Research Article

Analgesic Activity of Leaf Extracts of *Indigofera trifoliata*

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ABSTRACT

Most of the species belonging to the species *Indigofera* were used as an analgesic and anti inflammatory agents since time in memorial. The present research work focuses on leaf extracts of *Indigofera trifoliate* F. (Fabaceae) for their analgesic activity in albino rats. The analgesic activity of the aqueous and methanolic leaf extracts of *I. trifoliate* were evaluated by giving oral doses of 50, 100 and 200 mg/kg body weight using Tail flick response method and acetic acid induced writhing assay. The output of the analgesic activity showed dose dependence and significant ($P < 0.001$) increase in the analgesic activity at 90 min in tail flick response method, whereas the acetic acid induced writhing results are found to be significant for methanolic extract than that of aqueous extract. The analgesic effect produces by the extract is inferior to that of the standard drug used i.e Diclofenac sodium, when tested for the same. The results clearly states that the aqueous and methanolic extracts delayed the reaction time towards the thermally induced pain, reduced acetic-acid induced writhing, at higher doses the activity of both the extracts are significant ($P < 0.001$) compared with that of the reference drug. Hence, it is important to isolate the active principles for further testing the analgesic efficacy. The following secondary metabolites are identified by general chemical tests, from the above research work carried out it can be implicated that the flora should be explored still further for different secondary metabolites and other potential compounds as an alternate remedy for commercially available analgesic drugs. The natural system of medicine is gaining passion due to their fewer side effects than that of traditional medicines.

Keywords: *Indigofera tifoliata*, aqueous extract, methanolic extract, analgesic activity.

INTRODUCTION

Analgesic/ chronic pain is one of the major medical emergency yet to be attended due to unwanted side effects caused by the traditional drugs[1] mainly gastric mucosal depletion or ulcer causing effects by major class of analgesic drugs i.e NSAIDs, tolerance and dependence by most of the corticosteroids and opiate analgesics, and increase in the weight (obesity), or deposition of the fat by steroidal analgesic and anti inflammatory drugs[2]. So it is an alarming effect to explore alternate remedies which are biologically compatible, with fewer side effects[3]. Indigofera is a tropical and subtropical plant and which grows in the temperate region also[4]. It belongs to the family Fabaceae. It is widely distributed in Africa, Oman, Mauritius[5]. In India it is mostly distributed in Andhra Pradesh, Arunachal Pradesh, Assam, Bihar, Daman and Diu, Delhi, Goa, Gujarat, Haryana, Himachal Pradesh and other parts. Morphological features of the plant is erect or spreading, rarely prostrate or trailing, perennial herb or subshrub, 0.3–0.6(–1.2) m high, with woody rootstock or taproot; young stems ridged, green, strigose with dense, appressed, equally or unequally biramous hairs[6, 7]. Leaves digitately trifoliolate,. Indigofera is a large genus and most of the scientists and researchers have worked on this plant for their different phytochemical constituents and pharmacological activity[8, 9.]
Several of them and especially *Indigofera tinctoria* and *Indigofera suffruticosa* are used to produce the dye indigo [10]. Colonial planters in the Caribbeangrew indigo and transported its cultivation when they settled in the colony of South Carolina. Exports of the crop did not expand until the mid-to late 18th century[11]. When Eliza Lucas Pinckney and enslaved Africans successfully cultivated new strains near Charleston it became the second most important cash crop in the colony (after rice) before the American Revolution[12]. It comprised more than one-third the value of all exports. The chemical

