the control group. IV group animals were shown highly significant decrease in paw volume when compared with respect to I, II, III group animals (Table 1).

Table 1: Percentage inhibition of paw volume in CFA induced arthritis 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	V	Low dose, CFA (0.1 ml/rat, s.p) + EEMS (200 mg/kg, p.o.)	200 mg/kg	0.25 \pm 0.02	2.20 \pm 0.06	2.15 \pm 0.06	1.85 \pm 0.04 ^{**}
4	VI	High dose, CFA (0.1 ml/rat, s.p) + EEMS (400 mg/kg, p.o.)	400 mg/kg	0.27 \pm 0.02	1.97 \pm 0.14	1.95 \pm 0.08 [*]	1.32 \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

The percentage of inhibition of paw thickness in complete Freund's adjuvant induced arthritis rats was observed subsequently decreasing from 0th day to 28th day in II, III, IV groups when compared with the control group. There is a significant decrease in paw thickness about 0.44 \pm 0.06 when compared to other groups (Table 2).

Table No.2: Percentage inhibition of paw thickness in CFA induced arthritis rat

S. No	Group	Treatment	Paw thickness (cm) (Mean ± 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)	0.3±0.04	1.125±0.04 ^{###}	1.225±0.08 ^{###}	1.3±0.04 ^{###}	1.275±0.04 ^{###}
2	II	Standard, CFA (0.1 ml/rat, s.p) + Diclofenac sodium (15 mg/kg, p.o.)	0.3±0.05	0.8±0.04	0.775±0.04 ^{**}	0.7±0.04 ^{***}	0.45±0.08 ^{***}
3	III	Low dose, CFA (0.1 ml/rat, s.p) + EEMS (200 mg/kg, p.o.)	0.32±0.02	1.2±0.07	1.18±0.07	1.1±0.07	0.925±0.04 ^{**}
4	IV	High dose, CFA (0.1 ml/rat, s.p) + EEMS (400 mg/kg, p.o.)	0.25±0.04	1.12±0.04	0.95±0.04	0.9±0.07 ^{**}	0.55±0.06 ^{***}

All values are shown as mean ± 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

The percentage of inhibition of knee diameter in complete Freund's adjuvant induced arthritis rats was observed, decrease in knee diameter was gradually observed from 0th day to 28th day in II, III, IV groups when compared with the control group (Table 3).

Table No.3: Percentage inhibition of knee diameter in CFA induced arthritis rat

S.No	Group	Treatment	Knee diameter (cm) (Mean ± 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)	1.275±0.04	2.3±0.07 ^{###}	2.85±0.104 ^{###}	2.8±0.08 ^{###}	2.75±0.13 ^{###}
2	II	Standard, CFA (0.1 ml/rat, s.p) + Diclofenac sodium (15 mg/kg, p.o.)	1.3±0.07	2.02±0.07	2.22±0.07 ^{***}	1.77±0.04 ^{***}	1.45±0.02 ^{***}

3	III	Low dose, CFA (0.1 ml/rat, s.p) + EEMS (200 mg/kg, p.o.)	1.325±0.04	2.22±0.06	2.5±0.04*	2.35±0.08**	2.175±0.07**
4	IV	High dose, CFA (0.1 ml/rat, s.p) + EEMS (400 mg/kg, p.o.)	1.3±0.04	2.2±0.07	2.3±0.04**	1.8±0.04***	1.525±0.04***

All values are shown as mean ± 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

Haematological parameters were evaluated and it was observed decrease in the WBC count and ESR with the animals treated with EEMS while RBC count had shown an increase when compared to that of control group (Table 4).

