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Research Article

EVALUATION OF ANALGESIC AND ANTI-INFLAMMATORY ACTIVITY OF METHANOLIC EXTRACT OF *COCCULUS HIRSUTUS* LEAVES

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ABSTRACT
Inflammation and pain are the most common health problems treated with traditional remedies which mainly comprise medicinal plants. A number of natural products are used in the traditional medical systems in many countries. An alternative medicine for the treatment of various diseases is getting more popular. Many medicinal plants provide relief of symptoms comparable to that of obtained from allopathic medicines. Therefore agents of natural origin with very little side effects are required as substitute chemicals therapeutics. The methanolic leaf extract of *Cocculus hirsutus* (100& 200mg/kg) Linn (Menispermaceae) was investigated for its analgesic and anti-inflammatory effects in laboratory animals. The analgesic activity of the methanolic leaf extract of *Cocculus hirsutus* was investigated by eddy's hot plate model and acetic acid induced writhing in mice. Anti-inflammatory activity of *Cocculus hirsutus* was studied by both *in-vitro* and *in vivo* models. Human red blood cells membrane stabilization method was adopted for the *in-vitro* anti-inflammatory activity and for *in-vivo*, Carrageenan induced paw edema and cotton pellet induced granuloma in rats was employed. In eddy's hot plate analgesic study, both the doses of *Cocculus hirsutus* showed significant ($p<0.05$ and $p<0.01$ respectively) analgesic activity. In acetic acid induced writhing model, the onset of writhing was delayed and duration of writhing was shortened by the methanolic extract of *Cocculus hirsutus*.
In-vitro anti-inflammatory activity of the methanolic leaf extract of *Cocculus hirsutus* showed significant anti inflammatory activity in a concentration dependent manner. *Cocculus hirsutus* showed significant anti-inflammatory activity on both carrgeenan as well as cotton pellet induced granuloma models in rats. From the results, it was concluded that the methanolic leaf extract of *Cocculus hirsutus* possess analgesic and anti-inflammatory.
Key words: *Cocculus hirsutus,* Analgesic, Anti-inflammatory and HRBC.

INTRODUCTION

Inflammation or phlogosis is a pathophysiological response of mammalian tissues to a variety of hostile agents including infectious organisms, toxic chemical substances, physical injury or tumor growth leading to local accumulation of plasmic fluid and blood cells (Sobota et al., 2000). Although inflammation is a defense mechanism, the complex events and mediators involved in the inflammatory reaction can induce, maintain and aggravate many disorders. Hence, the employment of anti-inflammatory agents may be helpful in the therapeutic treatment of those pathologies associated with inflammatory reactions (Sosa et al., 2002). The clinical treatment of inflammatory diseases is dependent on drugs which belong either to the non-steroidal or steroidal chemical therapeutics. The nonsteroidal anti-inflammatory drugs (NSAIDs) such as aspirin, indomethacin and ibuprofen inhibit early steps in the biosynthesis pathway of prostaglandins by inhibition of COX enzymes and are the main drugs used to reduce the untoward consequences of inflammation (Albert et al., 2002). However, the side effects of the currently available anti-inflammatory drugs pose a major problem in their clinical use. For instance, NSAIDs cause several serious adverse effects like gastric injury and ulceration, renal damage, and bronchospasm due to their non-selective inhibition of both isoforms of the COX enzyme (Tapiero et al., 2002). The use of steroidal drugs as anti-inflammatory agents is also becoming highly controversial due to their multiple side effects (Van den Worm et al., 2001). Therefore, a need arises for the development of newer anti-inflammatory agents from natural sources with more powerful activity and with lesser side effects as substitutes for chemical therapeutics.

Cocculus hirsutus Linn (Family: Menispermaceae) is a widely growing plant found in the plains of India in dry localities. Indian tribes use various plant parts of this plant for a wide range of ailments, including constipation, kidney problems (Caius, 1986). 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). Leaf juice of this plant is used in the treatment of eczema (Masilamani, 1981). Hence there is a search for new Anti-inflammatory and Analgesic agent that retain therapeutic efficacy and yet are devoid of these adverse effects. Since not much study had been done to evaluate the biological activity of the plant, the present study is focused to evaluate the Anti-inflammatory and Analgesic activity of aerial parts of *Cocculus hirsutus*.

