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Research Article

Antiarthritic activity of leaf extracts of *Pamburus missionis Swingle*

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ABSTRACT

The current work was carried to examine anti -arthritic activity of leaves of *Pamburus missionis in* male albino rats by using CFA induced arthritis model . Acute toxicity study was carried out according to OECD 425 guidelines. The study was carried out for 28 days where the animals were treated with 200 and 400 mg/kg ethanolic extract of leaves of *Pamburus missionis* after inducing arthritis in rats by Freund's adjuvant. Further on 28th day the rats were subjected for the evaluation of inflammatory parameters like paw volume, paw thickness and Knee diameter. Blood was retracted from each animal of retro-orbital venous plexus of rats and it i s collected into vial containing EDTA which is subjected for haematological parameters viz., RBC count, WBC count, Hb, ESR and RA factor. Both inflammatory and antiarthritic activity parameters were made endorsed by radiographic images. Treatment with ethanolic extract of *Pamburus missionis* showed significant (P<0.05) report at a dose of 400mg/kg body weight showed most potent and significant activity, evidenced by analyzing the inflammatory parameters, haematological parameters and radiographical analysis. Hence the current study revealed that ethanolic extract of *Pamburus missionis* (Swing.) possesses antiarthritic activity, due to the medial of phytoconstituents present in the plant extracts.

Keywords: Anti-arthritic activity; Complete Freund's adjuvant; *Pamburus missionis*; Radiographic analysis.

INTRODUCTION

Indian tradition which relies on plants to treat inconstant bodily disorders and the Indian traditional systems of medicine, the Ayurveda, Siddha, and Unani, as well as the innumerable folk medicines utilize plants to treat various ailments, including arthritis (R. Arora *et al*., 2013). Musculo skeletal diseases like rheumatoid arthritis resulted in severe morbidity and and mortality rate (M.S. Baliga *et al*., 2013). Multifarious works had identified oral administration of curcumin to arthritic rats step downed elevated levels of the glycoprotein GpA72 thereby considerably dropped off the inflammation (R. Arora *et al*., 2013). The preliminary phytochemical testability of leaves of *Pamburus missionis S.* had shown presence of secondary metabolites, have a crucial role in medicine (Raveesha *et al.,* 2016). *Pamburus missionis* is a small thorny shurb commonly called as kattunaranthi in tamil belonging to the family Rutaceae. It is widely distributed in southern India. The leaf of plant was used rubrically for the treatment of fistula, joint swellings, rheumatism, fractures and piles. The l*eaves* 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 activitiy. Hence the current study demonstrates the evaluation of antiarthritic activity of ethanolic extract of *Pamburus missionis*.

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MATERIALS AND METHODS

Collection and authentification of *Pamburus missionis*

The leaves of *Pamburus missionis S.* was collected from Tirumala hills, Tirupati, Chittoor and it was authenticated by the Botanist Dr. Madhavashetty. Head of the Department, Department of Botany, SV University, Tirupati. The leaves of *P. missionis* were dried in shade, coarsely powdered and stored in air tight container.

Preparation of extract:

The dried leaves of *Pamburus missionis* were extracted with ethanol by soxhlet apparatus for 72hr and resulted extract was made concentrated by rotary evarporater, 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 conditi ons, i.e., room temperature 22 ± 2 ^{0}C, relative humidity 45-55% and light dark cycle 12:12 hours. The animals were fed with commercial pellet rat feed (Mysore feeds, Banga-

lore) and water ad *libitum*. All the animal experiments were strictly compiled with ethical standards of animal handling and approved by Institutional Animal Ethics Commitee.

Acute toxicity study of ethanolic extract of leaves of *Pamburus missionis*

Acute toxicity study was carried out according to the guidelines, Economic Co-operation Development (OECD guidelines 425) stated by committee for the purpose of control and supervision of experiments on animals (CPCSEA) Animals were divided in six groups. The animals were fasted for overnight. The ethanolic extract of *Pamburus missionis* were administered orally in doses of 200mg/kg and 2000mg/ kg of bodyweight to mices belonging to different groups and keenly kept under observation for 14 days for mortality rate and behavioural changes.

Anti-arthritic activity

Male albino rats of Wistar strain were disunited 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 *Pamburus missionis* 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 EEPM 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 breakdowned statistically by one way analysis of difference (ANOVA) method. Differences in mena were considered to be significant at (P<0.01).

RESULTS AND DISCUSSION

The objective of the present study to evaluate the antiarthritic activity of ethanolic extract of *Pamburus missionis* by examining the inflammatory parameters like percentage of inhibition of paw volume in complete freund's adjuvant induced arthritis rats. The decrease in paw volume was gradually observed from 0 th 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 which were given in Table. 1. The decrease in paw thickness was subsequently observed 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±0.06 when compared to other groups which were given in Table. 2. The decrease in knee diameter was gradually observed from 0th day to 28th day in II, III, IV groups when compared with the control group which were given in Table. 3.

