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Anti-inflammatory and anti-nociceptive activities of methanolic leaf extract of *Indigofera cassioides Rottl.* Ex. DC.

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ABSTRACT

Objective: To evaluate the anti-inflammatory and analgesic activities of methanolic leaf extract of *Indigofera cassioides* (*I. cassioides*) (MEIC) using various animal models. **Methods:** Anti-inflammatory and analgesic activities of MEIC was assessed by using different animal models. Anti-inflammatory activity of the extract was evaluated by using carrageenan-induced rat paw edema and cotton pellet granuloma method. Anti-nociceptive activity of the extract was evaluated for its central and peripheral pharmacological actions by using Eddy's hot plate method and acetic acid-induced writhing respectively. The study was carried out using dose of 200 & 400 mg/kg orally. Aceclofenac, aspirin and pentazocine was used as standard drugs to evaluate anti-inflammatory and analgesic activities, respectively. **Results:** Treatment with MEIC significantly ($P<0.001$) decrease the paw volume and weight of cotton pellet in the tested models. It also exhibit potent analgesic activity on chemical and thermal induced pain in mice. MEIC exhibit potent and dose dependent anti-inflammatory and analgesic activities in all the tested animal models. **Conclusions:** All the results obtained revealed that the extract MEIC showed potent anti-inflammatory and anti-nociceptive activity against all the tested models and the results obtained were comparable with the standards used. The activity of the extract may be due to the presence of terpenoids, flavonoids and other phytochemicals.

1. Introduction

In Indian system of medicine, a large number of drugs from herbal or mineral origin have been advocated for various types of diseases and other different unwanted conditions in humans[1]. Ayurvedic medicines are largely based upon herbal and herbomineral preparations and have specific diagnostic and therapeutic principles[2]. Inflammation is a disorder involving localized increases in the number of leukocytes and a variety of complex mediator molecules[3]. Prostaglandins are ubiquitous substances that indicate and modulate cell and tissue responses involved in inflammation. Their biosynthesis has also been implicated in the pathophysiology of cardiovascular diseases, cancer, colonic adenomas and Alzheimer's diseases[4,5]. Medicinal plants are believed to be an important source of new chemical substances with potential therapeutic effects[6,7]. The research into plants with alleged folkloric use as pain relievers, anti-inflammatory agents, should therefore be viewed as a fruitful and logical research strategy in the search for new analgesic and anti-inflammatory drugs.

Indigofera cassioides (*I. cassioides*) Rottl. Ex. DC. (Syn: Indigofera pulchella Roxb.) belonging to the family Fabaceae, is a large shrub, distributed throughout the hills of India. The flowers and leaves of the plant are reported to be antiscorbutic, diuretic and alternative. A decoction of the root is given for cough and its powder is applied externally for chest pains. The leaves and roots are used for swelling of the stomach[8,9]. The leaves are used by various ethnic groups and native medical practitioners to treat much kind of diseases such as arthritis, inflammation, tumor and liver diseases. In our earlier studies, the plant extract exhibited a potent antioxidant and antitumor activity[10,11]. In continuation of our previous studies, the present study was aimed to evaluate the anti-inflammatory and analgesic

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activities of methanol extract of *Indigofera cassioides* (MEIC) on various experimental models.

2. Materials and methods

2.1. Chemicals

Kappa carrageenan type III was obtained from Sigma, St. Louis, USA. Pentazocin was obtained from Ranbaxy, India. Aceclofenac and aspirin were obtained from Micro Labs, India. All other chemical used were of analytical grade.

2.2. Plant material and extraction

Leaves of *I. cassioides* were collected from the Yercaud hills in the month of November 2008. The plant was authenticated by Dr. G.V.S. Murthy, Joint Director, Botanical Survey of India, Coimbatore, Tamilnadu, India. A voucher specimen is preserved in our laboratory for future reference (Voucher No. P. Ch. IC 003/2008). The plant material was shade dried, pulverized and extracted (500 g) with 80% methanol at room temperature for 72 h. The extract was filtered and concentrated to dryness under reduced pressure and controlled temperature (40 ℃ to 50 ℃) in a rotary evaporator. The extract was a dark brown solid weighing 41 g (yield, 8.2 %) and was preserved in a vacuum desiccator at 4 ℃ until further use.

