TO EVALUATE ANTI-INFLAMMATORY AND ANALGESIC ACTIVITY OF LEAF OF *GYMNOSPORIA MONTANA-* A NATIVE OF GUJARAT

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ABSTRACT

Gymnosporia montana belonging to the family *Celastraceae* commonly known as Vikalo. It is a shrub or tree growing wild in dry areas and is commonly found in Gujarat. It is widely used in treating sore, ulcer, gastro-intestinal disorders, toothache, and dysentery. The dried powder of leaf of *Gymnosporia montana* was subjected to the phytochemical screening for the presence of various phytoconstituents like alkaloids, flavonoids, saponins, tannins, anthraquinones, and carbohydrates, etc. Effectiveness of methanolic extracts of leaf of *Gymnosporia montana* was evaluated (100, 200 and 300 mg/kg; p.o.) against carrageenan-induced rat paw edema, and cotton pellet-induced chronic inflammation. Further, methanolic fraction of the plant was also evaluated (200 mg/kg; p.o.) in tail flick model using rats as well as acetic acid-induced writhing response using mice. Pretreatment with methanolic fraction showed significant ($p < 0.05$) anti-inflammatory activity at 3 h when compared with the control group. Further, it also significantly ($p < 0.05$) reduced the increase in the weight of cotton pellet when compared with the control group and results were comparable with that of diclofenac (standard) treated group of animals. Pretreatment with methanolic fraction significantly ($p < 0.05$) prolonged the reaction time in rats in tail flick model as well as reduction in the number of mouse abdominal constrictions following acetic acid administration when compared with the control group. It can be thus concluded that the methanolic fraction of leaf of *Gymnosporia montana* showed significant anti-inflammatory activity and analgesic activity in experimental animals.

Keywords: *Gymnosporia montana,* anti-inflammatory, Vikalo

INTRODUCTION

Ethanomedicinally, one of the important plant such as *Gymnosporia montana* has been used in the treatment of Jaundice in tribal area[1]. *Gymnosporia montana,* a plant native in Gujarat, is a relatively insufficiently investigated for its pharmaceutical components and their activity, keeping this in view, the study is being undertaken to evaluate the plant. *Gymnosporia montana*, belonging to the family *Celeastraceae* commonly known as Vikalo is a shrub or tree growing wild in dry areas[2]. The roots, stem, barks and leaves are used in medicine. The ground bark is applied with mustard oil to kill hair lice. The root is used in gastrointestinal troubles especially dysentery, in South Africa. The plant has been traditionally useful in treating ulcer, gastro-intestinal disorders, toothache, dysentery[3], antispasmodic[4] and hepatoprotective[5] effect was also reported. In South Africa, leaves are used as a vermifuge for children. They have also found utility in the treatment of Jaundice. Wood and leaf ash are reported to be used in Nigeria as substitute for salt. Leaf ash mixed with ghee is applied for treatment of sores. Decoction of the leafy twigs has been used as mouth wash and to relieve toothache. In traditional system of medicine the root, stem, and leaves are valued for their medicinal properties but major medicinal properties are attributed to the leaf of *Gymnosporia montana*. The present study was undertaken to evaluate anti-inflammatory and analgesic activity of leaf of Gymnosporia montana- A native of Gujarat. The present study was undertaken to evaluate the anti-inflammatory activity of different extracts of *Gymnosporia montana* for its effects against acute and chronic inflammatory models and the analgesic activity of most potent and active methanolic fraction of *Gymnosporia montana* against tail flick and acetic acid-induced writhing response using experimental animals.

MATERIALS AND METHODS

Collection and authentification of plant material The plant *Gymnosporia montana* were collected from the Vijapur, Gandhinagar, Gujarat, India during November 2008 and were authenticated by Dr. S.K.Patel, Head of the Botany Department, Government Science College, Gandhinagar. The voucher specimen KB/O8/0011 was deposited in K.B.Institute of pharmaceutical Education and Research, Gandhinagar, Gujarat, India.

Preparation of different extract

The selected plant parts of *G. montana* was separated and dried under sunlight. Dried powdered passed through sieve of 60 mesh (#) size and stored in airtight containers and then used for present work. Shade dried leaf powder was extracted successively with petroleum ether (60-80), ethyl acetate, and three different dose of 70% methanol. The extraction was carried out by

soxhlet assembly, for 6-8 hours. Then the solvent was filtered and repeat the process for three times in the same manner. The extracts were concentrated and dried under controlled temp of 60^0 C on a water bath and reported the % yield respectively. Dried extracts of the leaves was used for further investigation.

