Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

Evaluation of the antinociceptive properties from *Indigofera tinctoria* leaves extracts

*Saravana Kumar A., [1]Gandhimathi R., [1]Mohana Lakshmi S., [1]Rahul Nair., [1]Ashok.Kumar.C.K

Sree Vidyanikethan College of Pharmacy, Sree Sainath Nagar, Chandragiri (M), Tirupati, Andhra Pradesh, India-517102.

Abstract

The analgesic activity the ethanol extract of *Indigofera tinctoria* Linn. (Fabaceae) leaves (EEIT) was investigated in chemical models of nociception in mice. EEIT at doses of 100 200 and 400mg/kg p.o produced an inhibition of 21.71%, 42.62% and 72.38%, of the abdominal writhes induced by acetic acid in mice. In the formalin test, the administration of 100,200 and 400mg/kg *p.o* had no effects in the first phase (0–5 min) but produced a dose-dependent analgesic effect on the second phase (15–40 min) with inhibitions of the licking time of 26.62%, 46.5% and 60.07%, respectively. In hot plate method, EEIT at the dose of 400mg/kg showed the mild analgesic and EEIT 100 and 200 mg/kg showed non significant analgesic effect due to peripheral analgesic activity. Based on the results of this study, we suggest that the peripheral analgesic effect of *Indigofera tinctoria* may be attributed to inhibition of prostaglandin release and other mediators involved. Further studies are needed to evaluate the mechanism of action of the analgesic activity of the *Indigofera tinctoria.*

Key words: antinociceptive activity, chemical models, *Indigofera tinctoria,* peripheral analgesic activity.

Introduction

Pain is part of life, present throughout human development, from birth to death. It is associated with various diseases, inflammatory conditions, tissue trauma and surgical interventions. Pain is essential for survival because it acts as a protective and alarm mechanism. According to the International Association for the Study of Pain, pain is an unpleasant sensory and emotional experience associated with potential or actual tissue damage, or described in terms of such damage [1]. Etiology of pain is the encoding and processing of noxious stimuli by the nervous system. When a noxious stimulus is applied to normal tissue, physiological nociceptive pain occurs and withdrawal reflexes are activated. Pathophysiological nociceptive pain occurs when tissue is inflamed or injured; it may appear as spontaneous pain, as hyperalgesia and can be effectively treated with nonsteroidal anti-inflammatory drugs and opiates [2,3]. But development of dependence and incidence of side effects on clinical evaluation make their efficacy arguable. This has been the basis for the development of new analgesic drugs, which includes herbal drugs.

Indigofera tinctoria Linn. (Family: Fabaceae) was one of the original sources of indigo dye. It has light green pinnate leaves and sheafs of pink or violet flowers, cultivated extensively in Northern India, especially in Bengal, Bihar, Orissa, Sind and southern India, Madras. The plant is a legume, so it is rotated into fields to improve the soil [4]. The plant has been extensively used in ayurveda & siddha used for Tikita rasam, katu rasam, ushna veeryam, katu, vipaka, anthelmintic, anti periodic. Roots used for anti poison, giddiness, colic, gonorrhoea, hair tonic. Leaves used for Jaundice, produce complexion, vatha fever, gout. In Unani used for haemostatic, sedative, piles, healer of ulcers, diuretic, dropsy.

* **For Correspondence**
A. Saravana Kumar M.Pharm., (Ph.D)
Assistant Professor
Department of Pharmacology
Sree Vidyanikethan College of Pharmacy
Sree Sainath Nagar, Chandragiri (M)
Tirupathi , Andhra Pradesh, India-517102
E-mail: sarganjune1@gmail.com

Decoction of the leaves used in bites and strings of venomous insects and reptiles, to relieve the pain and also burn and scalds. [5]. The whole plant of *Indigofera tinctoria* Linn. contains glycoside indican, indigotine, indiruben, galactomannan composed of galactose and mannose, 2.5% of alkaloids, rotenoids and flavanoids [6]. and their pharmacological activities hepatoprotective[7], antidyslipidemic[8], antiproliferative[9] , antileukaemia[10] and plants as a Source of Anti-Cancer agents[11] were reported. However there are no reports on the analgesic activity of the plant leaves. Hence, the present study was designed to verify the claims of the native practitioners.

