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Research Article

EFFECT OF *COCCULUS HIRSUTUS* LEAVES EXTRACT ON FREUNDS COMPLETE ADJUVANT AND FORMALDEHYDE INDUCED ARTHRITIS

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ABSTRACT
Cocculus hirsutus Linn (Menispermaceae) leaves known as chilahinta in ayurveda and kattukudi in sidha is an important medicinal plant belonging to family menispermaceae .the leaves are used to treat several disease like eczema, dysentery and urinary problem . In the present study ethanolic extract of *Cocculus hirsutus leaves* was used for investigation of antiarthritic activity by using freunds complete adjuvant and formaldehyde as inducer. Animals treated with ethanolic extract of cocculus hirsutus leaves at the dose 200mg/kg and 400mg/kg showed significant decrease in arthritis in all models. The results suggests that the extract at 400mg/kg showed significant reduction ($p<0.001$) in paw diameter when compared with control group of animal. These findings indicate that cocculus hisutus *leaves* extract shows significant antiarthritic activity.
KEYWORDS: arthritis, *Cocculus hirsutus*, freund's complete adjuvant.

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic systemic inflammatory disease associated with long-term disability and premature mortality. Many medicines are clinically prescribed for treating this hard-to-cure illness. Conventional medicine, including treatment with steroids, nonsteroidal anti-inflammatory drugs (NSAIDs) and such biological agents as tumor necrosis factor alpha (TNF-α) and interleukin-1beta (IL-1β) antagonists (Fleischmann et al., 2004), has shown only limited success against RA (American College of Rheumatology, 1996; Chandrashekara et al., 2002). Such therapies are helpful in controlling the symptoms of acute RA, but their effects on chronic, prolonged RA are unsatisfactory. Moreover, the adverse effects of drug therapy are significant and include gastrointestinal disturbances, infections and cardiovascular risks (Scheiman, 2001; Mangge et al., 2003; Rubbert-Roth and Perniok, 2003; Ortiz, 2004). The inflammatory process of RA is reportedly associated with an increase of the pro-inflammatory cytokines TNF-α and IL-1β (Dayer, 2003; Fleischmann et al., 2004; Shin et al., 2003).

Cocculus hirsutus Linn (Menispermaceae) is commonly known as Jal-jammi (Chopra et al., 1958). It is a climber found in tropical and sub-tropical regions of India. A decoction of the leaves is taken in eczema, dysentery and urinary problem. Leaves and stem are used for treating eye diseases. Roots and leaves are given for Sarsaparilla, as diuretic and in gout (Nadkarni, 1982). Ethanolic extract of whole plant showed the presence of isoquinoline alkaloid d-trilobine and dl-coclaurine (Jaganatha, 1961), Cohirsinine (Viquaruddin, 1991), Jamtinine (Viquaruddin, 1992) cohirsutine (Viquaruddin, 1993). Aerial parts of the plant reported to be used as a diuretic, laxative (Ganapathy et al., 2002) and root extract showed analgesic and anti-inflammatory effect (Nayak, 1993). Leaf juice of this plant is used in the treatment of eczema (Masilamani, 1981).

The Literature review revealed that antioxidant, Hypoglycaemic acitivity. Since not much study had been done to evaluate the biological activity of the plant, the present study is focused to evaluate the antiarthritic activity of *Cocculus hirsutus leaves*.

MATERIAL AND METHODS

Plant material

The leaves of *Cocculus hirsutus* were collected from Vaikalmedu, Erode (Dist), tamilnadu in the month of august 2009. The plant was identified and authenticated by the experts in the botnical survey of india coimbatore, Tamil Nadu, India (No. BSI/SC/5/23/08-09/Tech.1754). The leaves were shade dried, pulverized by a mechanical grinder and stored in a well-closed container for further extraction

Preparation of the extract

The leaves were dried in shade at room temperature and coarsely powdered. The ethanolic extract was prepared using ethanol by maceration process. The grinded leaves is macerated with absolute ethanol for 7 days following the process called simple maceration. After 7 days of maceration, evaporation of solvent was done to obtain semisolid product which was used for further studies.