Phytochemical test

Phytochemical analysis of the extract was performed using standard methods. Tests for alkaloids, flavanoids, saponins, tannins, anthraquinone, and carbohydrates were carried out [6] and were confirmed by qualitative analysis and also by thin-layer chromatography.

Drugs and chemicals

All different organic solvents used for extraction under study were obtained from S.D. Chem. Pvt. Ltd., A.R. grade. Fresh drug solutions were prepared in 1% CMC and administered orally. The carrageenan was obtained from Sigma Chemical Co. (St Louis, MO).

Animals

Swiss mice (18-22 g) and Wistar albino rats (200-250 g) of either sex and were selected for the study. Animals were fed a standard chow diet and water that was freely available under standard condition of a 12 h dark-light cycle, 60±10% humidity and a temperature of 21.5 ±1^0c. Coprophagy was prevented by keeping the animals in cages with gratings on the floors. These experiments complied with the guidelines of CPCSEA in our laboratory for animal experimentation.

Methodology

Anti-inflammatory activity

Carrageenan –induced pedal edema in rats

The animals were randomly divided into following groups of six animals each.

Group-I (Control): Animals received only aqueous suspension of 1% CMC as vehicle without any treatment.

Group-II (Drug treatments): Animals received petroleum ether (300mg/kg, p.o.), ethyl acetate (300mg/kg, p.o.) and methanolic extracts (100,200 and 300 mg/kg, p.o.)

Group-III (Standard): Animals received diclofenac sodium (5mg/kg; i.p.) as a reference standard.

Different plant extracts (100, 200 and 300 mg/kg, p.o.), standard and vehicles were administered in animals one hour prior to the injection of carrageenan. A subplanter injection of 0.1 ml of carrageenan in 0.9 % sodium chloride was administered in the left hind paw of different groups

of rats. The paw volume was measured just before and 1, 2, 3, 4, 5 and 6 h after administration of carrageenan by the volume displacement method, using a plethysmometer. The average foot swelling in drug treated as well as standard group was compared with that of control and the percentage inhibition of edema was determined, as shown here: % Inhibition = $\{1-(Vd-Vp/Vc-Vp)\} \times 100$

Here, Vd-Vp = Difference in paw volume after carrageenan and initial paw volume for drug treated animals. And Vc-Vp = Difference in paw volume after carrageenan and initial paw volume for control animals[7].

Cotton pellet-induced granuloma formation in rats

The animals were randomly divided into different groups of six animals each as shown in earlier model. Sterile cotton pellets (50±1 mg) soaked in 0.2 ml of distilled water containing cephalosporin was implanted subcutaneously bilaterally in axillae under light ether anesthesia[8]. Animals received different treatments for seven consecutive days starting from the day of cotton pellet implantation. The animals were anaesthetized again on the 8th day and cotton pellets were removed surgically, freed from extraneous tissue, incubated at 37^0 C for 24 h, and dried at 60^0C to constant weight. The weight of cotton pellet before implantation was subtracted from the weight of the dried dissected pellets. Thus any, increment in the dry weight of the pellets was taken as a measure for granuloma formation. The mean weight was calculated for pellets from the group of rats receiving drugs and compared with the mean values for the control[9].

Analgesic activity

Two assay models, viz. tail flick and acetic acid-induced writhing response, were employed to detect analgesic activity.

Tail flick method in rats

Wistar albino rats of either sex 200-250 g were selected for the study. Before the study, rats were screened for sensitivity test by placing the tip of the tail on the radiant heat source. Any animals who failed to withdraw its tail in five sec were rejected from the study. The animals were randomly divided into following groups of six animals each.

Group-I (Control): Animals received only aqueous suspension of 1% CMC as vehicle without any treatment.

Group-II **(Drug treatment)**: Animals received active fraction of methanol (200 mg/kg, p.o.).

Group-III (Standard): Animals received morphine (10mg/kg, i.p.) as a reference standard.

One hour after the administration of vehicle, methanolic fraction and reference standard, the tail of the rat was placed on the nichrome wire of an analgesiometer and the time taken by the animal to withdraw (flick) its tail from the hot wire was taken as the reaction time. Analgesic activity was measured at 0 and 30 min after the administration of the test drugs. The cut-off time for determination of latent period was taken as 30 sec to avoid injury to the skin[10].

Acetic acid-induced writhing in mice

Swiss albino mice of either sex 20-25 g were selected for the study. The animals were randomly divided into three groups of six animals each as described in tail flick method. Here, aspirin (25 mg/kg, i.p.) was taken as a reference standard. One hour after the administration of vehicle, methanolic fraction and aspirin, the injection of acetic acid 0.6% v/v (10ml/kg; i.p.) was given and thereafter, the numbers of writhes were observed for up to 30 min. Reduction in number of writhing by any treatment as compared to vehicle treated animal was considered as a positive analgesic response, which was expressed as percent protection. % Protection= Wc-Wt / Wc X100, Where, Wc=Mean writhing of the control group and Wt= Mean writhing of the treated group[11].