MATERIALS AND METHODS

Chemicals and Reagents

The chemicals used in the present study were Carrageenan (S. D. Fine Chemicals Limited, Bombay), indomethacin (IPCA, Bombay), Naproxen (Ranbaxy, Gurgaon), Pentazocine (Neon labs, Mumbai), Diclofenac (Biochem pharma, Mumbai).

Plant Material

The fresh leaves of *Cocculus hirsutus (*L.*)* Diels was collected during the month of December 2008 from Vaikalmedu, Erode (Dist), Tamilnadu. The plant was identified and authentified by the Botanical survey of India, Coimbatore, Tamilnadu (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 Extract

The dried powdered plant material was extracted with methanol in a Soxhlet extraction apparatus. The solvent was removed under reduced pressure and semi solid mass was obtained.

Animals

Male Swiss albino mice weighing 20-25gm and male Wistar rats weighing150-200gm were used for this study. The animals were obtained from animal house, IRT Perundurai medical college,

Erode, Tamilnadu, India. On arrival, the animals were placed randomly and allocated to treatment groups in polypropylene cages with paddy husk as bedding. Animals were housed at a temperature of 24±2°C and relative humidity of 30-70%. A12:12 light: day cycle was followed. All the animals were allowed to free access to water and fed with standard commercial pelleted chaw (M/s.Hindustan Lever Ltd., Mumbai). All the experimental procedures and protocols used in this study were reviewed by (IAEC) Institutional Animal Ethics Committee (688/2/C-CPCSEA) of Nandha College of pharmacy and were in accordance with the guidelines of the IAEC.

Analgesic Activity

Eddy's Hot plate method in mice

The hot plate assay method was employed for the purpose of preferential assessment of possible centrally mediated analgesic effects of methanolic extract of *Cocculus hirsutus*. The central analgesic drug, Pentazocine, was used for positive control group. In this experiment, four groups (n=6) of Swiss albino mice (20–25 g) were placed on a hot plate maintained at room temperature for 15 min. Food was withdrawn on the preceding night of the experiment. Group-1 normal control (0.5% CMC p.o.), and group-2 Pentazocine (30mg/kg, i.p.), whereas groups-3 and 4 animals received methanolic extract of *Cocculus hirsutus* (100 and 200 mg/kg, p. o respectively). Each animal was then individually placed gently on Eddy's hot plate at 55°C. Latency to exhibit nociceptive responses such as licking paws or jumping off the hot plate, were determined 15, 30, 45, 60, and 90 min after administration of the test drug or vehicle (Jeane Silva *et al*., 2003).

Acetic acid induced writhing response in mice

This method was used to preferentially evaluate possible peripheral effects of methanolic extract of *Cocculus hirsutus* as analgesic substance. Groups of four Swiss albino male mice (n=6) were fasted overnight prior to the start of the experiment, with free access to water. The peripheral analgesic drug, Indomethacin (5mg/kg), was used as a positive control. Group-1 normal control (0.5% CMC p.o), and group-2 Indomethacin (5mg/kg, p.o.), whereas groups 3 and 4 animals received methanolic extract of *Cocculus hirsutus* at doses of 100 and 200 mg/kg was administered orally (p.o), to mice. 30 min after treatment, the mice were injected intra peritoneally with 0.1 ml of 1% acetic acid solution to induce the characteristic writhings. 5 min after acetic acid administration, the mice were then placed in an observation box, and the number of writhings was counted in a 5min period. The response of the extract and Indomethacin treated groups were compared with those of animals in the control group (Mate *et al.,* 2008, Jeane Silva *et al*., 2003).