aniline, from which many important dyes are derived, was first synthesized from *I. suffruticosa* (syn. *I. anil*, whence the name aniline). Several species of this group are used to alleviate pain. The herbs are generally regarded as an analgesic with anti-inflammatory activity, rather than ananodyne. *Indigofera articulata* Gouan (Arabic *Khedaish*) was used for toothache, and *Indigofera oblongifolia* Forsskal (Arabic "Hasr") was used as an anti-inflammatory for insect stings, snakebites, and swellings [13,14] . *Indigofera suffruticosa* and *Indigofera aspalthoides* have also been used as anti-inflammatories. A patent was granted for use of *Indigofera arrecta*extract to relieve ulcer pain.The Maasai people of Kenya use parts of *Indigofera brevicalyx* and *I. swaziensis* as toothbrushes. In Indonesia, especially Sundanese ethnic traditionally use *Indigofera tinctoria* L called as "tarum" as dye for batik.
So due to versatile pharmacological activities of the natural sources especially this genus it is necessary to find out alternate remedy for side effects caused by traditional system of medicine. The active principle which is responsible for the activity has to be found out by different chromatographic techniques.

PLAN AND OBJECTIVE

The design and objective of the present research work focuses on the identification of the analgesic activity of aqueous and methanolic leaf extracts of *Indigofera trifoliate* and to find out most potent extract among them.

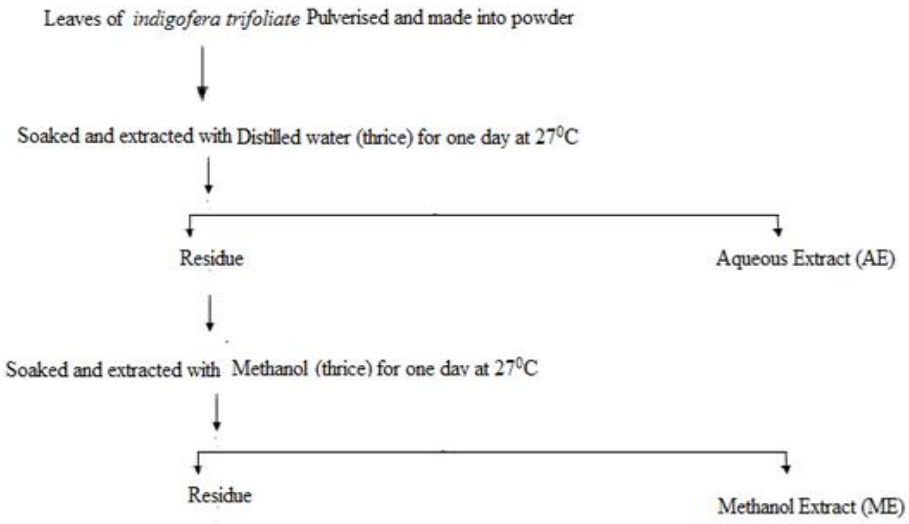
MATERIALS AND METHODS

Collection and authentication of plant material

The leaves of the plant, *Indigofera trifoliate* growing in the local areas in and around Visakhapatnam and Vizianagaram of Andhra Pradesh state were collected during the month of September-October. It was identified and authenticated by Dr. S.B.Padal, Dept. of Botany, Andhra University and Sample specimen was kept in our laboratory for future reference. Plant material was garbled at first to remove all the dust particles and unwanted material then it was washed thoroughly, initially with tap water and then with distilled water and then allowed to dry in shade. The dried plant material was pulverized to fine powder and stored at room temperature in air tight container until used further.

Preparation of Plant extracts

To 500 grams of *Indigofera trifoliate* leaf powder, 2litres of each solvent, viz. methanol and Distilled water was added consequently for preparing the extracts in increasing solvent polarity. (flow chart-1).Extraction with the solvent was done for one day at 27^0C, after maceration the supernatant of each solvent was recovered by filtering through whatmann filter paper. This process was repeated thrice and the respective solvent from the supernatant was evaporated in a Rota vapor to obtain crude extracts which are to be stored at 4^0c until used for evaluation.
The codes are given as follows
ME- Methanol extract of *Indigofera trifoliate,*
AE- Aqueous extract of *Indigofera trifoliate.*



Schematic representation of showing extraction procedure from leaves of *Indigofera trifoliate*

Experimental animals

White wistar albino rats weighing 150-250grams were obtained from Mahaveer enterprises, Hyderabad, Telangana. The animals are brought with at most care to the laboratory and they are allowed to acclimatize to the environmental condition and laboratory conditions. They are fed with water and food *ad libitum*. The experimental animals were treated according to the rules of institutional and international guidelines guiding the use of experimental animals. The animal ethical committee registration number: 1048/a/07/CPCSEA.