Materials and Methods

Plant collection

The leaves of *Indigofera tinctoria* Linn. was collected from abirami botanicals of Tuticorin, Tamilnadu. It was identified and authenticated by Prof. Jayaraman, Taxonomist, Tambaram, Chennai, Tamilnadu, India. The voucher specimen (IT-P-08-S5) of the plant was deposited at the college for further reference.

Preparation of extracts

The leaves of plants were dried in shade, separated and made to dry powder. It was then passed through the 40 mesh sieve. A weighed quantity (220gm) of the powder was subjected to continuous hot extraction in Soxhlet Apparatus. The extract was evaporated under reduced pressure using rotary evaporator until all the solvent has been removed to give an extract sample. The ethanolic extract of *Indigofera tinctoria* Linn. (EEIT) yielded thick violet semi-solid residues. Percentage yield of MEIT was found to be 18.9% w/w.

Preliminary phytochemical screening

The phytochemical examination of the EEIT was performed by the standard methods [12].

Animals used

Male albino mice (20-25g) were obtained from the animal house in C.L. Baid Metha College of Pharmacy, Chennai. The animals were maintained in a well-ventilated room with 12:12 hour light/dark cycle in polypropylene cages. The animals were fed with standard pellet feed (Hindustan Lever Limited., Bangalore) and water was given *ad libitum*. Ethical committee clearance was obtained from IAEC (Institutional Animal Ethics Committee) of CPCSEA (Reference No: IAEC/XIII/06/CLBMCP/2008-2009 Dated on 4-09-2008.

Acute Toxicity Study

The acute toxicity of 90% ehanolic extract of *Indigofera tinctoria* was determined as per the OECD guideline no. 423 (Acute Toxic Class Method). It was observed that the test extract was not mortal even at 2000mg/kg dose. Hence, $1/20^{th}$ (100mg/kg), $1/10^{th}$ (200mg/kg) and $1/5^{th}$ (400mg/kg) of this dose were selected for further study [13]**.**

Antinociceptive activity

Acetic acid-induced writhing in mice:

The writhing test was carried out as described by Koster *et al.* (1959) [14]. Male Albino mice weighing 20 to 25 gm were divided into five groups of eight animals each. The first group received vehicle control (1% w/v SCMC, 1ml/100 g) whereas Group-II, III and VI received 90% ethanolic extract of the *Indigofera tinctoria* (L.) (EEIT) (100, 200 and 400 mg/kg body weight) *p.o* Group-V received standard drug (indomethacin, 10mg/kg) *p.o*, respectively. The muscular contraction was induced by an intraperitoneal injection of 0.6% acetic acid solution (0.25 ml/animal) 30 min after the treatment. The responses of extract treated groups were compared with those of animals receiving indomethacin 10mg/kg (as standard drug), as well as with the controls.

Formalin induced pain

The formalin test was carried out as described by Hunskaar and Hole (1987) [15]. Male Albino mice weighing 20 to 25 gm were divided into five groups of eight animals each. All groups of animals were injected subcutaneously with 20µl of formalin into the dorsal hind paw. The Group I received vehicle control (1% w/v SCMC, 1ml/100 g) whereas Group-II, III and IV received 90% ethanolic extract of the *Indigofera tinctoria* (L.) (100, 200 and 400 mg/kg body weight p.o) and Group-V received standard drug (indomethacin, 10mg/kg) p.o, respectively 30 min before formalin injection. The time the mice spent licking or biting the injected paw or leg was recorded. On the basis of the response pattern described by Tjolsen *et al.* (1992) [16], two distinct periods of intensive licking activity were identified and scored separately. The first period (early phase) was recorded 1- 5min after the injection of formalin and the second period (late phase) was recorded 20–40 min after the injection. The percentage inhibition of licking was calculated by the formula: $(C-T)/C\times100$ where C represents the vehicle treated control group value for each phase and T represents the treated group value for each phase.