Anti-inflammatory Activity

***In-Vitro* Anti-inflammatory Activity**

The human red blood cell membrane stabilization method was used for this study. The blood was collected from healthy human volunteer who was not taken any NSAID's for 2 weeks prior to the experiment and mixed with equal volume of Alsever solution (2% Dextrose, 0.8%Sodium citrate, 0.5% Citric acid & 0.42% Nacl) and centrifuged at 3,000 rpm for 20 min. The Packed cells were washed with Isosaline and a 10% suspension was made. Various concentrations of methanolic extract of *Cocculus hirsutus* were prepared (100, 200 mcg/ml) using with CMC and to each concentration 1ml of phosphate buffer, 2ml of hypo saline & 0.5ml of HRBC suspension were added. It is incubated at 37°c for 30 min and centrifuged at 3,000 rpm for 20 min. The hemoglobin content of the supernatant solution was estimated spectrometrically at 560nm. Diclofenac (mcg/ml) was used as reference standard and a control was prepared omitting the extracts (Rajendran Vadivu *et al*., 2008).

***In-Vivo* Anti-inflammatory Activity**

Cotton Pellet induced granuloma method in rats

Cotton pellets, weighing 5 mg each were sterilized. Under ether anesthesia, the pellets were introduced subcutaneously through a skin incision on the back of the animals. Starting from 30 min after the implantation of cotton pellet for all the rats, 0.5% CMC to the normal control group, 25 mg/kg of Naproxen to the positive control group and 100, 200 mg/kg of the methanol extract of *Cocculus hirsutus* to the test groups was administered daily for 5 days (a daily p.o. administration). On the fifth day, the animals were sacrificed with chloroform, the granuloma was removed and the weights were determined (Perez *et al*., 2005).

Carrageenan-Induced Paw Edema in rats

For this experiment, the male rats (120-150g) were divided into four groups (n = 6). The first group received 0.5% CMC (10ml/ kg p.o.), while the second group received Indomethacin (8mg/kg p.o). The third and the fourth groups were treated with the methanol extract of *Cocculus hirsutus* (100 and 200 mg/ kg p.o.) respectively. Acute inflammation was produced by the sub plantar administration of 0.1 ml of 1% Carrageenan (in 1% CMC w/v) in the right hind paw of the rats. The paw thickness was measured at 0 min, 30 min, 60 min, 120 min and 240 min after Carrageenan injection by using verniar calipers (Vasudevan *et al.,* 2006). The animals were pretreated with the drug 1 hour before the administration of Carrageenan (Perez *et al*., 2005).

Statistical Analysis

All the results were expressed as mean ± standard error mean (S.E.M.). Data were analyzed using one-way ANOVA followed by Dunnett's *t*-test. The analysis was carried out using Graph pad software of version 4. $p<0.05$ was considered as statistically significant.

RESULTS

Analgesic Activity

Hot plate Method in Mice

The analgesic activity of methanolic extract of *Cocculus hirsutus* assessed using hot plate method in Swiss albino mice was illustrated in Table.1 Methanolic extract of *Cocculus hirsutus* showed significant analgesic activity at 100 and 200mg/kg, p.o dose. Analgesic activity was comparable with the standard drug Pentazocine. Among the two doses 200mg/kg showed maximum analgesic activity at reaction time 90min (8.6 ± 1.7) is slightly lower than the standard drug Pentazocine (10.6±0.2).

Acetic Acid-induced Writhing response in Mice

The analgesic effect of methanolic extract of *Cocculus hirsutus* leaves on acetic acid induced writhing was shown on Table 2. Injection of acetic acid into control mice produced 66.67 ± 0.5 writhes. Pretreatment with methanolic extract of *Cocculus hirsutus* at doses of 100 and 200 mg/kg reduced the number of writhes 36.33 ± 0.5 (45.51% protection) and 23.67 ± 0.7 (64.50% protection) respectively. Among the two doses 200 mg/kg showed the slightly lower analgesic activity than standard drug Indomethacin 19.67 ± 0.5 (70.50 % protection). It was observed that the onset of writhing was delayed and duration of writhing was shortened.