Screening of analgesic activity of *Indigofera trifolium* by *Tail flick method*[15,16,17]

The method followed to determine the analgesic activity of *I.trifoliata* is radiant heat tail-flick method. Analgesiometre is used for this experiment. The rats are placed such that their tail protrudes out, the radiant heat produced is applied over the tail with the help of the instrument. The time taken by the animal to flick the tail is determined as the reaction time. The power passing through the nichrome wire is kept constant at 5 ampere. The tail is kept at 1.5 cm from the heat source and the application of the heat is maintained with in 2cm from the starting root of the tail. The reaction time and the strength of the current is monitored carefully so as to avoid any damage or the injury to the tissue of the tail during the experiment.

Screening of suitable animals for the experiment

Total number of albino rats used for this experiment was 15 weighing between 150-250grams. These total rats were grouped in three groups containing 5 albino rats in each group. The rat withdraws its tail normally in 3-6 seconds when exposed to heat which is termed as flick reaction time, if any animal fails to flick its tail in that stipulated reaction time those rats were rejected for the study. The rats which are normal and suitable for the experiment were labeled accordingly for the identification purpose.

Preparation of Standard drug

Diclofenac sodium is used as a standard drug in this method. 400mg diclofenac sodium tablets were brought and powdered. Then 40ml of distilled water is added to get 10mg of diclofenac in 1ml of solution. Group I is maintained as standard group which received 0.1ml/ 100 gram body weight of albino rats. The drug is administered orally with the help of a cannula. Diclofenac is used as a control in this experiment.

Analgesic activity Screening

Analgesiometre is used for recording the response of flicking of tail by heating the nichrome wire as a source of heat. The tail of each rat is labeled with a marker. The initial flicking of the tail by the rat was observed for three times at an interval of 15 minutes. The rats which could not elicit response were rejected for the study of analgesic activity. The initial tail flicking responses of each rat are considered as control readings. The drug solution was administered orally with the help of cannula.

Group I was maintained as a standard group which received the standard drug diclofenac sodium orally. Group II received test drug (Methanolic extract). Group III received the test drug (Aqueous drug). The dose was calculated as per the body weight of the rats accordingly. The rats were administered with a dose of 0.25ml, 0.20ml, 0.15ml, 0.25 ml as per 250gms, 200gms, 200gms, 175gms and 250gms respectively. The tail flicking reaction time is noted at an interval of 30 minutes for about 180minutes. The time is recorded between the onset of stimulus and flicking of the tail. The heat id maintained at constant range to avoid the damage of the tissue of the tail.

Screening of Analgesic activity of *Indigofera trifolium* by Acetic acid induced writhing method [18,19]

For the acetic acid induced writhing assay method total number of rats taken is 25 which were divided as 5 rats per group. The writhing were induces as per the standard method. The standard group received the standard drug 100mg/kg body weight of diclofenac sodium. Control group received 0.3ml saline solution and the test groups received 50,100 and 200 mg/kg of *I.trifoliata* extracts intra peritoneal. Firstly the experimental rats are allowed to fast for 16hrs and after that the treatment is started. After one hour of the treatment the rats were administered with 0.3ml of 6% acetic acid which acts as a source for the contraction of the abdominal muscle (Writhing). The stretching contractions and jerking of the hind limb was noted between 5 to 30 minutes after the administration of the acetic acid. The standard group rats are compared with that of the test and control group. Inhibition of the writhing activity was taken as the mark of analgesia and the percentage of writhing inhibition is calculated

$$\% \text{ Inhibition of writhing} = \frac{\text{Mean writhing by control -- Mean writhing by test}}{\text{Mean writhing by control}} \times 100$$

RESULTS AND DISCUSSIONS

Tail flicking of the rat is recorded before administering of the drug at 30 minutes interval and after administering of the drug up to 180 minutes. The recording is done carefully without getting damage to the tissue of the tail. The reaction time to flick the tail and the time intervals are well elucidated in Fig I.