2.3. Animals

Male Wistar albino rats (150-200 g) and Swiss albino mice (20-25 g) were procured from Venkatershwara Enterprises, Bangalore, Karnataka, India, and used throughout the study. They were housed in microlon boxes in a controlled environment [temperature (25±2)℃ and 12 h dark/light cycle] with standard laboratory diet and water ad libitum. The study was conducted after obtaining Institutional Animal Ethical Committee (IAEC) clearance.

2.4. Anti-inflammatory activity

2.4.1. Carrageenan-induced rat paw edema

The rats were divided into four groups (n=6). The different groups were treated orally with MEIC (200 & 400 mg/kg), Aceclofenac (10 mg/kg), and vehicle control (0.3% CMC, 1 ml/kg/p.o.). The administration of extract and drugs was 1 h prior to injection of 0.1 mL of 1% freshly prepared suspension of carrageenan in normal saline in the right hind paw sub plantar of each rat. The paw volume was measured initially and then at 1, 2 and 3 h after the carrageenan injection by using plethysmometer. The anti-inflammatory effect of MEIC was calculated by the following equation: -

Anti-inflammatory activity (%) = (1-Vt/Vc) × 100

Where Vt represents the paw volume in drug treated animals and Vc represents the paw volume of control groups animals[12].

2.4.2. Cotton pellet-induced granuloma

The animals were divided into four groups of six animals in each group. The rats were anaesthetized and sterile cotton pellets weighing (10±1) mg was implanted subcutaneously into both sides of the groin region of each rat. Group I served as control and received the vehicle (0.3% CMC, 1 mL/kg/p.o.). Group II animals received aceclofenac at a dose of 10 mg/kg for the same period. MEIC at the concentration of 200 & 400 mg/kg was administered orally to groups III and IV animals for seven consecutive days from the day of cotton pellet implantation. On 8th day the animals were anaesthetized and the pellets together with the granuloma tissues were carefully removed and made free from extraneous tissues. The wet pellets were weighed and then dried in an oven at 60 ℃ for 24 h to constant weight, after that the dried pellets were weighed again. Increment in the dry weight of the pellets was taken as a measure of granuloma formation. The antiproliferative effect of MEIC was compared with control [13].

2.5. Analgesic activity

2.5.1. Acetic acid induced writhing test

The writhes were induced by intraperitoneal injection of 0.6 %v/v acetic acid (80 mg/kg). Group I served as control (0.3% CMC, 1 mL/kg/p.o.) and group II animals received aspirin at a dose of 300 mg/kg. Two different doses of MEIC (200 & 400 mg/kg) were administered orally to the group III and group IV. The extract and standard drug was administered 30 min before chemical stimulus. The number of muscular contractions was counted over a period of 20 min and is expressed as writhing numbers[14].

2.5.2. Hot plate method

The hot plate method in rats was performed by the method of Eddy and Leimbach[15]. The evaluated parameters were the latency time for paw licking and jumping responses on exposure to the hot plate surface, kept at (55±1) ℃. The animal was kept in the hot plate until it lifted one of its hind paws. For this method, the animals were divided into four groups of six animals each. Group I served as control (0.3% CMC, 1 mL/kg/p.o.). Group II received pentazocin at a dose of 5 mg/kg. Group III and IV received MEIC at a dose of 200 & 400 mg/kg orally. All the treatments were given 30 min before the thermal stimulus and the response was determined at 60, 120 and 180 min.

2.6. Statistical analysis

All values were expressed as mean±SEM. Statistical analysis was performed with one way analysis of variance (ANOVA) followed by Tukey Kramer Multiple Comparison test. P values < 0.05 were considered to be statistically significant when compared to control.

3. Results

3.1. Carrageenan induced rat paw edema

The result of MEIC against carrageenan-induced paw edema is shown in Table 1. MEIC (200 & 400 mg/kg) gave significant (P<0.001) reduction of rat paw edema at all assessment times in dose dependent manner. The extract showed maximum inhibition of 66.32% at the dose of 400 mg/kg after 3 h of drug treatment in carrageenan-induced paw edema whereas the standard drug showed 60% of inhibition.