Anti-inflammatory Activity

In *vitro* Anti-inflammatory Activity

The methanolic extracts of the leaves of *Cocculus hirsutus* were studied for *in vitro* anti inflammatory activity by HRBC membrane stabilization method. The methanolic extract of *Cocculus hirsutus* leaves showed significant anti inflammatory activity in a concentration dependent manner. Methanolic extract at a concentration of 200mcg/ml showed 53.7% protection of HRBC in

hypotonic solution. All the results were compared with standard Diclofenac, which showed 65.2% protection.

***In Vivo* Anti-inflammatory Activity**

Cotton Pellet-induced Granuloma Method in rats

The Anti-inflammatory effect of the methanolic extract of *Cocculus hirsutus* assessed using Cotton pellet induced granuloma method in Wister rats was illustrated in Table 4. The methanolic extract of *Cocculus hirsutus* showed significant Anti-inflammatory activity at 100 and 200mg/kg, (p.o.), dose. After 6 days, the mean dry weight of granulomatous tissue surrounding the threads was significantly lower for the group treated with *Cocculus hirsutus* extract as compared to the control group. Among the two doses 200mg/kg showed maximum decreased formation of granuloma tissue. The results indicate that *Cocculus hirsutus* at the dose levels of 100 mg/kg and 200 mg/kg produced a significant decrease the weight of the granuloma 55.2 ± 0.5 (57.4% inhibition) and 41.4 ± 0.5 (68.3% inhibition) respectively. Among the two doses 200 mg/kg showed the slightly lower reduced weight of granuloma than standard drug Naproxen 31.8 ± 0.2 (76.0 % inhibition) (Perez *et al*., 2005).

Carrageenan-Induced Paw Edema in rats

The anti-inflammatory effect of the methanolic extract of *Cocculus hirsutus on* and Indomethacin on the Carrageenan induced hind paw edema as shown in Table.5 the methanolic extract of *Cocculus hirsutus* at doses 100 mg/kg and 200 mg/kg, produced a significant effect against Carrageenan induced inflammation after 4.0 h of the administration. The dose of 200 mg/kg exhibited a significant inhibition of 44.95 % after 2.0 h, the effect increased at 4.0 h (54 %). Anti-inflammatory activity of methanolic extract of *Cocculus hirsutus* was significant and similar to that of Indomethacin (8 mg/kg).

DISCUSSION

The inflammation is a complex process, which is frequently associated with pain and involves several events, such as the increase of muscular permeability, increase of granulocytes and mono nuclear cells migration, as well as the granulomatous tissue proliferation (Andrade *et al*., 2007). Pain is subjective experience, which is difficult to define exactly even though we all experience it. Pain distinguished as two types, peripheral or neurogenic pain may involve the following pathological states: peripheral nociceptive afferent neurons which are activated by noxious stimuli and central mechanism which is activated by afferent inputs pain sensation (Mate *et al.,* 2008).

The hot plate test was selected to investigate central antinociceptive activity because it had several advantages particularly the sensitivity to strong antinociceptive and limited tissue damage. Prostaglandins and bradykinins were suggested to play an important role in pain. Phenolic compounds are reported to inhibit prostaglandin synthesis (Alcarez and Ferrandiz., 1987). A number of Phenolic compounds have been reported to produce analgesic activity (Hosseinzadeh *et al*., 2002). As phytochemical tests showed presence of Phenolic compounds in methanolic extract of *Cocculus hirsutus*, they might suppress the formation of prostaglandin and bradykinins (Kou *et al*., 2005; Hosseinzadeh *et al*., 2002).

Acetic acid is known to trigger the production of noxious substances within the peritoneum, which induces the writhing response (Bartolini *et al*., 1987). The effect of the extracts against the noxious stimulus may be an indication that it depressed the production of irritants and thereby reduction in number of writhes in the animals. The writhing induced by chemical substances is due to sensitization of nociceptors by prostaglandins (Berkenkopf and Weichmann., 1988; Vasudevan *et al*., 2006). The abdominal constriction response induced by acetic acid is a sensitive procedure to establish peripherally acting anti-nociceptives. This response is thought to involve local peritoneal receptors (Chakraborty *et al*., 2004). This result indicates that the analgesic effect of methanolic extract of *Cocculus hirsutus* might be mediated by inhibiting the synthesis or action of prostaglandins.