The standard group (group I) tail flicking initially recorded was 3.5 seconds, which upon administering of standard dose (diclofenac) gradually increased up to a mean of 8.3 seconds at 120 minutes time interval. Then tail flicking reaction time gradually decreased to 5.9 seconds.

The test group (Methanol extract) tail flick was initially recorded at 3.5 seconds, which gradually increased after administration of the test dose up to 90 minutes interval. The flicking of the tail decreased to 3.7 seconds at the end of 180 minutes time interval.

Another test group (Aqueous extract) tail flicking initially recorded at 2.7 seconds, Which gradually increased upon addition of the test dose up to 60 minutes and gradually decreased to 2.8 seconds at 180 minutes time interval.

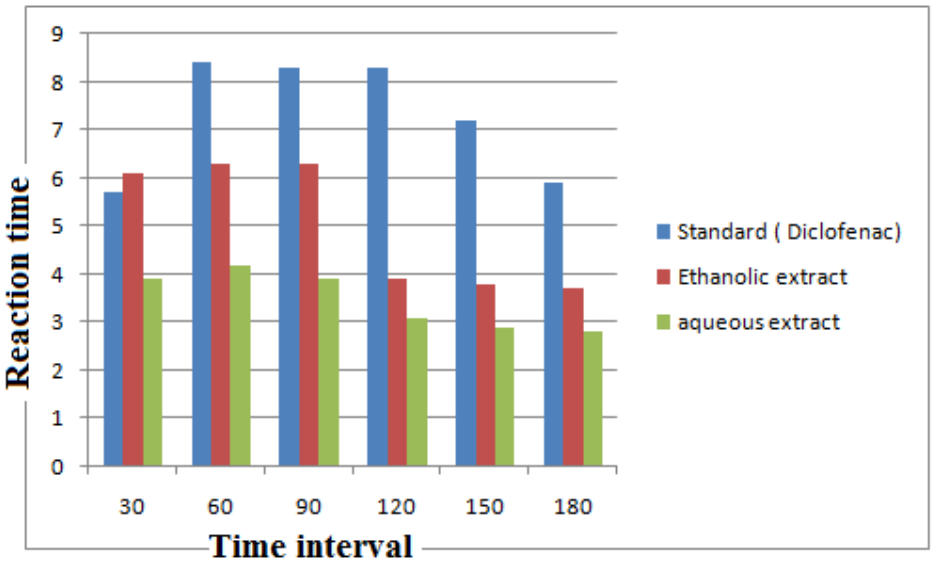


Fig. I: Responses recorded between the Standard drug (Diclifenac) and methanol and aqueous Extracts of *I.trifoliata*

Statistical Analysis

The standard group (Group I) showed a mean increase of 2.2 seconds before administration of the drug. The student t test showed that the variation is statistically significant. The time of reaction of tail flicking has increased to 8.3 seconds up to 120 minutes after administration of the drug with a mean difference of 4.8 seconds ($P<0.001$). But at 180 minutes there is a decline in the reaction time with a mean difference of 2.4 seconds. So statistically all the results shown that the mean increase in the flick time of the tail were highly significant which is well illustrated in Table I.

The test dose (methanol extract) group II showed a mean increase of 2.7 seconds initial flicking of tail. After administration of the drug (0.1ml / 100gm body weight) equivalent to the standard drug (Diclofenac sodium) the tail flick reaction time drastically increased and remained constant until 90 minutes, this showed that the increase in the mean reaction time is highly significant($P<0.001$).Later the reaction time declined with a mean reaction time of 0.3 seconds.