Hot plate reaction time in mice

The *hot plate* method was carried out as described by Turner (1965) [17]. Male Albino mice weighing 20 to 25 gm were divided into five groups of eight animals each. Group I received vehicle control (1% w/v SCMC, 1ml/100 g p.o) Group-II and, III and IV received 90% ethanolic extract of the *Indigofera tinctoria* (L.) (100, 200 and 400 mg/kg body weight p.o) and Group V received standard drug morphine (5 mg/kg s.c) 30 min before the thermal pain stimulus. Mice were screened by placing them on a hot plate maintained at 55±1 °C and recorded the reaction time in seconds for licking of hind paw or jumping. A cut-off time of 40s was selected to avoid tissue damage.

Statistical analysis

The data were expressed as mean ± standard error mean (S.E.M).The Significance of differences among the group was assessed using one way and multiple way analysis of variance (ANOVA). The test followed by Dunnett's test p values less than 0.05 were considered as significance.

RESULTS

Phytochemical screening

The results of preliminary phytochemical screening of the ethanoloic extract of *Indigofera tinctoria* (L.) revealed that presence of alkaloids, flavonoids, carbohydrates, glycosides, tannins, terpeniods, phenols and absence of saponins and steroids.

Table-1. Analgesic effect of ethanol extract of *Indigofera tinctoria* (L) (EEIT) in the acetic acid-induced writhing test [a]

Group	Design of treatment	Number of writhings[b]	Inhibition (%)
I	Control(1% w/vSCMC,1ml/100g)	37.3±1.6	–
II	EEIT (100mg/kg b.w, *p.o*)	30.2 ± 1.4	21.71
III	EEIT (200mg/kg b.w, *p.o*)	21.4 ±1.5*	42.62
IV	EEIT (400mg/kg b.w, *p.o*)	10.3 ± 1.4**	72.38
V	Indodomethacin (10mg/kg b.w, *p.o*)	10.8 ±1.6**	71.04

[a] After 30 minutes EEIT treatment, mice were injected *i.p.* with 0.6% (v/v) acetic acid. [b] Values are mean±S.E.M. (N=8).
* $P<0.05$, ** $P<0.01$ significant compared with control values (ANOVA followed Dunnett's test).

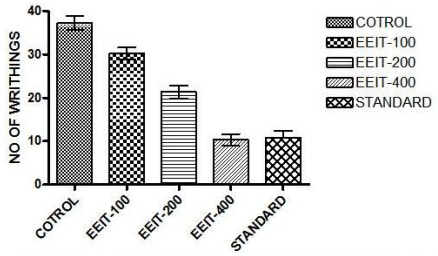

Figure-1. Analgesic effect of ethanol extract of *Indigofera tinctoria* (L) (EEIT) in the acetic acid induced writhing test

Acetic acid-induced writhing in mice:

Table-1 and Figure-1 shows the pain behavior of writhing response, which is presented as cumulative abdominal stretching response. The treatment of animals with EEIT extract (100 and 200mg/kg/ body weight *p.o*) produced a significant and dose dependent inhibition (21.71 and 42.62%) of the control writhes. The inhibition writhing response by EEIT (400 mg/kg) (72.38%) was more than to that produced by Indomethacin (10 mg/kg) (71.34%).

Formalin induced pain:

EEIT (200 mg/kg, 400 mg/kg) produced significant ($P < 0.01$) inhibition in the late phase of formalin induced pain (46.5 and 60.07%) respectively (Table-2 and Figure-2). The positive control indomethacin (10 mg/kg) also produced significant ($P < 0.01$) inhibition in the late phase (65.81%).

