Pharmacognosy Magazine
ISSN: 0973-1296

PHCOG MAG.
An official Publication of Phcog.Net

PHCOG MAG.: Research Article
Effect of *Celastrus paniculatus* Willd. seed on adjuvant induced arthritis in Rats

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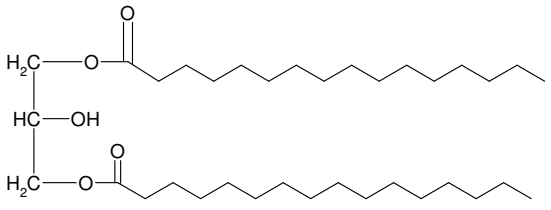
ABSTRACT - In this study, the anti-arthritic effect of oral administration of petroleum ether, alcoholic extracts of *Celastrus paniculatus* Willd seed on Freund's adjuvant arthritis has been studied in Wistar albino rats. The body weight loss that was found during the arthritic condition was corrected on treatment with petroleum ether, alcoholic extracts of *Celastrus paniculatus* Willd seed. The swelling of the paw during the secondary lesions was also markedly reduced. Hematological parameters like haemoglobin content, total WBC count, ESR and RBC were also estimated. The results indicated that the seed of *Celastrus paniculatus* is endowed with anti-arthritic activity.

KEYWORDS - *Celastrus paniculatus*, Anti-arthritic, Freund's complete adjuvant, ESR

INTRODUCTION

Celastrus paniculatus Willd (Family-Celastraceae) is a large deciduous climber, distributed through the hilly parts of Himalaya at an altitude of 1200 m, South Gujarat, Central India, Burma and China. *C. paniculatus* has been claimed in traditional literature to be valuable against a wide variety of diseases. The use of seeds of *C. paniculatus* in the treatment of a number of ailments, including antirheumatic, aphrodisiac, emetic, laxative and nervine tonic. Decoction is beneficial in gout, leprosy, paralysis, anti-inflammatory and arthralgia (1-3). The seed oil mainly contains palmitic acid, oleic acid, linoleic acid, linolenic acid and their glycerol esters mainly ∝,∝'-dipalmitoyl glycerol [Figure 1]. The oil also contains sesquiterpene alkaloids viz. Celapanin, Celapanigin and Celapagin [Figure 2] (4).

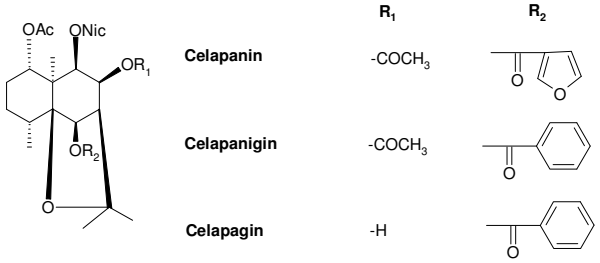
Figure 1 - *Glycerol ester in C. paniculatus*



α, α' Dipalmitoyl glycerol

In recent years, the extracts of flowers, seeds, leaves and barks of *C. paniculatus* have been extensively studied for many potential uses including anti-oxidant (5), analgesic and anti-inflammatory (6) and anti-anxiety activity (7). Adjuvant induced arthritis is a chronic crippling, skeletomuscular disorder having nearest approximation to human rheumatoid arthritis for which no medicine is available effecting a permanent cure in the recent times.

Figure 2 - Sesquiterpene alkaloids in C. paniculatus



The modern drugs both steroidal and non-steroidal anti-inflammatory drugs are used for the amelioration of the symptoms of the disease, however they offer only temporary relief and also produce severe side effects (8). In the indigenous system of medicine, the seed oil of *C. paniculatus* is reported to be useful in the treatment of rheumatoid arthritis (9). However, so far no systematic study has been reported regarding the anti-arthritic activity of extracts of *C. paniculatus* seeds. In the present study, an effort has been made to establish the scientific validity for the anti-arthritic activity of *C. paniculatus* seed extracts using adjuvant induced arthritis.

MATERIALS AND METHODS

Plant material

The seeds of *C. paniculatus* were collected from local market of Belgaum, Karnataka, India and authenticated at the Department of Botony, R.L.S. Institute, Belgaum, Karnataka by Prof. R.S. Goudar. A voucher specimen of the plant has been deposited in K.L.E.S's College of Pharmacy, Belgaum under the number K.L.E/2005/Tech.887. The seeds were dried in shade and coarsely powdered (40 mesh size).