3.2. Effect of MEIC on cotton pellet granuloma

The effects of MEIC and aceclofenac on the proliferative phase of inflammation are shown in Table 2. A significant (P<0.001) reduction in the weight of the cotton pellets was observed with MEIC (200 & 400 mg/kg) compared with the vehicle treated rats. However the degree of reduction was less than the effect caused by the standard drug aceclofenac.

3.3. Effect of MEIC on acetic acid induced writhings in mice

The extract (200 & 400 mg/kg) dose dependently reduced acetic acid induced writhings in mice. Maximum reduction was observed at the dose of 400 mg/kg. The reduction was statistically significant (P<0.001) when compared to control. The results were showed in Table 3.

3.4. Effect of MEIC on hot plate method

The animals pretreated with MEIC (200 & 400 mg/kg) showed a dose dependent increase in latency of response in the hot plate method. The increase in the latency responses were significant (P<0.001). However the latency period was less than the effect caused by the standard drug pentazocine. The results were displayed in Table 4.

Table 1

Effect of methanol extract of *I. cassioides* on Carrageenan induced paw edema in rats.

Design of treatment	Paw volume (mL)				Percentage inhibition after 3 h
	0 h	1 h	2 h	3 h	
Group-I (0.3% CMC 1 mL/kg/p.o)	0.21±0.01	0.27±0.01	0.49±0.03	0.95±0.03	–
Group-II (Aceclofenac, 10 mg/kg/p.o)	0.21±0.01	0.24±0.01	0.28±0.01[a]	0.38±0.02[a]	60.00
Group-III (MEIC, 200 mg/kg/p.o)	0.22±0.01	0.27±0.01	0.31±0.01[a]	0.38±0.01[a]	60.00
Group-IV (MEIC, 400 mg/kg/p.o)	0.23±0.01	0.26±0.01	0.28±0.01[a]	0.32±0.02[a]	66.32

n=6; Data were expressed as Mean±SEM; [a]P<0.001 vs Control; Data were analyzed by One way ANOVA followed by Tukey Kramer Multiple Comparison test.

Table 2

Effect of methanol extract of *I. cassioides* on cotton pellet granuloma in rats.

Design of treatment	Wet weight (mg)	% inhibition	Dry weight (mg)	% inhibition
Group-I (0.3% CMC, 1 ml/kg/p.o)	203.80±6.24	–	56.17±2.17	–
Group-II (Aceclofenac, 10 mg/kg/p.o)	63.17±2.19[a]	69.00	25.50±2.08[a]	54.60
Group-III (MEIC, 200 mg/kg/p.o)	76.67±2.67[a]	62.37	38.80±2.13[a,b]	30.87
Group-IV (MEIC, 400 mg/kg/p.o)	65.00±1.88[a]	68.11	34.30±1.75[a]	38.94

n=6; Data were expressed as Mean±SEM; [a]P<0.001vs Control; [b]P<0.01 vs Aceclofenac; Data were analyzed by One way ANOVA followed by Tukey Kramer Multiple Comparison test.

Table 3

Effect of methanol extract of *I. cassioides* on chemical stimulus induced (acetic acid induced writhing) pain in mice.

Design of treatment	No of writhings	% reduction
Group-I (0.3 % CMC, 1 mL/kg/p.o)	105.66±3.20	–
Group-II (Aspirin, 300 mg/kg/p.o)	49.67±2.99[a]	53.00
Group-III (MEIC, 200 mg/kg/p.o)	66.83±2.23[a,b]	36.75
Group-IV (MEIC, 400 mg/kg/p.o)	50.00±2.15[a,c]	52.68

n=6; Data were expressed as Mean±SEM; [a]P<0.001vs Control; [b]P<0.001 vs Aspirin; [c]P<0.001 vs MEIC 200 mg/kg; Data were analyzed by One way ANOVA followed by Tukey Kramer Multiple Comparison test.

Table 4
Effect of methanol extract of *I. cassioides* on thermal stimulus induced pain in mice.