Animals

Wistar rats (150 – 200 g) and Swiss albino mice (18 – 22 g) of either sex were used in this study. They were maintained under controlled temperature (23 ± 2°C) and relative humidity (40 – 60%) with standard environmental conditions of 12/12 light/dark cycle in the Departmental animal house. They were housed in polypro-pylene cages with free access of food and water ad libitum. The cages were cleaned daily by changing the sawdust bedding. The experimental protocol was approved by Institute's animal ethical committee (688/2/C-CPCSEA); care and use of laboratory animals were confirmed to national guidelines.

ANTIARTHRITIC STUDIES

a. Prophylactic model

Animals were randomly divided into four groups of five animals each (n=5). Group I served as a Control received 0.5% CMC, Group II received Indomethacin (10mg/kg p.o.) served as a standard, Group III and IV received the ethanolic extract of *Cocculus hirsutus* at the dose of 200mg/kg and 400mg/kg p.o. respectively. Rats were made arthritic by

single intra-dermal injection of 0.1 ml of Freund's complete adjuvant (1 mg dry heat killed Mycobacterium tuberculosis per millilitre sterile paraffin oil) into a foot pad of the left hind paw. Drug treatment was started from the initial day i.e. from the day of adjuvant injection (0day), 30 minutes before adjuvant injection and continued till 21st day (Bendele *et al*., 2001). The paw thickness was measured every 4th day till 21st day by using digital verniar calipers (Vasudevan *et al*., 2006).

b. Therapeutic model

Animals were randomly divided into four groups of five animal each (n=5). Group I served as a control received 0.5% CMC, Group II received Indomethacin (10 mg/kg p.o.) served as a standard, Group III and IV received ethanolic extract of *Cocculus hirsutus* at the dose of 200mg/kg and 400mg/kg p.o. respectively. Wistar rats were made arthritic by single intra-dermal injection of 0.1 ml of Freund's complete adjuvant (containing 1.0 mg dry heat killed Mycobacterium tuberculosis per milliliter sterile paraffin oil) into a foot pad of the left hind paw of rats. Drug treatment was started from 8th day i.e. from the day of adjuvant injection and continued till 28th day (Bendele *et al*., 2001). The paw thickness was measured every 7th day till 21st day by using digital verniar calipers (Vasudevan *et al*., 2006).

c. Formaldehyde induced arthritis model

Animals were divided into following Group I served as a control received 0.5% CMC, Group II received indomethacin (10 mg/kg p.o.) served as a standard, Group III and IV received ethanolic extract of *Cocculus hirsutus* at the dose of 200mg/kg and 400mg/kg p.o. respectively. Rats were injected with 0.1 ml of formaldehyde solution in the sub-planter surface of the left hind paw, on the first and third day of the test. Ethanolic extract of *Cocculus hirsutus* (200 & 400mg/kg) and Indomethacin (10 mg/kg) were administered orally once a day for 10 days. The rat paw thickness was measured daily for 10 days. The percent inhibition of the mean increase in the paw edema of each group was calculated on the tenth day and compared with the control. The rat paw thickness was measured using digital verniar calipers (Vasudevan *et al*., 2006).

RESULTS

Prophylactic effect of ethanolic extract of Cocculus hirsutus (EECH) on Freund's complete adjuvant induced arthritis in rats.

Effect of EECH (200 mg/kg, 400mg/kg) and Indomethacin (10mg/kg) on paw thickness in c Freund's complete adjuvant induced arthritis in rats was shown on table.1. In the control group paw thickness showed changes up to 8th day and slightly decreased on day 21. The paw thickness of Indomethacin treated group significantly ($p<0.01$) decreased as compared to control group. ethanolic extract of *Cocculus hirsutus* (400mg/kg) treated group rat showed significant reduction in paw thickness only on 21st day. Ethanolic extract of *Cocculus hirsutus* (200mg/kg) did not produced any significant reduction on paw thickness. Percent inhibition of paw thickness of ethanolic extract of *Cocculus hirsutus* (400mg/kg) and Indomethacin was 65% and 76% respectively.