Preparation of extract

The petroleum ether extract of seed was prepared using petroleum ether (40-60^{0}C) by soxhlet method at a temperature of 40-60^{0}C (10). The ethanolic extract was prepared using ethanol by soxhlet method at a temperature of 40-60^{0}C. The extract were concentrated under vacuum and dried over anhydrous sodium sulphate. The petroleum ether extract yielded oil that was reddish brown in colour. The ethanolic extract yielded semisolid, viscous, dark coloured mass. A suspension of petroleum ether extract in 1% tween 80 (11) and ethanolic extract in 1% (w/v) gum acacia (12) was prepared for oral administration by gastric intubation method.

Pharmacological screening for anti-arthritic activity

Animal selection

For acute toxicity studies and anti-arthritic activities, Wister albino rats weighing between 150 g to 200 g were selected. The animals were acclimatized to standard laboratory conditions (temperature 25 ± 2ºC) and maintained on 12 h light, 12 h dark cycle. They were provided with regular rat chow (Lipton India Ltd., Mumbai, India) and drinking water ad libitum. The animal care and experimental protocol were in accordance with the Institutional Animal Ethical Committee (IAEC).

Acute toxicity studies

The acute oral toxicity study (13) was carried out as per the guideline set by the Organization for Economic Co-operation and Development (OECD) received from the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). One tenth of the medium lethal dose (LD_{50}) was taken as an effective dose (14).

Freund's Adjuvant induced Arthritis

Freund's Adjuvant induced Arthritis model (15) was used to assess the anti-arthritic activity in albino rats. Animals were randomly divided into four groups of six animals each (n=6). Group I served as control received 1% tween 80, Group II received Diclofenac sodium (10 mg/kg p.o.) served as reference standard and Group III and IV received the crude extracts of seeds of petroleum ether and alcohol p.o. respectively.

Arthritis was induced by injecting a 0.05 ml (0.5% w/v) suspension of killed *Mycobacterium tuberculosis* bacteria homogenized in liquid paraffin into the left hind paw. Drug treatment was started from the initial day i.e. from the day of adjuvant injection (0 day), 30 minutes before adjuvant injection and continued till 21st day. Paw volume was measured on 4th, 8th, 14th and 21st day by using plethismometer. The mean changes in injected paw edema with respect to initial paw volume, were calculated on respective days and % inhibition of paw edema with respect to untreated group was calculated using following formula.

$$i = \left(1 - \left(\frac{\Delta V_{Treated}}{\Delta V_{Untreated}} \right) \right) \times 100$$

Where,

i = % inhibition of paw edema

$\Delta V_{Treated}$ *= Mean change in paw volume of treated rat*

$\Delta V_{Untreated}$ *= Mean change in paw volume of untreated rat*

The changes in body weight were recorded daily. At 22nd day blood was withdrawn through retroorbital vein puncture of all groups by anaesthetizing the animals with diethyl ether and the biochemical parameters like haemoglobin content, total WBC count, ESR and RBC were analysed.

Statistical analysis

The experimental results are represented as Mean ± SEM (Standard Error of Mean). Statistical analysis was performed by one-way ANOVA followed by Dunnet's 't' test. P< 0.05 were considered significant.

RESULTS

From the acute toxicity study, the LD_{50} cut-off dose for pet-ether extract and alcoholic extract were found to be 5000 mg/kg and 3000 mg/kg body weight respectively. Hence, the therapeutic doses were taken as 500 mg/kg and 300 mg/kg body weight for pet-ether and ethanolic extracts respectively.

In adjuvant-induced arthritis model rats developed a chronic swelling in multiple joints with influence of inflammatory cells, erosion of joint cartilage and bone destruction and remodeling. These inflammatory changes ultimately result in the complete destruction of joint integrity and functions in the affected animal (16).

Table 1 - Mean changes in paw volume and percentage inhibition of paw volume in adjuvant-induced arthritis in rats

Group	Changes in Paw Volume				% Inhibition of Paw Volume			
	4th day	8th day	14th day	21st day	4th day	8th day	14th day	21st day
Control	5.26 ± 0.26	5.34 ± 0.20	5.24 ± 0.21	5.13 ± 0.22	0	0	0	0
Standard	4.63** ± 0.15	3.95** ± 0.25	3.13** ± 0.29	1.31** ± 0.18	11.41	25.84	40.59	74.87
Petroleum Ether Extract (500 mg/kg)	4.69** ± 0.22	4.10** ± 0.24	3.19** ± 0.12	1.61** ± 0.11	10.91	23.31	38.74	68.32
Alcoholic Extract (300 mg/kg)	4.89 ± 0.11	4.41 ± 0.17	3.66** ± 0.13	2.87** ± 0.10	6.23	17.43	29.86	43.97

*All values are in Mean ± SEM. ; p<0.05 = Significant, ** p<0.01 = More significant vs. Control; n = 6*

Table 2 - Effect on haematological parameters in adjuvant induced arthritis in rats.