Design of treatment	Reaction time (s)			
	0 h	1 h	2 h	3 h
Group-I(0.3% CMC, 1 mL/kg/p.o)	2.53±0.14	2.45±0.08	2.53±0.10	2.58±0.09
Group-II(Pentazocine, 10 mg/kg/i.p)	2.40±0.13	7.41±0.35[a]	9.95±0.41[a]	8.38±0.25[a]
Group-III(MEIC, 200 mg/kg/p.o)	2.70±0.07	3.65±0.15[b,a]	5.98±0.24[a,c]	3.90±0.21[b,c]
Group-IV(MEIC, 400 mg/kg/p.o)	2.30±0.20	3.98±0.45[a,c]	5.80±0.21[a,c]	3.08±0.25[c]

n=6; Data were expressed as Mean±SEM; [a]P<0.001, [b]P<0.01 vs Control; [c]P<0.001 vs Pentazocine; Data were analyzed by One way ANOVA followed by Tukey Kramer Multiple Comparison test.

4. Discussion

The most widely used primary test for screening of anti-inflammatory agents is carrageenan induced edema in the rat paw hind paw[12]. The development of edema in the paw of the rat after injection of Carrageenan is believed to be biphasic event. The initial phase observed during the first hour is attributed to the release of histamine and serotonin; the second phase is due to the release of prostaglandin-like substances[16]. Based on this, it could be argued that the suppression of the first phase may be due to inhibition of the release of early mediators, such as histamine and serotonin, and the action in the second phase may be explained by an inhibition of cyclooxygenase[17]. Ueno et al[18] found that the injection of carrageenan into the rat paw induces the liberation of bradykinin, which later induces the biosynthesis of prostaglandins and other autocoids, which are responsible for the formation of the inflammatory exudates. Besides, in the carrageenan induced rat paw edema model, the production of prostanoids has been through the serum expression of COX-2 by a positive feedback mechanism[19]. There fore, it is suggested that the mechanism of action of MEIC may be related to prostaglandin synthesis inhibition.

The cotton pellet granuloma method has been widely employed to assess the transductive, exudative and proliferative components of chronic inflammation and is a typical feature of established chronic inflammatory reaction. The fluid absorbed by the pellet greatly influences the wet weight of the granuloma and dry weight correlates well with the granuloma of the granulomatous tissue formed[17,20]. Administration of MEIC at the doses of 200 & 400 mg/kg significantly reduced the granulomatous tissue formation when compared to control.

With respect to the writhing test, the research group of Deraedt et al[21] described the quantification of prostaglandins by radioimmunoassay in the peritoneal exudates of rats, obtained after intraperitoneal injection of acetic acid. They found high levels of prostaglandins PGE2 α and PGF2 α during the first 30 min after acetic acid injection. Nevertheless, it was found that the intraperitoneal administration of acetic acid induces the liberation not only of prostaglandins, but also of the sympathetic nervous system mediators[22, 23]. Thus the results obtained for the writhing test using acetic acid are similar to those obtained for the edematogenic test using carrageenan, since MEIC was effectively inhibiting the writhings in mice in dose dependent manner. The results were comparable with the group treated with aspirin. Therefore, anti-inflammatory substances may also be involved in the peripheral analgesic activity.

It is known that non-steroidal anti-inflammatory drugs usually do not increase the pain threshold in normal tissues, whereas local anesthetics and narcotics do[24]. However, the hot plate test was undertaken to verify if MEIC would have any central analgesic effect. The results for the group treated with MEIC showed significant activity when compared to control group and nearly equal to the group treated with pentazocin (5 mg/kg). Hence, it is assumed that MEIC has significant analgesic effect on the central nervous system.

Preliminary phytochemical screening indicated the presence of flavonoids in MEIC. Selected phenolic compounds and flavonoids were shown to inhibit both the cyclooxygenase and 5-lipxygenase pathways[25-27]. This inhibition reduces the release of arachidonic acid. The exact mechanism by which flavonoids inhibit these enzymes is not clear. Quercetin, in particular, inhibits both cyclooxygenase and lipoxigenase activities, thus diminishing the formation of these anti-inflammatory metabolites[28,29]. Another anti-inflammatory feature is the ability of flavonoids to inhibit eicosanoid biosynthesis. Eicosanoids, such as prostaglandins, are involved in various immunological responses and are the end products of the cyclooxygenase and lipoxigenase pathways. Flavonoids also inhibit both cytosolic and membranal tyrosine kinases which play key roles in the signal transduction pathway that regulates cell proliferation[30-32]. Another anti-inflammatory property of flavonoids is their suggested ability to inhibit neutrophils degranulation. This is the direct way to diminish the release of arachidonic acid by neutrophils and other immune cells [33, 34]. From the above discussion, the methanol extract of *Indigofera cassioides* exhibited significant anti-inflammatory and analgesic activity.