Therapeutic effect of ethanolic extract of *Cocculus hirsutus* (EECH) on Freund's complete adjuvant induced arthritis in rats.

Effect of ethanolic extract of *Cocculus hirsutus* (200 mg/kg, 400mg/kg) and Indomethacin (10mg/kg) on paw thickness in Freund's complete adjuvant induced arthritis in rats was shown on table.2. The paw thickness of Indomethacin treated group significantly ($p<0.001$) decreased as compared to control group. Ethanolic extract of *Cocculus hirsutus* (400mg/kg) treated group rat showed significant reduction in paw thickness after 7th day. The effect of ethanolic extract of *Cocculus hirsutus* (200mg/kg) on paw thickness was less significant. Percent inhibition of paw thickness of ethanolic extract of *Cocculus hirsutus* (400mg/kg) and Indomethacin was 55.7% and 65% respectively.

Effect of ethanolic extract of *Cocculus hirsutus* (EECH) on formaldehyde induced arthritis in rats.

Effect of effect of ethanolic extract of *Cocculus hirsutus* (200 mg/kg, 400mg/kg) and Indomethacin (10mg/kg) on paw thickness in Formaldehyde induced in rats was shown on table.3. Oral treatment of effect of ethanolic extract of *Cocculus hirsutus* (400mg/kg) and indomethacin (10mg/kg) significantly reduced the paw edema induced by formaldehyde in rats. On 2nd day on wards indomethacin showed significant reduction in paw oedema ($P<0.01$). Effect of ethanolic extract of *Cocculus hirsutus* (400mg/kg) showed the similar effect only on 9th day and EECH (200mg/kg) on 10th day. Percent inhibition of edema of EECH (200, 400mg/kg) and Indomethacin was 53.2%, 62.5% and 71.6% respectively.

DISCUSSION

Arthritis is a chronic inflammatory disease that affects several parts of the joints including the cartilage, synovium, tendon and muscles. In the present study, rats were selected to induce arthritis because rats develop a chronic swelling in multiple joints with an influence of inflammatory cells, erosion of joints cartilage and bone destruction (Patil et al., 2007). Freunds complete Adjuvant (FCA) induced models are extensively used to study the pathogenesis of rheumatoid arthritis for testing therapeutics. One of the reasons for the wide utilization of this model is due to strong correlation between the efficacy of the therapeutic agents in animal model and in rheumatoid conditions in human. In adjuvant arthritis, bacterial peptidoglycan and muramyl dipeptide are responsible for arthritic induction. It occurs through cell mediated autoimmunity by structural mimicry between mycobacteria and cartilage proteoglycans in rats (Kumar et al., 2008).

In the present study the prophylactic effect EECH on FCA induced arthritis in rats showed inhibitory effect on arthritis. RA is a chronic cytokine mediated destructive inflammatory polyarticular joint disease, characterized by massive synovial proliferation, systemic and local inflammation resulting in cartilage and bone destruction (Meera et al. 2008; Mohr et al., 1976). In the present study adjuvant induced arthritis in rats demonstrated the inhibiting effect of EECH. Chronic inflammation involves the release of number of mediators like cytokines, granulocyte, colony stimulating factor, platelet derived growth factor (PGDF), monocyte and interferon. Paw edema is major factor in assessing the degree of inflammation and therapeutic efficacy of the drugs. (Meera et al., 2008; Sakuma et al., 2001). Paw edema in Adjuvant induced arthritis in rats is known due to involve of inflammation. After FCA injection on the rat hind paw, a pronounced swelling and hyperalgesia appeared with no involvement of contra lateral paw. This response is considered as primary response. The mediators of chronic inflammation are responsible for pain, severe destruction of bone and cartilage that can lead to severe disability (Kumar et al. 2008).