Hot plate reaction time in mice

As shown in Table-3 and Figure-3, the EEIT showed significant ($P < 0.05$) analgesic activity at the dose of 400mg/kg b.w p.o. Morphine sulphate at 5 mg/kg significantly showed its maximum protective effect of 29.78±1.2 ($P < 0.01$) after 30min compared to (19.16±1.4) ($P < 0.05$) 400 mg/kg of the EEIT. But EEIT 100 and 200mg/kg produced non significant protection (12.57±0.9 and 15.52±0.4) from thermal induced pain.

Discussion and Conclusion

The ethanol extract of *Indigofera tinctoria* (L) (EEIT) given orally at doses of 100, 200 and 400 mg/kg significantly inhibited the acetic acid-induced writhing response in a dose-dependent manner. Acetic acid, which is used as an inducer for writhing syndromes and, causes analgesia by releasing of endogenous substances, which then excites the pain nerve endings; the abdominal constriction is related to the sensitization of nociceptive receptors to prostaglandins [18]. It is well known that after administration of acetic acid there is the liberation of several mediators such as cytokines and eicosanoids. Also the arachidonic acid is liberated from membrane after phospholipase A2 activity leading to the production of prostaglandins and leukotrienes [14,19]. In this method extracts could act inhibiting phospholipase

Table 2. Analgesic effect of ethanol extract of *Indigofera tinctoria* (L) (EEIT) in the formalin test [a]

Groups	Design of treatment	Licking(s) [b]		Inhibition (%)
		0-5min	15-40min	
I	Control(1%w/vSCMC,1ml/100g)	55.5± 2.6	121.7±3.5	–
II	EEIT (100mg/kg b.w, *p.o*)	54.5±2.4	89.3±3.3*	26.62
III	EEIT (200mg/kg b.w, *p.o*)	54.6±2.7	65.1±2.7**	46.5
IV	EEIT (400mg/kg b.w, *p.o*)	52.4± 2.2	48.6±2.3**	60.07
V	Indomethacin(10mg/kg b.w, *p.o*)	53.8± 2.3	41.6± 2.6**	65.81

[a] After 30 minutes EEIT treatment, mice were injected *s.c* with 20µl of formalin into the dorsal hind paw. [b] Values are expressed as mean±S.E.M. (N=8). * P<0.05, ** P<0.01 significant compared with control values (ANOVA followed y Dunnett's test).

A2, or even blocking cyclooxigenases (COX-1 and/or COX-2). This result indicates that the analgesic effect of EEIT might be mediated by its peripheral effect such as blockage the stimulus propagation in the pain nervous fibers or blockage in the eicosanoid system.

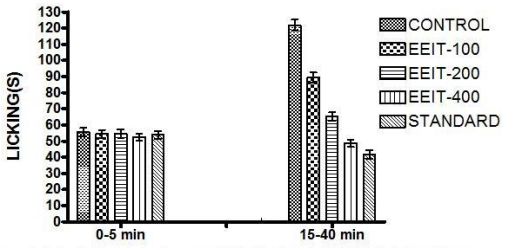

Figure-2. Analgesic effect of ethanol extract of *Indigofera tinctoria* (L) (EEIT) in the formalin test

The formalin test is a valid and reliable model of nociception and is sensitive for various classes of analgesic drugs. Formalin test produced a distinct biphasic response and different analgesics may act differently in the early and late phases of this test. Therefore, the test can be used to clarify the possible mechanism of antinociceptive effect of a proposed analgesic effect. Experimental results demonstrated that substance P and bradykinin participate in the early phase, while histamine, serotonin, prostaglandins, nitric oxide and bradykinin are involved in the late phase of the formalin test [16]. Centrally acting drugs such as opioids inhibit both phases equally [20]. But peripherally acting drugs such as aspirin, indomethacin and dexamethasone only inhibit the late phase. The late phase seems to be an inflammatory response with inflammatory pain that can be inhibited by anti-inflammatory drugs [21, 22]. The effect of *Indigofera tinctoria* on the late phase of formalin test suggests that its activity may be resulted from its peripheral action or anti inflammatory action when compared with indomethacin activity.