Statistical analysis

The results were expressed as Mean ± SEM. The significance of difference between mean values for the various treatments was tested using one way analysis of variance (ANOVA) followed by Tukey's multiple range test[12]. Data showing P values less than 0.05 ($P<0.05$) was considered as statistically significant.

RESULTS AND DISSCUSION

Plant under study exhibited anti-inflammatory activity against carrageenan-induced rat paw edema as shown in table 1.

Table 1: Effect of different extracts of *Gymnosporia montana* on carrageenan-induced rat paw edema

Treatment	Edema at 3 h volume (ml)	% Inhibition
Control	1.33±0.084	-
Diclofenac sodium (5mg/kg)	0.46±0.024*	68.42
Petroleum ether (300mg/kg)	0.63±0.010	52.90
Ethyl acetate (300mg/kg)	1.05±0.042	21.05
methanol (100mg/kg)	0.70±0.013	47.36
methanol (200mg/kg)	0.44±0.006*	69.17
methanol (300mg/kg)	0.49±0.010	63.15

All values represent mean ± SEM, n=6 in each group. * $P < 0.05$, when compared with the control group (ANOVA, followed by Tukey's multiple range test), $F_{tab} = 2.75$; F_{cal} (6, 35) = 70.51 (Edema at 3 h, volume in ml).

Results were checked by using three different dose of methanolic fraction (100, 200, and 300mg/kg) of leaf of *Gymnosporia montana* based on dose dependant manners. From all different fractions methanolic fraction at dose of 200mg/kg (0.44± 0.006) and reference standard (0.46±0.014) showed significant reduction in edema formation as compared to control group (1.33±0.084).

Further, the methanolic fraction at the dose of 200mg/kg and diclofenac sodium treated group showed significant suppression of granulation tissue formation in cotton-pellet implanted rats. The % inhibition of granuloma formation with methanolic (200mg/kg) and diclofenac sodium treated animals was found to be 60% and 62% respectively as shown in table 2.

Table 2: Effect of different extracts of *Gymnosporia montana* on cotton pellet-induced granuloma in rats

Treatment	Weight of dry cotton pellets granuloma (mg)	% Inhibition
Control	186.17±6.184	-
Diclofenac sodium (5mg/kg)	075.00±3.088*	62.00
Petroleum ether (300mg/kg)	116.50±5.537	37.00
Ethyl acetate (300mg/kg)	139.00±5.305	25.00
methanol (100mg/kg)	130.17±3.156	30.08
methanol (200mg/kg)	088.67±2.883*	60.07
methanol (300mg/kg)	101.00±4.243	45.00

All values represent mean ± SEM, n=6 in each group. * $P < 0.05$, when compared with the control group (ANOVA, followed by Tukey's multiple range test), F tab = 2.37; F cal (6, 35) = 67.15 (Wt of dry cotton pellets granuloma in mg.

Based on above findings, the analgesic activity was performed only in methanol fraction using tail flick and writhing tests. Oral administration of methanol fraction significantly prolonged the reaction time in rats when compared with the control group. The mean numbers of tail flick latent period in animals have been shown in table 3. Morphine (10 mg/kg, i.p.) showed significant analgesic action at 30 min of time interval. Results of drug treated animals were comparable with that of morphine group. As can be seen from the results, it is clear that methanol raised the pain threshold, indicating that the methanol fraction might be centrally acting after 30 minutes from the administration time.

Table 3: Effect of methanolic fraction (200 mg/kg, p.o.) of *Gymnosporia montana* on mean tail-flick latent period

Treatment	Retention time in seconds
Control	2.67±0.33
methanolic fraction	7.83±0.30*
Morphine (10 mg/kg, i.p.)	26.00±1.81*

All values represent mean ± SEM, n=6 in each group. * $P<0.05$ indicate significant activity as compared to control group (ANOVA, followed by Tukey's multiple range test), F tab =3.68, F cal (2, 25) =124.96(Retention time).

The analgesic activity was performed only in 200 mg/kg methanolic fraction using tail flick and writing tests. Oral administration of methanolic fraction significantly prolonged the reaction time in rats when compared with the control group. The mean numbers of tail flick latent period in animals. Morphine (10 mg/kg, i.p.) showed significant analgesic action at 30 min of time interval. Results of drug treated animals were comparable with that of morphine group. As can be seen from the results, it is clear that 200 mg/kg methanolic fraction raised the pain threshold, indicating that the methanolic fraction might be centrally acting after 30 minutes from the administration time.