The test dose (aqueous extract) group III showed a mean increase of 1.2 seconds initial flicking of tail. After administration of the drug (0.1ml / 100gm body weight) a slight increase in the mean reaction

time until 60 minutes has occurred, this showed that the increase in the mean reaction time is not statistically significant (P<0.5).Later the reaction time declined with a mean reaction time of 0.2 seconds.

Group II and Group III showed a mean increase in the tail flicking time when compared with that of standard at 30 minutes after administration of the drug is about 2.2 and 2.7 seconds respectively. The readings showed a gradual increase in the mean reaction time up to 90 minutes. The test dose (methanolic extract) also produced identical effectiveness when compared with that of the standard dose. But as the time progressed there is a drastically decrease in the mean reaction time. The comparison between the standard and the test group II showed statistical significant reading (P<0.001). The above results implicate that the methanolic extract dose produced similar effect as that of the standard upto a time interval of 90 minutes. The mean reaction time comparison between the Standard dose , methanolic extract and aqueous extract are well illustrated in the Fig 2.

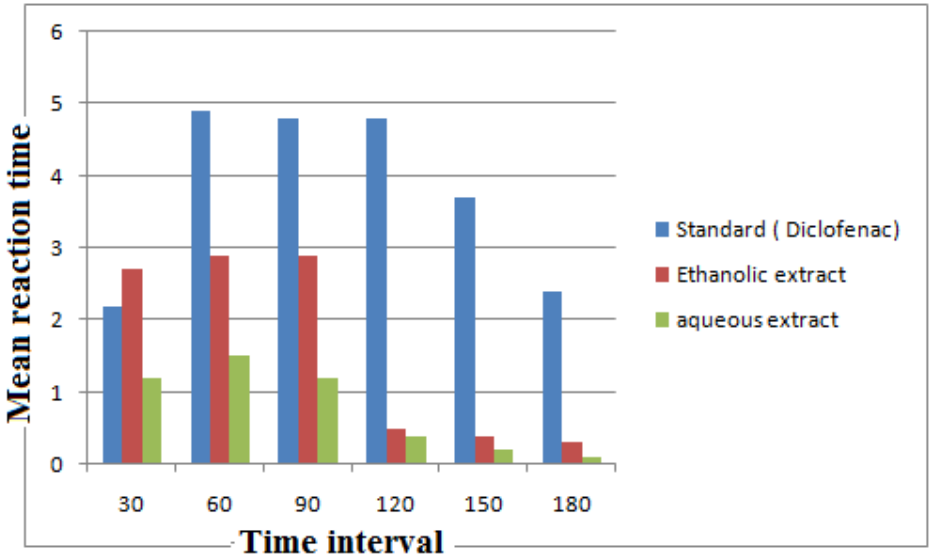


Fig. II: Mean reaction time recorded between the Standard drug (Diclofenac) and methanol and aqueous extracts of *I.trifoliata*

Table I illustrates the following

1) The initial reaction time of flicking of tail of the albino rats.
2) Tail flicking reaction time after administration of the drug
3) Mean reaction time of the tail flick obtained from the data.

Table I: Illustrating the recordings of tail flicking of albino rats in Group I (Standard), Group II (Methanolic extract), Group III (aqueous extract)

S.No	Time interval (in minutes)	Initial flick time			After drug administration			Mean reaction time		
		G-I	G-II	G-III	G-I	G-II	G-III	G-I	G-II	G-III
1	30	3.5	3.4	2.7	5.7± 1.01	6.1± 2.01	3.9± 1.24	2.2	2.7*	1.2
2	60	3.5	3.4	2.7	8.3± 0.14	6.3± 0.15	4.2± 1.81	4.8	2.9*	1.5
3	90	3.5	3.4	2.7	8.3±.0.84	6.3± 0.14	3.9± 1.29	4.8	2.9*	1.2
4	120	3.5	3.4	2.7	8.3± 0.83	3.9± 1.14	3.1± 1.09	4.8	0.5	0.4
5	150	3.5	3.4	2.7	7.2± 0.69	3.8± 1.82	2.9± 1.70	3.7	0.4	0.2
6	180	3.5	3.4	2.7	5.9± 0.71	3.7± 0.92	2.8 ± 1.61	2.4	0.3	0.1