The hot plate test has been found to be suitable for evaluation of centrally acting analgesics [23]. It is known that non-steroidal anti-inflammatory drugs usually do not increase the pain threshold in normal tissues, whereas local anesthetics and narcotics drugs are increase the pain threshold [24]. The results for the group-treated with EEIT did not differ significantly from those obtained for the negative control group. On the other hand, group-treated with morphine (5 mg/kg, s.c) showed a highly significant result. Hence, it is assumed that EEIT has no analgesic effect on the central nervous system that would contribute to its peripheral analgesic effect. Therefore, despite of the mild effect observed for EEIT at the dose of 400mg/kg for this test in relation to the control group. Based on the results of this study, we suggest that the peripheral analgesic effect of *Indigofera tinctoria* may be attributed to inhibition of prostaglandin release and other mediators involved. Further studies are necessary to fully elucidate the mechanism of action of the analgesic activity of the *Indigofera tinctoria.*

A number of flavonoids have been reported to produce analgesic activity. Also, there are few reports on the role of tannins in analgesic activity [25]. Hence, the present analgesic activity of *Indigofera tinctoria* may attribute to the presence of flavonoids and tannins. Tannins and flavonoids were detected in the

Table-3. Effect of ethanol extract of *Indigofera tinctoria* (L) (EEIT on hot plate reaction time in mice

Group	Design of treatment	Mean Latent Time Initial [b]	After 30 min[b]
I	Control(1%w/vSCMC,1ml/100g)	10.43 ± 0.8	10.36±0.7
II	EEIT (100mg/kg b.w, *p.o*)	10.03 ± 0.7	12.57±0.9[ns]
III	EEIT (200mg/kg b.w, *p.o*)	10.74 ± 0.6	15.52±0.4[ns]
IV	EEIT (400mg/kg b.w, *p.o*)	11.93 ± 0.7	19.16±1.4*
V	Morphine (5 mg/kg s.c)	11.98 ±0.6	29.78±1.2**

[a]After 30 minutes EEIT treatment, mice were placed on hot plate. [b]Values are expressed as mean±S.E.M. (N=8) and units are in sec. ns – non significant,* P<0.05, and ** P<0.01 significant compared with control values (ANOVA followed Dunnett's test).

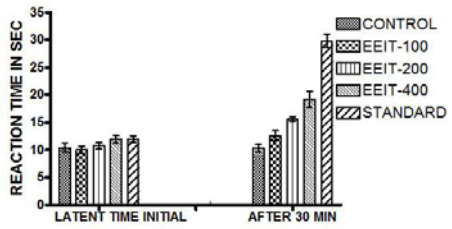

Figure-3. Analgesic effect of ethanol extract of *Indigofera tinctoria* (L.) (EEIT on hot plate reaction time in mice

preliminary phytochemical screening of *Indigofera tinctoria,* suggesting the possibility of those constituents may contribute the analgesic activity of the plant.

In conclusion, this study has demonstrated with pharmacological models that *Indigofera tinctoria* has analgesic effect attributed to the plant in folklore medicine. However, more experiments were needed to find out the mode of action and identify the compounds responsible for the analgesic effect.

Acknowledgement

Authors are sincerely thankful to Dr. S. Venkatraman, M.Sc., M.D., Ph.D., Director and Mr.P.Muralidharan, M.Pharm. (Ph.D), Department of Pharmacology, C.L. Baid Metha Foundation for Pharmaceutical Education and Research, for their contribution and facilities provided regarding our Research work.

References

[1] Rocha, A.P., Kraychete, D.C., Lemonica, L., Carvalho, L.R., Barros, G.A.M., Garcia, J.B.S., Sakata, R.K., Rev. Bras. Anestesiol., 2007, 57 (1), 94–105.