In addition to this, oral administration of methanolic fraction significantly reduced the number of writhing or mouse abdominal constrictions induced by 0.6% acetic acid when compared with the control group. Reduction in number of writhes in methanolic fraction and aspirin was 77.05% and 88.15% respectively as shown in table 4.

Table 4: Effect of methanolic fraction (200 mg/kg, p.o.) of *Gymnosporia montana* on acetic acid-induced writhing response in mice

Treatment	Average number of writhes in 30 min	% Inhibition
Control	23.17±1.06	-
methanolic fraction	13.17±0.69*	77.05
Aspirin (25 mg/kg; i.p.)	03.00±0.36*	88.15

All values represent mean ± SEM, n=6 in each group. * $P<0.05$ indicate significant activity as compared to control group (ANOVA, followed by Tukey's multiple range test), F tab =3.68, F cal (2, 25) = 115.22(writhing).

The present study demonstrated significant anti-inflammatory and analgesic activity of methanolic fraction of leaf of *Gymnosporia montana* in experimental animals.

Amongst the many methods used for screening of anti-inflammatory drugs, one of the most commonly employed techniques is based upon the ability of such agents to inhibit the edema produced in the hind paw of the rat after injection of phlogistic agents such as carrageenan[13]. Carrageenan rat paw edema is a suitable test for evaluating anti-inflammatory drugs, which has been frequently used to assess the anti-edematous effect of natural products[14 & 15]. The probable mechanism of action of carrageenan-induced paw edema is bi-phasic, the first phase is attributed to the release of histamine, 5-HT and kinins in the first hour; while, the second phase is related to the release of prostaglandin like substances in 2-3 h[16]. It has been reported that the second phase of edema is sensitive to most clinically effective anti-inflammatory agents[17]. Pretreatment with methanolic fraction showed significant (p<0.05) reduction in edema formation as compared to control and standard group. In the cotton pellet granuloma model, inflammation and granuloma

develops during a period of several days. This method has been useful for evaluation of steroid and non-steroid anti-inflammatory drugs. It has been considered that cotton pellet-induced granuloma is closely related to the formation of antibodies. This model is an indication for the proliferative phase of inflammation. Inflammation involves infiltration of macrophages, neutrophils and proliferation of fibroblasts[18], which are basic source of granuloma formation. Our observations showed that the methanolic fraction significantly inhibited granuloma formation in rats. Multiplications of small blood vessels as well as proliferation of fibroblasts are the characteristic features at the repair phase of inflammation. Such proliferating cells penetrate the exudates, producing a highly vascularized reddened mass known as granulation tissue[19]. Hence, the decrease in the weight of cotton pellets indicated the reduction in the proliferative activity of phenolics[20] in methanolic fraction of leaf *Gymnosporia montana.* They may act by reducing the effect of inflammatory mediators. They have been shown to inhibit the migration of leucocytes in experimental models[21]. A number of phenolics have been reported to possess anti-inflammatory activity [18].

Antinociceptive drugs are generally classified into centrally or peripherally acting with respect to the site of action. In the present study, antinociceptive effect was performed using tail flick test and writhing test in experimental animals[22 & 23]. In order to distinguish between the central and peripheral analgesic action of *Gymnosporia montana,* acetic acid induced writhing response in mice was used to examine the effect. This method is not only simple and reliable but also affords rapid evaluation of peripheral type of analgesic action. Several chemicals can induce writhing response in animals. Acetic acid induces writhing response by activating the chemosensitive nociceptors in animals, while tail flick response is considered to be selective for opioid like substances[24 & 25]. The radiant heat test on tail of rats is very effective to estimate the efficacy and potency of central acting morphine –like analgesics drugs. It has been reported that narcotic agents like morphine and centrally acting analgesics could inhibit thermally induced nociceptive responses in animals[26 & 27].

It is well known that peripherally acting components reduce or inhibit writhing induced by acetic acid and centrally acting drugs elicit protective effect on thermal painful stimuli [28]. Number of phenolics [29 & 30] was reported to possess antinociceptive activity. The abdominal constriction is related to the sensitization of nociceptive receptors to prostaglandin[31]. It is therefore, possible that *Gymnosporia montana* exerts an analgesic effect probably by inhibiting synthesis of

prostaglandin[32] and thereby its actions. Thus, *Gymnosporia montana* (methanolic fraction) exhibited significant analgesic activity, which could involve central mechanisms.

CONCLUSION

It is concluded that, methanolic fraction of *Gymnosporia montana* possesses significant anti-inflammatory and analgesic activity. The mechanism if action of this plant might be attributed to both central and peripheral components of the pain.

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