According to our results of investigation, EECH 400mg/kg dose showed significant, more reliable and effective activity by suppressing the paws oedema. The activity exhibited by extract was in dose dependent manner. In addition increased sensitivity of the affected paw to pressure or flexion and extension of the inflamed paw joints.

The arthritis in rats is associated with spontaneous behaviors such as protection of the affected paw, evidenced by curving or elevation of the paw, as well as avoidance of supporting the body on the paw. In RA musculoskeletal pain after report an undesired reduction of their daily activity level. This was due to impact of pain on daily functioning is generally expressed as a disability (Verbunt et al., 2008).

Paw thickness are also used for assessment of RA. Paw diameter decreased significantly in EECH and Indomethacin treated rats.

In the present study the therapeutic effect EECH on FCA induced arthritis in rats showed significant inhibitory effect on arthritis. The latent secondary response occurs after few days and is characterized by joint swelling. The secondary response could be due to the liberation and over production of bradykinin, prostaglandins, and kinins in paw tissue which accompanies leukocyte migration (Kumar et al., 2008). The inhibition of the increase in the hind paw swelling may be associated with inhibition of cell infilteration, neutrophill infiltration and bone erosion. In inflammatory process, there are fenestration of the microvasculature, leakage of the elements of the blood into interstitial spaces and migration of leuckocyte into inflamed tissue (Mythilypriya et al., 2007). The initial inflammatory response was developed within few hours, but more critical clinical signals emerged from the 10^{th} post-inoculation day and thereafter the alterations remain detectable for several weeks. Standard drug Indomethacin and EECH(400mg) treated rats showed significantly decreased paw volume from day 14^{th} after inoculation of adjuvant. In the present investigation the arthritic rats showed a soft tissue swelling that was noticeable around the ankle joints during the acute phase of arthritis and was due to be edema of particular tissues such as ligaments and joint capsules. The swelling has been found to be increasing in the initial phase of inflammation and then becomes constant in two weeks. The change in paw volume has been found to associate with an increase in granulocyte and monocytes. Because, the activation of macrophages results in the production of several cytokines including IL-1, IL-6, interferon-γ and TNF-α which have been implicated in immune arthritis (Mythilypriya et al., 2007).

Inflammation in rats experimentally study may attribute to the ability to inhibit various chemical mediators of inflammation like histamine and 5-HT during initial phase (Meera et al., 2008; Harsh, 2000). Paw swelling is apparently simple sensitive and quick procedure for evaluating the degree of inflammation and therapeutic effect of drugs. Chronic inflammation involves the release of number of mediators like cytokines (IL-1B, TNF), interferons and PGDF. These mediators are responsible for the pain, destruction of bone and severe disability (Ericet al., 1996; Patil et al., 2007). *In vivo* and *in vitro* anti-inflammatory effects have been reported for several flavonoids (Clavin et al., 2007).

In the formaldehyde induced arthritis test, the peak inhibitory effect (62.5) was produced by the extract at the dose of 400mg/kg. The mechanism of the anti-inflammatory effect of EECH in formaldehyde induced arthritis in rats may depend on the neutralization of the active globulins (Vasudevan, 1999). Swelling around ankle joint and paw of arthritic rat was considered to be due to edema of particular tissue such as ligament and capsule. The reduction of paw edema and soft tissue thickening at depth site could probably due to the effect EECH.

Thus, in the light of above facts, it can be demonstrated that the EECH may serve as an effective anti-arthritic drug and the effect might be speculated due to phytochemicals such as triterpenoids, alkaloids and flavonoids. This study warrants the investigation to isolate and identify the active principles and to investigate the exact mechanism of action of EECH against arthritis.