All these data obtained in this study point to possibly developing the methanol extract of *Indigofera cassioides* as a novel and potential agent in the management of inflammation and pain which are probably mediated via inhibition of various autocoids formation and release. Further detailed investigation is underway to determine the exact phytoconstituents that are responsible for these activities.

Conflict of interest statement

We declare that we have no conflict of interest.

References

[1] Brekhmann II, Dardimov IV. New substances of plant origin which increase nonspecific resistance. *Annu Rev Pharmacol* 1969; **21**: 219–226.

[2] Patwardhan B, Hopper B. Ayurvedic and future drug development. *J Alter Complement Med* 1992; **19**: 9–10.

[3] Mantri P, Witiak DT. Inhibition of cyclooxygenase and 5–lipoxygenase. *Curr Med Chem* 1994; **1**: 328–355.

[4] Smith WL, De Witt DL. Biochemistry of prostaglandins endoperoxide H synthase–2 and their differential susceptibility to NSAIDs. *Sem Nephrol* 1995; **15**: 179–194.

[5] Lipsky P. The clinical potential of COX–2 specific inhibitors. *Am J Med* 1999; **106**: 51S–57S.

[6] Farnsworth NR. Screening plants for new medicines. In: Wilson EO. *Biodiversity Part II.* Washington: National Academy Press; 1989, p. 83–97.

[7] Eisner T. Chemical prospecting. A call for action. In: Borman FH, Kellert SR. *Ecology, Economic and Ethics, The broken cycle*. New Haven CT: Yale University Press; 1996.

[8] Nadkarni AK. *Indian Materia Medica.* Popular Prakasan Pvt. Ltd: Bombay; 1993, p. 680.

[9] Kirtikar KR, Basu BD. *Indian Medicinal Plants*. Dehradun: International Book Publishers; 1993, p. 714–715.

[10] Senthil Kumar R, Rajkapoor B, Perumal P. Antioxidant activities of *Indigofera cassioides* Rottl. Ex. Dc. using various *in vitro* assay models. *Asian Pac J Trop Biomed* 2012; **2**(4): 256–261.

[11] Raju Senthil Kumar, Balasubramanian Rajkapoor, Perumal Perumal. *In vitro* and *In vivo* anticancer activity of *Indigofera cassioides* Rottl. Ex. DC. *Asian Pac J Trop Med* 2011; **4**(5): 379–385.

[12] Sulaiman MR, Perimal EK, Akhtar MN, Mohamad AS, Khalid MH, Tasrip NA, et al. Anti–inflammatory effect of zerumbone on acute and chronic inflammation models in mice. *Fitoterpia* 2010; **81**: 855–858.

[13] Jothimanivannan C, Kumar RS, Subramanian N. Anti–inflammatory and analgesic activities of ethanol extract of aerial parts of *Justicia gendarussa* Burm. *Int J Pharmacol* 2010; **6**(3): 278–283.

[14] Purnima A, Koti BC, Tikare VP, Viswanathaswamy A, Thippeswamy A, Dabadi P. Evaluation of analgesic and antipyretic activities of *Centratherum anthelminticum* (L) kuntze seed. *Ind J Pharm Sci* 2009; **71**: 461–464.

[15] Nikajoo LT. Analgesic activity of aqueous and alcohol root extracts of *Pergularia daemia* (forsk.) chiov. *Int J Pharm Pharm Sci* 2009; **1**: 33–37.

[16] Brito ARMS, Antonio MA. Oral anti–inflammatory and antiulcerogenic activities of a hydroalcoholic extract and partitioned fractions of *Turnera ulmifolia* (Turneraceae). *J Ethnopharmacol* 1998; **61**: 215–228.