Table No.4: Haematological parameters in CFA induced arthritis rat

Group	Treatment	On 28 th day Mean ± SEM				
		RBC (x 10 ⁶ / mm ³)	WBC (x 10 ³ / mm ³)	Hb (g/dl)	ESR (mm/hr)	RA Factor (IU/ml)
I	Control, CFA (0.6 mg/rat, intra plantar)	3.31±0.08###	13.38±1.3###	10.73±0.33###	10.67±0.45###	160±0.00
II	Standard, CFA (0.6 mg/rat, intra plantar) + Diclofenac sodium(15 mg/kg, p.o.)	5.50±0.12***	7.01±0.09***	13.94±0.17***	3.5±0.21***	15±5.0***
III	Low dose, CFA (0.6 mg/rat, intra plantar) + EEMS (200 mg/kg, p.o.)	4.21±0.09**	9.4±0.52*	11.5±0.04*	2.30±0.08**	7.175±0.07**
IV	High dose, CFA (0.6 mg/rat, intra plantar) + EEMS (400 mg/kg, p.o.)	5.01±0.09***	7.39±0.14***	12.3±0.04**	1.78±0.04***	9.225±0.04**

All values are shown as mean ± 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

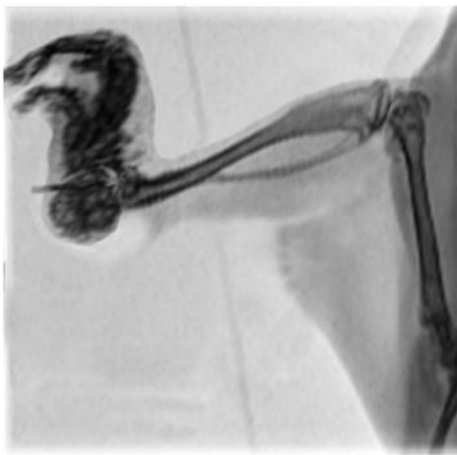
Interpretation of IR, NMR and Mass spectra for the isolated compound was performed and the presence of functional groups, mass number and structural data was analysed (Table 5).

Table 5: Physicochemical character and Structural interpretation of IR, NMR & Mass Spectra for the isolated compound

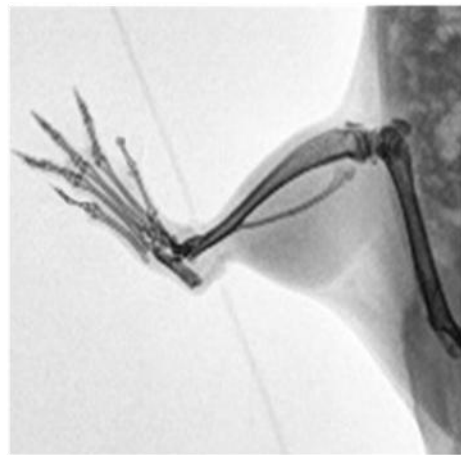
Code of the Compound	Appearance	IR (KBr) cm^{-1}	^1H NMR	Mass
EEMS	Pale yellowish	Keto(C=O) (std 1725) od 1720 Stretching, Ester (C-O) (std 1200) od 1224 Stretching, Ether (-O-) (std 1200-1000) od 1183 Stretching, Alcoholic OH (std 3600-3000) od 3312 Stretching, Alcoholic (C-O) (std 1100-1000) od 1070 Stretching	Methyl proton δ 1 -2 (6H), Hydroxy proton δ 1-4 (6H), Alkyl proton δ 1-4 (6H)	309.9 -ve ion impact

The radiological report which was evidenced both haematological and immunological parameters (Fig. 1).

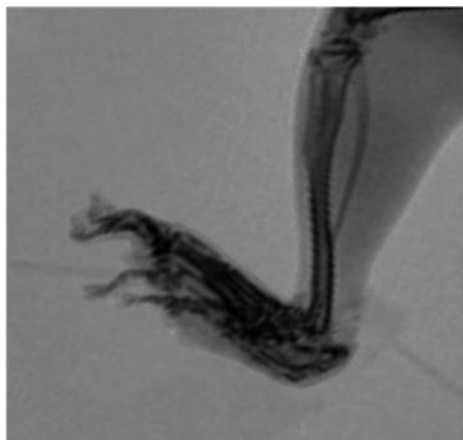
Figure 1: Radiological analysis of ethanolic leaf extract of *Myxopyrum smilacifolium* B.