The centrally acting Analgesic activity of the extract was also corroborated in our study by the tail immersion test results. The fact that in thermal stimuli (hot plate & Tail immersion tests); the anti nociceptive effect should be shown by acting centrally on opioid receptors. Since the drug had shown the analgesic activity in tail immersion test, it seems that the methanolic extract can act centrally. Taking this in to consideration the methanolic extract of *Cocculus hirsutus* possess peripheral and central analgesic properties.

The extracts exhibited membrane stabilization effect by inhibiting hypotonicity-induced lyses of erythrocyte membrane. The erythrocyte membrane is analogous to the lysosomal membrane (Chou, 1997) and its stabilization implies that the extract may as well stabilize lysosomal membranes. Stabilization of lysosomal membrane is important in limiting the inflammatory response by preventing the release of lysosomal constituents of activated neutrophil such as bactericidal enzymes and proteases, which cause further tissue inflammation and damage upon extra cellular release (Murugasan, 1981). Some of the NSAIDs are known to possess membrane stabilization properties which may contribute to the potency of their anti inflammatory effect. Though the exact mechanism of the membrane stabilization by the extract is not known yet, hypo tonicity induced hemolysis may arise from shrinkage of the cells due to osmotic loss of intracellular electrolyte and fluid components. The extract may inhibit the processes, which may stimulate or enhance the efflux of these intracellular components (Rajendran Vadivu et al., 2008). It is known that the inflammatory granuloma is a typical response of a chronic inflammatory process and it has been established that the dry weight of the pellets is well correlated with the granulomatous tissue. The chronic inflammation occurs by means of the development of proliferative cells. These cells can be either spread or in granuloma form. The *Cocculus hirsutus* extract showed significant anti-inflammatory activity in cotton pellet induced granuloma and thus found to be effective in chronic inflammatory conditions. It reflected its efficacy in inhibiting the increase in the number of fibroblasts and synthesis of collagen and mucopolysaccharide during granuloma tissue formation (Olajide *et al*., 2000).

Obtained results showed that methanolic extract of *Cocculus hirsutus* has anti-inflammatory activity on an acute inflammatory process like in carrageenan induced paw edema in rat paws. It is well known that leukocytes migration to the injured tissues is an important aspect of the inflammatory process. Histamine and serotonin are responsible for the immediate inflammation response, whereas Kinins and prostaglandins mediate prolonged response. The anti inflammatory effect of methanolic extract of *Cocculus hirsutus* in rats with carrageenan-induced paw was significant (Perez *et al.*, 2005).

CONCLUSION

The results of the study shows that the methanolic extract of *Cocculus hirsutus* leaf posses peripheral and central analgesic activity in animal model. The *Cocculus hirsutus* leaf extract shows in vitro anti-inflammatory activity on HRBC and in vivo anti-inflammatory activity on acute and chronic anti-inflammatory activity models in rats.

Further detailed study on *Cocculus hirsutus* plant using different flogestic agents in this area will enable us to understand the mechanism of action underline the above mention activity.

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Table.1 Analgesic effect of methanolic extract of *Cocculus hirsutus* (100 & 200mg/kg), leaves on heat stimulating response in the hot plate test in Swiss albino male mice.

Treatment group	Dose (mg/kg)	Reaction time (min)				
		15	30	45	60	90
Control (Vehicle)	10	2.0 ± 0.3	2.6 ± 0.4	2.3 ± 0.2	1.7 ± 0.2	1.3 ± 0.2
Pentazocine	30	5.6 ± 0.5[b]	6.0 ± 0.3[b]	6.3 ± 0.7[b]	8.6 ± 0.7[b]	10.6 ± 0.2[b]
MCH-I	100	3.3 ± 0.4[(ns)]	3.3 ± 0.4[(ns)]	3.7 ± 0.2[(ns)]	5.3 ± 0.8[c]	6.6 ± 0.4[b]
MCH-I I	200	4.3 ± 0.4[c]	5.0 ± 0.3[b]	5.3 ± 0.2[b]	6.0 ± 0.3[c]	8.6 ± 1.7[b]

The data represent the Mean ± SEM (n=6),
$p<0.05^{(c)}$, $p<0.01^{(b)}$, $p<0.001^{(a)}$ compared to corresponding control.
MCH-methanolic extract of *Cocculus hirsutus* (p.o).