[2] Schaible, H.G., Richter, F., Patho physiology of pain. Langenbecks Arch Surg, 2004, 389 (4), 237–243.

[3] Basbaum, A., Bushnell, C., Pain: basic mechanisms: World Congress on Pain (10th, San Diego, Calif) *Pain,* 2002, 1, 3–7.

[4] *Indigofera tinctoria* (L.). http://en.wikipedia.org/wiki/Indigofera_tinctoria.

[5] Nadkarni, A.K., *Indian Materia Medica.* 1926, 1, 680-682.

[6] Chopra, R.N., Nayer, S.L., Chopra, I.C., *Glossary of Indian Medicinal Plants*, National Institute of Science Communication 1996, pp.141.

[7] Singh, B., Saxena, A.K., Chandan, B.K., Bhardwaj, V., Gupta, V.N., Suri, O.P., and Handa, S.S., *Phytotherapy Reserch,* 2001, 15, 294-297.

[8] Narender, T., Tanvir, K., Anjupuri, and Ramesh Chander, *Bioorganic & Medicinal Chemistry Letters,* 2006, 16, 3411-3414.

[9] Kameswaran, R., and Ramanibai, R., *Journal of Biological Sciences*, 2008, 1-7.

[10] Hoessel, R., Eisenbrand, G., and Meijer, L., *Nature cell biology,* 1999, 1, 60-67.

[11] Cragg, G.M., and Newman, Developed under the Auspices of the UNESCO, *Encyclopedia of Life Support System (EOLLS)*, Oxford, UK 2006.

[12] Harbone, J.P., Phytochemical methods, a guide to modern technique of plant analysis *(Chapmann and Hall, London),* 1973, pp.1-271.

[13] OECD, 2002. Acute oral toxicity. Acute oral toxic class method guideline 423 adopted 23.03.1996. In: Eleventh Addendum to the, OECD, guidelines for the testing of chemicals organisation for economical co-operation and development, Paris, June, 2000.

[14] Koster, R., Anderson, M., and De Beer, E.J., *Acetic acid analgesic screen*, Federation Proceedings, 1959, 18, 412–420.

[15] Hunskaar, S., and Hole, K., *Pain,* 1987, 30, 103–114.

[16] Tjolsen, A., Berge, O.G., Hunskaar, S., Rosland, J.H., and Hole, K., *Pain,* 1992, 51, 5–17.

[17] Turner, R.A., In: Turner, R., Ebborn, P. (Eds.), *Analgesics: Screening Methods in Pharmacology.* Academic Press, New York, 1965, pp. 100.

[18] Chen, Q., *Methodology in pharmacological study on Chinese Materia Medica*, People's Medical Publishing House, Beijing, 1993, pp. 360.

[19] Martinez, V., Thakur, S., Mogil, J.S., Tach´e, Y., Mayer, E.A., *Pain,* 1999, 81, 179- 186.

[20] Shibata, M., Ohkubo, T., Takahashi, H., and Inoki, R., *Pain*, 1989, 38, 347–352.

[21] Hunskaar, S., and Hole, K., *Pain*. 1987; 30: 103–114.

[22] Rosland, J.H., Tjolsen,, A., Maehle, B., and Hole, K., *Pain,* 1990, 42, 235–242.

[23] Dhara, A.K., Suba, V., Sen, T., Pal, S., Chaudhuri, A.K.N., *Ethnopharmacology* 2000, 51, 17.

[24] Ferreira, S.H., Lorenzetti, B.B., Castro, M.S.A., Correa, F.M.A., Dumonde, D.C.,

Jasani, M.K. (Eds.), *The Recognition of Anti-rheumatic Drugs*, MTP Press Limited, St. Leonard House, Lancaster, 1978, pp. 25–37.

[25] Hossinzadeh, H., Ramezani, M., Fedishei, M., Mahmoudi M., *Phytomedicine,* 2002, 9, 135-141.