CONCLUSION

Ethanolic extract of *Cocculus hirsutus* leaves were evaluated for anti-arthritic activity in rats and mice. The ethanolic extract of *Cocculus hirsutus* leaves possess anti-arthritic activity on dose dependant manner. Anti-arthritic activity of *Cocculus hirsutus* may be due to the presence of phyto-constituents such as triterpenoids, flavonoids and saponins. Finding from the present study justify traditional use of *Cocculus hirsutus* in Indian Ayurvedic medicine in the treatment of inflammation and arthritis condition.

Further investigations are needed to identify and isolate the active chemical constituent responsible for the exact mechanism of action against inflammation and arthritis.

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Table1: Prophylactic effect of EECH (200 mg/kg, 400mg/kg) and Indomethacin (10mg/kg) on paw thickness in Freund's complete adjuvant induced arthritis in rats.

Groups	Dose (mg/kg)	Mean difference in rat paw thickness (mm)				%inhibition
		4th Day	8th Day	14th Day	21st Day	
Control	--	4.92±0.21	5.11±0.23	4.90±0.20	4.77±0.25	--
Indomethacin	10	2.97±0.15	1.42±0.20 *	1.27±0.19**	1.11±0.34**	76%
EECH(200mg)	200	3.27±0.16	3.09±0.12	2.85±0.19	2.40±0.20	48%
EECH(400mg)	400	3.15±0.21	2.67±0.18	1.77±0.20*	1.61±0.24*	65%

Values are expressed as mean ±S.E.M. (n=5), (***p<0.001, ** p<0.01,*p<0.05) as compared to control.

Table 2: Therapeutic Effect of EECH (200 mg/kg, 400mg/kg) and Indomethacin (10mg/kg) on paw thickness in Freund's complete adjuvant induced arthritis in rats.

Groups	Dose (mg/kg)	Mean difference in rat paw thickness (mm)				%inhibition
		7th Day	14th Day	21th Day	28st Day	
Control	--	3.45±0.19	3.35±0.16	3.28±0.19	2.98±0.14	--
Indomethacin	10	3.80±0.17	2.98±0.26*	2.01±0.05*	1.07±0.08**	65%
EECH(200mg)	200	4.02±0.12	3.82±0.08	3.02±0.09	2.22±0.06	25%
EECH(400mg)	400	3.92±0.09	3.05±0.09	2.73±0.15*	1.32±0.09*	55.7%

Values are expressed as mean ±S.E.M. (n=5), (***p<0.001, ** p<0.01,*p<0.05) as compared to control.

Table3: Effect of EECH (200 mg/kg, 400mg/kg) and Indomethacin (10mg/kg) on paw thickness in formaldehyde induced arthritis in rats.

Day	Mean difference in rat paw thickness (mm)			
	control	Indomethacin (10mg/kg)	EECH(200mg)	EECH(400mg)
1st day	3.71 ±0.21	2.62±0.23	2.82±0.20	2.79±0.25
2nd day	3.49±0.04	2.48± 0.03*	2.65±0.05	2.57±0.04
3rd day	3.39±0.15	2.15±0.20*	2.58±0.19	2.52±0.34
4th day	3.62± 0.07	2.32±0.03*	2.74± 0.04	2.64±0.04
5th day	3.40±0.16	2.11±0.12*	2.52±0.19	2.42±0.20
6th day	3.36±0.21	1.58±0.18*	2.38±0.20	2.22±0.24
7th day	3.29±0.03	1.27± 0.04*	2.12± 0.02	1.89±0.33
8th day	3.25±0.03	1.09± 0.02*	1.75±0.03	1.32±0.06
9th day	3.12± 0.08	0.98±0.05*	1.51±0.02	1.27±0.07*
10th day	3.01±0.07	0.72±0.05*	1.40±0.04*	1.15±0.05*
% inhibition	--	**71.6**	**53.2**	**62.5**

Values are expressed as mean ±S.E.M. (n=5), (***p<0.001, ** p<0.01,*p<0.05) as compared to control.