Group	Changes in haematological parameters (Mean ± SEM)			
	Total WBC Count (cells/cu. mm)	RBC Count (million/cu.mm)	Hb (gm%)	ESR (mm/hr)
Control	7.85±0.08	5.20±0.10	13.73±0.25	4.05±0.17
Standard	7.15±0.11**	5.22±0.12	14.08±0.12	3.25±0.12**
Pet. Ether extract	7.03±0.08**	5.58±0.14	14.50±0.13**	3.22±0.15**
Alcohol extract	7.25±0.11**	5.37±0.09	14.10±0.12	3.50±0.17*

*All values are in Mean ± SEM ; *p<0.05 = Significant,*
*** p<0.01 = More significant vs. Control; n = 6*

Table 3 - Changes in body weight in adjuvant induced arthritis in rats

Group	Mean body weight (gm)		Mean changes in body weight (±SEM)
	Before induction	on 21st day	
Control	158.3	166.6	8.33±1.667
Standard	155.8	195.5	40±2.582**
Pet. Ether extract	150.8	174.2	23.33±4.595**
Alcohol extract	151.6	162.5	10.83±1.537

*All values are in Mean ± SEM ; *p<0.05 = Significant,*
*** p<0.01 = More significant vs. Control; n = 6*

The pet-ether extract inhibited the rat paw oedema by 68.32%, alcoholic extract 43.97% whereas diclofenac sodium produce 74.87% inhibition of rat paw oedema after 21 days (Table 1). As shown in Table 2 standard drug, pet-ether and alcoholic extracts have shown the increase in Haemoglobin content compare to control. The total WBC counts were remarkably increased in adjuvant-induced rats (Table-2 Control group).

However, *C. paniculatus* seed extracts and standard drug treated group significantly decreased ($P<0.01$) the total WBC count. The ESR count, which drastically increased in arthritic control group, has been remarkably counteracted by the standard and extracts, restoring it back to normal thus justifying its significant roles in arthritic conditions.

The loss of body weight observed during the arthritis condition. The standard drug petroleum ether and alcoholic extract treatment significantly increased the body weight.

DISCUSSION

In the present study, rats were selected to induce arthritis because rats develop a chronic swelling in multiple joints with influence of inflammatory cells, erosion of joint cartilage and bone destruction. It has close similarities to human rheumatoid disease (17). The determination of paw swelling is apparently simple, sensitive and quick procedure for evaluating the degree of inflammation and the therapeutic effects of drugs. Chronic inflammation involves the release of number of mediators like cytokines (IL-IB and TNF-$\propto$), GM-CSF, interferon's and PGDF. These mediators are responsible for the pain, destruction of bone and cartilage that can lead to severe disability (18). However, standard drug, petroleum ether and alcoholic extract significantly suppressed the swelling of the paws.

In arthritis condition there is a mild to moderate rise in WBC count due to release of IL-IB inflammatory response IL-IB increases the production of both granulocyte and macrophages colony stimulating factor (18,19). In the present study, the migration of leucocytes into the inflamed area is significantly suppressed by the standard drug and petroleum ether extract as seen from the significant decrease in total WBC count.

Erythrocyte sedimentation rate (ESR) is an estimate of the suspension stability of RBC's in plasma. It is related to the number and size of the red cells and to the relative concentration of plasma proteins, especially fibrinogen, $\propto$ and β globulins. Increase in the rate is an indication of active but obscure disease processes. The acute phase proteins in ESR and C-reactive protein (CRP) share the property of showing elevations in the concentration in response to stress or inflammation like injection, injury, surgery and tissue necrosis. The ESR count, which drastically increased in arthritic control group has been remarkably counteracted by the standard, extracts and back to normal thus justifying its significant role in arthritic conditions. (19)

Changes in body weight have also been used to assess the course of the disease and the response to therapy of anti-inflammatory drugs (20). As the incidence and severity of arthritis increased, the changes in the body weights of the rats also occurred during the course of the experimental period. The loss of the body weight during arthritic condition was also supported by earlier observation (21) on alterations in the metabolic activities of diseased rats. Earlier findings suggest that absorption of ^{14}C- glucose and ^{14}C- leucine in rats intestine was reduced in the case of inflamed rats (22). But on the treatment with anti-inflammatory drugs, the decrease in absorption was nullified (23) and it shows that the anti-inflammatory drugs correct the decreased/deranged absorption capacity of intestine during inflammation. The increased body weight during treatment of standard drug, petroleum ether and alcoholic extracts may be due to the restoration of absorption capacity of intestine.

From the results observed in the current investigation, it may be concluded that the petroleum ether extract, alcoholic extract of seed of *C. paniculatus* possesses potentially useful anti-arthritic activity since it was active in both the inflammation models and adjuvant.

ACKNOWLEDGEMENTS

We thank the Principal, K. L. E. Society's College of Pharmacy, Belgaum for providing the facilities to carry out this study.

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