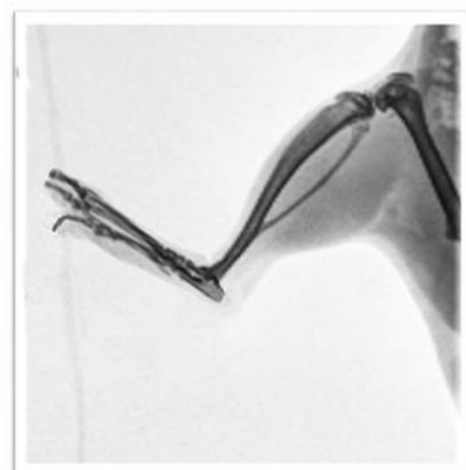
a. Control



b. Standard



c. EEMS-200mg/Kg



c. EEMS-400mg/Kg

Radiological images of A) Control group B) Standard group C) EEMS 200mg/kg D) EEMS 400mg/kg.

The data were expressed as Mean \pm SEM and the results were analyzed statistically by one way analysis of difference (ANOVA) method for six rats in each group. Differences in mean were considered to be significant at (P<0.01).

DISCUSSION

The result of current study indicates that EEMS extract exhibits anti-arthritis activity in rats with Freund's adjuvant induced arthritis. The complete form of CFA leads to production of certain immunoglobulin thereby induction of CFA results in swelling which lasts for weeks. Natural remedies for the treatment of rheumatoid arthritis were gaining the importance. *Xanthium strumarium* showed significant (P < 0.001) acute anti-inflammatory activity that it reduced the edema volume induced by Carrageenan administration (Mithun Vishwanath K. Patil *et al.*, 2012). *C.purpurea* may be a potential preventive or therapeutic candidate for the treatment of inflammation and arthritis (Gopal V.Bihania *et al.*, 2014). Thymoquinone administration brought down arthritic scoring and histology of bones by involvement in RA pathogenesis. (Sadiq Umar *et al.*, 2012). Acute toxicity studies revealed the non-toxicity nature of EEMS at the dose of 2000mg/kg. The current investigation was revealed that the effect of EEMS after injecting the animals with CFA suspension resulted in arthritis which is evidenced by visible clinical signs viz., edema, swelling at 7th day. In control group the signs of arthritis were continued to grow where as the animals treated with EEMS, the inflammatory symptoms were reduced. Rats with Ibuprofen 15mg/kg showed significant prevention in the paw volume on 14th day (P<0.01), 21st (P<0.001) and 28th (P<0.001) day as compared to the control group. Rats treated with EEMS at low dose (200mg/kg) showed significant prevention of paw volume on 21st (P<0.01) and 28th day (P<0.01) respectively where as the treatment with EEPM at high dose (400mg/kg) P.O showed significant prevention on 14th day (P<0.05), 21st day (P<0.001) and 28th day (P<0.001) respectively. The decreased level in the WBC count and ESR was observed with the animals treated with EEMS while RBC count had shown an increase when compared to that of control.

CONCLUSION

The study outcome was that, the ethanolic extract of *Myxopyrum smilacifolium Blume* posses a significant antiarthritic. Immunological, haematological and radiological analysis revealed the prominent antiarthritic activity of EEMS extract. The interpretation of isolated compound by Infrared, Nuclear Magnetic Resonance and Mass spectra revealed the presence of irridoid glycoside, Myxopyroside because of which the EEMS extract shows antiarthritic activity.

REFERENCES

1. Arora., R, Malhotra., P, Sharma., A, Haniadka., R, Yashawanth., HS, Baliga., MS. (2013). Medicinal efficacy of Indian Herbal remedies for the treatment of arthritis. Bioactive Food Dietary Interventions for Arthritis and Related Inflammatory Diseases, 601-617.