Haematological parameters were evaluated. The decrease in the WBC count and ESR was observed with the animals treated with EEPM while RBC count had shown an increase when compared to that of control group which were given in Table. 4. The data were expressed as Mean±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). Both haematological and immunological parameters were evidenced by radiological report which was shown in Figure 1.

The result of current study indicates that EEPM extract exhibits anti-arthritic 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 srtumarian showed significant (P < 0.001) acute anti - inflammatory activity that it reduced the edema volume induced by Carrageenan administration (Mithun Vishwanath K. Patil *etal*., 2012). C.purpurea may *be* a potential preventive or therapeutic candidate for the treatment of inflammation and arthritis (Gopal V.Bihania*etal*., 2014). Thymoquinone administration brought down arthritic scoring and histology of bones by involvement in RA pathogenesis. (Sadiq Umar *etal*., 2012). Acute toxicity studies revealed the non-toxicity nature of EEPM at the dose of 2000mg/kg. The current investigation was revealed that the effect of EEPM 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 EEPM, 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 EEPM 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

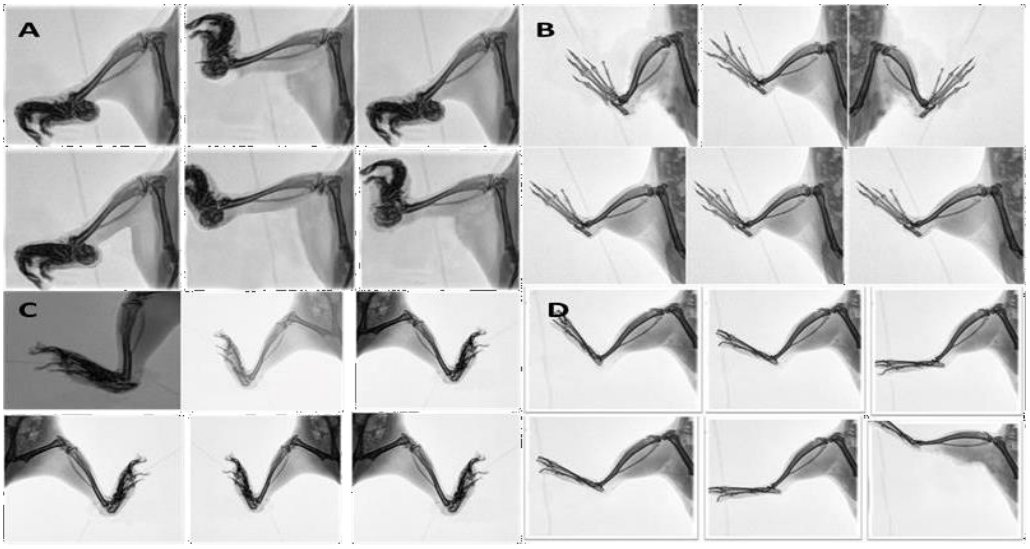

Figure 1: Radiological observations of ethanolic leaf extract of P. missionis

Table 1: Percentage constrained of paw volume in CFA induced arthritis rat

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

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

Table 2: Percentage constrained of paw thickness in CFA induced arthritis rat

S. No	Group	Treatment	Paw thickness (cm) (Mean ± SEM) on				
			0th day	7th day	14th day	21st day	28th 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) + EEPM (200 mg/kg, p.o.)	0.325±0.02	1.2±0.07	1.2±0.07	1.1±0.07	0.925±0.04**
4	IV	High dose, CFA (0.1 ml/rat, s.p) + EEPM (400 mg/kg, p.o.)	0.275±0.04	1.125±0.04	0.975±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

Table 3: Percentage constrained of knee diameter in CFA induced arthritis rat

S.No	Group	Treatment	Knee diameter (cm) (Mean ± SEM) on				
			0th day	7th day	14th day	21st day	28th 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) + EEPM (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) + EEPM (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

Table 4: Haematological observations in CFA induced arthritis rat

Group	Treatment	On 28th 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) + EEPM (200 mg/kg, p.o.)	4.25±0.09**	10.4±0.52*	11.78±0.143*	8.9±0.23**	70±10.0***
IV	High dose, CFA (0.6 mg/rat, intra plantar) + EEPM (400 mg/kg, p.o.)	5.16±0.09***	7.33±0.14***	12.13±0.209**	4.47±0.12***	20±0.00***

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

treated with EEPM while RBC count had shown an increase when compared to that of control.

CONCLUSION

The study outcome was that, the ethanolic extract of *Pamburus missionis* posses a significant antiarthritic activity . Immunological, haematological and radiological analysis revealed the prominent antiarthritic activity of EEPM extract. Thus further investigations were have to be made to find out the phytoconstituents responsible for antiarthritic activity along with mechanism of action.

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