Table.2 Analgesic effect of methanolic extract of *Cocculus hirsutus* (100 & 200 mg/kg) and Indomethacin (5 mg/kg) on Acetic acid induced writhing test in Swiss albino male mice

Treatment group	Dose (mg/kg)	Total number of writhes	%Inhibition
Control (Vehicle)	10 ml/kg	66.67 ± 0.5	--
Indomethacin	5	19.67 ± 0.5[b]	70.50
MCH-I	100	36.33 ± 0.5[b]	45.51
MCH-II	200	23.67 ± 0.7[b]	64.50

The above data represents the mean ± SEM (n=6);
$p<0.05^{(c)}$, $p<0.01^{(b)}$, $p<0.001^{(a)}$ compared to corresponding control.
MCH-methanolic extract of *Cocculus hirsutus* (p.o).

Table.3 *In vitro* anti-inflammatory activity of methanolic extract of *Cocculus hirsutus* by HRBC Membrane stabilization method.

Treatment Group	Concentration (mcg/ml)	Absorbance (540nm)	%Inhibition
Control	--	0.461 ± 0.001	--
Diclofenac	50	0.164 ± 0.001[b]	65.2
MCH-I	100	0.283 ± 0.001[b]	38.5
MCH-II	200	0.216 ± 0.001[b]	53.7

The above data represents the mean ± SEM (n=6);

Table.4 Anti-inflammatory effect of *Cocculus hirsutus* methanol extract (100 mg/kg & 200 mg/kg), and Naproxen (25 mg/kg) on Cotton pellet-induced granuloma in rats

Treatment group	Dose (mg/kg)	Weight of cotton pellet (mg)	(%) Inhibition
Control	10ml/kg	130.7 ± 2.6	--
Naproxen	25	31.8 ± 0.2[b]	76.0
MCH-I	100	55.2 ± 0.5[b]	57.4
MCH-II	200	41.4 ± 0.5[b]	68.3

The data represent the mean ± S.E.M (n=6),
$p<0.05^{(c)}$, $p<0.01^{(b)}$, $p<0.001^{(a)}$ compared to corresponding control.
MCH-methanolic extract of *Cocculus hirsutus* (p.o).

Table.5 Anti inflammatory activity of methanolic extract of *Cocculus hirsutus* (100 & 200 g/kg) and indomethacin (8 mg/kg) On Carrageenan induced paw edema method in Wister rats.

Treatment Group	Dose (mg/kg)	Paw thickness (mm) 0 min	30 min	60 min	120 min	240min	%inhibition
Control	10 ml/kg	4.79 ± 0.06	5.09 ± 0.03	6.11±0.02	7.23±0.05	8.63 ±0.01	-
Indomethacin	8	4.21 ± 0.06[b]	4.01 ± 0.01[b]	4.0 ± 0.05[b]	3.65 ± 0.04[b]	3.53 ± 0.02[b]	59
MCH-I	100	4.68± 0.12[ns]	4.54 ± 0.09[b]	4.38 ± 0.04[b]	4.31 ± 0.01[b]	4.25± 0.18[b]	50.3
MCH-II	200	4.6 ± 0.03[ns]	4.38 ± 0.04[b]	4.22 ± 0.06[b]	3.98 ± 0.1[b]	3.95 ± 0.1[b]	54

The data represent the Mean ± SEM (n=6),
$p<0.05^{(c)}$, $p<0.01^{(b)}$, $p<0.001^{(a)}$ compared to corresponding control.
MCH-methanolic extract of *Cocculus hirsutus* (p.o).

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