2. Raveesha Peeriga, and Chandrasekhar Kothapalli Bonnth. (2016) Pharmacognostical investigation and phytochemical screening of leaves of *Myxopyrum smilacifolium* B. Pharmacognosy Journal, 8(2), 159-164.
3. Kiew., R. (1984). The genus *Myxopyrum* L. (Oleaceae). *Blumea*, 29(2), 499-512.
4. Praveen., RP, Ashalatha Nair., S, (2001). Antimicrobial efficacy of methanolic extract of root, callus and fruit extracts of *Myxopyrum smilacifolium*. International Journal of Applied Sciences and Biotechnology, 64, 632-633.
5. Praveen., RP, Ashalatha Nair., S. (2015) Antioxidant potential of methanolic extract of root, callus and fruit extracts of *Myxopyrum smilacifolium*. International Journal of Biological and Pharmaceutical Research, 6(1), 51-55.
6. Gopalakrishnan., S, Rajameena., R, Vadivel., E. (2012). Phytochemical and Pharmacognostical studies of the leaves of *Myxopyrum serratum* A.W.Hill. Journal of Chemical and Pharmaceutical Research, 4(1), 788-794.
7. Vijaya kumar Harshani., NK, Wimalasiri., WR, Johannes., R. (1994). Coumarins and an indole alkaloid from *Pamburus missionis*. *Phytochemistry*, 36(4), 879-881.
8. David., LD, Kyong-Hwi., Park. (1975) Flavones and diterpenes of *Pamburus missionis*. *Phytochemistry*, 14, 1617-1620.
9. Jayasree., S, Vijayakumar., N, Suseela Gomathi., K, Mary Helen., PA. (2015). Study on antimicrobial activity and chemical profiling of *Pamburus missionis* (Night) Swingle. International Journal of Pharmacy & Pharmaceutical Research, 2(3), 118-129.
10. Pavithra., PS, Sreevidya., N, Rama., SV. (2009) Antibacterial activity and chemical composition of essential of *Pamburus missionis*. Journal of Ethnopharmacology, 124, 151-153.
11. Gopal., VB, Supada., RR, Subhash., LB. (2014). Anti-arthritis activity of methanol extract of *Cyathocline purpurea* (whole plant) in Freund's complete adjuvant-induced arthritis in rats. Biomedicine & Aging Pathology, 4, 197-206.
12. Vinod Nair., Surender Singh., Gupta., YK. (2011). Evaluation of the disease modifying activity of *Colchicum luteum* Baker in experimental arthritis. Journal of Ethnopharmacology, 133, 303-307.
13. Gurpreet kaur, Sarwat Sultana. (2012). Evaluation of antiarthritic activity of isoeugenol in adjuvant induced arthritis in murine model. Food and Chemical Toxicology, 50, 2689-2695.
14. Bandawane., DD, Beautikumari., SS, Gate., SS, Patel., AN. (2014). Evaluation of anti-arthritis activity of ethyl acetate fraction of *Cassia auriculata* Linn. leaves. Biomedicine & Aging Pathology, 4, 105-115.



15. Gullaiya., S. (2012). Modulation of disease related immune events by demethoxycurcumin against autoimmune arthritis in rats. *Biomedicine & Aging Pathology*, 3, 7-13.
16. Patil., MVK. (2012). Anti-arthritic and anti-inflammatory activity of *Xanthium srtumarium* L. ethanolic extract in Freund's complete adjuvant induced arthritis. *Biomedicine & Aging Pathology*, 2, 6-15.
17. Dhaval patel, Ginpreet Kaur, Mrunal Ghag Sawant, Pradeep Deshmukh. (2013). Herbal Medicine – A natural cure to arthritis. *Indian Journal of Natural Products and Resources*, 4(1), 27-35.
18. Amandeep kaur, Parminder Nain, Jaspreet Nain. (2012). Herbal plants used in treatment on Rheumatoid arthritis: A Review. *International Journal of Pharmacy & Pharmaceutical Sciences*, 4(4), 44-57.
19. Sonam Pandey. (2015) Preliminary phytochemical screening and invitro antibacterial activity of *Bauhinia variegata* Linn. against human pathogens. *Asian Pacific Journal of Tropical Disease*, 5(2), 123-129.