[17] Olajide OA, Awe SO, Makinde JM. Effect of the aqueous extract of Bridelia ferruginea stem bark on Carrageenan–induced edema and granuloma tissue formation in rats and mice. *J Ethnopharmacol* 1999; **66**: 113–117.

[18] Ueno A, Naraba H, Ikeda Y. Intrinsic prostacyclin contributes to exudation induced by bradykinin or Carrageenan: a study on the paw edema–induced in preceptor – deficient mice. *Life Science* 2000; **66**: 155–160.

[19] Nantel F, Denis D, Gordon R. Distribution and regulation of cyclooxygenase – 2 in carrageenan–induced inflammation. *Braz J Pharmacol* 1999; **128**: 853–859.

[20] Olajide OA, Awe SO, Makinde JM. Studies on the anti–inflammatory, antipyretic and analgesic properties of the *Aistonia boonei* stem bark. *J Ethnopharmacol* 2000; **71**: 179–186.

[21] Deraedt R, Jouquey S, Delevallee F, Flahaut M. Release of prostaglandins E and F in an algogenic reaction and its inhibition. *Eur J Pharmacol* 1980; **61**: 17–24.

[22] Hokanson GC. Acetic acid for analgesic screening. *J Nat Prod1* 978; **41**: 497–498.

[23] Duarte JDG, Nakamura M, Ferreira SH. Participation of the sympathetic system in Acetic acid induced writhing in mice. *Braz J Med Biol Res* 1988; **21**: 341–343.

[24] Ferreira SH, Lorenzetti BB, Castro MSA, Correa FMA. Antialgic effect of aspirin like drugs and the inhibition of prostaglandin synthesis. In: Dumonde DC, Jasani MK eds. *The recognition of anti–rheumatic drugs*. Lancaster: MTP Press Limited, St. Leonard House; 1978, p. 25–37.

[25] Ferrandiz ML, Nair AG, Alcaraz MJ. Inhibition of sheep platelet arachidonate metabolism by flavonoids from Spanish and Indian medicinal herbs. *Pharmazie* 1990; **45**: 206–208.

[26] Ferrandiz ML, Alcaraz MJ. Anti–inflammatory activity and inhibition of arachidonic acid metabolism by flavonoids. *Agents Actions* 1991; **32:** 283–288.

[27] Laughton MJ, Evans PJ, Moroney MA, Hoult JR, Halliwell B. Inhibition of mammalian 5–lipoxygenase and cyclooxygenase by flavonoids and phenolic dietary additives. Relationship to antioxidant activity and to iron ion–reducing ability. *Biochem Pharmacol* 1991; **42**: 1673–1681.

[28] Robak J, Gryglewski RJ. Bioactivity of flavonoids. *Pol J Pharmacol* 1996; **48**: 555– 564.

[29] Kim HP, Mani I, Iversen L, Ziboh VA. Effects of naturally occurring flavonoids and bioflavonoids on epidermal cyclooxygenase and lipoxigenase from guinea pigs. *Prostaglandins Leukot Essent Fatty Acids*, 1998; **58**: 17–24.

[30] Damas J, Bourdon V, Remacle–Volon G, Lecomte J. Pro–antiinflammatory flavonoids which are inhibitors of prostaglandin biosynthesis. *Prostaglandins Leukot Med* 1985; **19**: 11–24.

[31] Hertog MG, Kromhout D, Aravanis C. Flavonoids intake and long term risk of coronary heart disease and cancer in the seven countries study. *Arch Intern Med* 1995; **155**: 381–386.

[32] Formica JV, Regelson W. Review of the biology of quercetin and related bioflavonoids. *Food Chem Toxicol* 1995; **33**: 1061–1080.

[33] Hoult JR, Moroney MA, Paya M. Actions of flavonoids and coumarins on lipoxigenase and cyclooxygenase. *Methods Enzymol* 1994; **234**: 443–454.

[34] Tordera M, Ferrandiz ML, Alcaraz MJ. Influence of anti–inflammatory flavonoids on degranulation and arachidonic acid release in rat neutrophils. *Z Naturforsch* 1994; **49**: 235–240.