*Significant (P<0.001)

Writhing Assay by Acetic acid method

The results of acetic acid induced writhing in albino rats are illustrated in Table II. The methanolic extract showed percentage inhibition of writhing (30 minutes) of 53. 96, 61.90, 68.25 writhing responses at 50mg, 100mg, 200mg dose. While the aqueous extract showed percentage inhibition of writhing responses 55.28, 58.73, 63.24 at 50mg, 100mg, 200mg dose. The mean percentage

inhibition of the standard drug was found to be 50.71. The results obtained are found to be of high significance (P<0.001) when compared with that of the control and standard doses. The results were well illustrated in table II.

Table II: Effect of acetic acid induced writhing in albino rats when administered with standard dose, Methanolic and Aqueous extracts at different doses of *I.trifoliata*

Experimental group	No. Of writhing (30 minutes)		% inhibition of writhing	
	Methanolic extract	Aqueous extract	Methanolic extract	Aqueous extract
Diclofenac sodium (100mg/kg b.w)	31.2± 1.2	31.2± 1.2	50.7	50.7
Control(0.3 ml normal saline)	63.1±1.34	63.1±1.33		
I.trifoliata (50mg)	29.1±0.40	30.01±1.19	53.6	55.3
I.trifoliata (100mg)	24.3±0.12	26.1±1.02	61.9	58.73
I.trifoliata (200mg)	20.08±0.15	23.8±3.2	68.2	63.49

CONCLUSIONS

Thermally induced tail flick reaction time of the albino rats was found significant by administration of different extracts of *I.trifoliata.* The standard drug administered is diclofenac sodium. After administration of the doses at an interval of 90 minutes the test doses produces significant effect same as that of the standard but during the course of time the flick reaction time of the tail gradually decreased. The methanolic extract showed significant effect than that of aqueous extract. The results conclude that methanolic extract delays the reaction time and increase the susceptibility towards the pain by delaying the tail flick reaction time. The phytochemical constituents responsible for the analgesic activity has to be found out as to derive a new moiety as an alternate to the conventional dosage forms.

Acetic acid induced abdominal contraction method has been used to evaluate peripherally acting analgesics. In acetic acid induced method pain is generated indirectly via endogenous mediators like prostaglandin, which stimulates peripheral nociceptive neurons. These neuronal fibers are sensitive to both narcotics and non steroidal anti-inflammatory drugs. Methanolic extract of *indigofera trifolata* inhibited the acetic acid induced pain with potency compared to the diclofenac sodium. *Indigofera trifolata* is dominated by terpenoids may have potent analgesic activity. Terpenoids are reported to be inhibitors of NF-κB signaling just like aspirin and other NSAIDS. These evidences reveal that terpenoids may be responsible for the analgesic activity of methanolic extract of *Indigofera trifolata*.

The observations confirm that methanolic and aqueous extract of the leaf of the plant has analgesic activity. From this study, it is concluded that *indigofera trifolata* may be useful in treating pain with no visible signs or symptoms of toxicity in normal rats indicating a high margin of safety. The extracts exhibited analgesic activity comparable to that of a standard drug. The traditional use of *indigofera trifolata* to treat pain is supported by laboratory results from this study, suggesting a need to isolate and evaluate active constituents responsible for the exhibited biological activity.

FUTURE SCOPE

In various traditional medicinal systems a number of natural products are used to relieve the symptoms of pain. These studies have identified that compounds such as alkaloids, phenols, tannins, fixed oils and flavonoids are responsible for analgesic activity. The observed analgesic activity of this plant could have resulted from the combined activity of these compounds present in the extract.

Traditional system of medicines causes side effects like teratogenicity, discoloration of skin, hypersensitive reactions. Natural products are found to be most promising and viable alternative for conventional medicines in the treatment of pain and analgesia.

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