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# Evaluation of antinociceptive effects of *Tragia plukenetii*: A possible mechanism

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## Abstract

*Tragia plukenetii* R.Smith. (Euphorbiaceae) is an erect, prostate herb with sparsely hispid stinging hairs. In the present study, ethanolic extract and its fractions of *T. plukenetii* aerial parts were evaluated for antinociceptive and central nervous system (CNS) depressant effects. Among all the extracts, chloroform extract has produced significant analgesic activity at a test dose of 250 mg/kg in acetic acid induced writhing test and Eddy's hotplate test. The analgesic effect of chloroform extract (68.83% inhibition) is comparable with aspirin (72.09% inhibition) in acetic acid induced writhing test. Chloroform extract significantly increased the latency time in hotplate test. In the study of CNS depressant effect, the chloroform extract was found to produce a significant ($P < 0.01$) reduction of the exploratory capacity and depressant effect in locomotor activity. From the point of CNS depressant and good protective effect on chemical and thermal pain stimuli, indicates that *T. plukenetii* chloroform extract may have morphinomimetic properties. The naloxone is not able to alter the *T. plukenetii* induced antinociceptive effect in writhing and hotplate test. Thus, the observed antinociceptive activity of *T. plukenetii* might have resulted from the activation of peripheral receptors.

**Key words:** Antinociceptive activity, aspirin, central nervous system depressant activity, morphine, naloxone, *Tragia plukenetii*

## Introduction

Analgesia is an ill-defined, unpleasant sensation, usually evoked by an external and internal noxious stimulus. Analgesics are drugs that selectively relieve pain by acting on the central nervous system or on peripheral pain mechanisms, without significantly altering consciousness.[1] For centuries, medicinal plants are the basis for the treatment of various diseases. The use of herbal medicines world-wide has provided an excellent opportunity to India to look for therapeutic lead compounds from our ancient system of Ayurveda, which can be utilized for development of new drugs.[2] Nearly, 80% of the world population still depends on plant based traditional medicine for their primary healthcare and almost threefourth of the natural products used world-wide are derived from medicinal plants.[3] Since the use of herbal drugs remains a good alternative to allopathic agents, it is important to conduct scientific evaluation of as many traditionally used medicinal plants as possible with reference to the modern system of medicine.[4,5]

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*Tragia plukenetii* R.Smith (Euphorbiaceae) is a herb or under shrub grows to 1m height, branched, with sparsely hispid stinging hairs, very commonly found in hedges and in forest outskirts throughout the year [Figure 1]. It is a commonly known as small Climbing nettle.[6] The ethanolic extract of *T. plukenetii* is reported to possess significant antihyperglycemic properties in both glucose and alloxan induced diabetic rats at a dose of 300 mg/kg.[7] The methanolic extract of *Tragia involucrata* was studied in different experimental models and was revealed that the extract possess significant analgesic and anti-inflammatory activity.[8]

In view of the reported analgesic properties of other *Tragia* species, the present study was conducted to evaluate the antinociceptive effects of *T. plukenetii* and the action mechanism on several experimental models in mice. The activity on CNS was also investigated in order to examine the antinociceptive activity related to central depressant action.

## Materials and Methods

### Plant material

The whole plant of *T. plukenetii* was collected from Vallur Mandal of Cudapah District, Andhra Pradesh, in the month of December, 2009. The botanical identification of the plant

was performed and authenticated by Prof. Rama Krishna, Head, Department of Botany, P.G. College of Sciences, Saifabad, Hyderabad. A voucher specimen (TRP-303-09) is being maintained in the laboratory of Pharmacognosy and Phytochemistry, G. Pulla Reddy College of Pharmacy, Hyderabad. The roots were separated and aerial parts were cleaned, cut, air dried, made free from adhering debris and grounded into powder. The dried powder material was passed through sieve#60 and stored in an air tight container.

## Chemicals

All the chemicals, reagents, solvents used were of analytical grade. Aspirin, morphine, diazepam and naloxone were purchased locally.

## Preparation of extract

The shade dried aerial parts powder (470 g) was extracted with 80%aqueous ethyl alcohol by cold maceration for 7 days. The yield of crude aqueous ethanolic extract was 9.26% (43 g). To the concentrated ethanolic extract (30 g), 300 ml of water was added and fractionated with petroleum ether (4 × 250 ml), chloroform (4 × 250 ml), ethylacetate (4 × 250 ml) and *n*-butanol (4 × 250 ml). The yield of petroleum ether, chloroform, ethylacetate, *n*-butanol and left over aqueous extracts were 0.63%, 0.42%, 0.53%, 1.48% and 6.38% (w/w), respectively. All the organic extracts were tested for the presence of various phytoconstituents using standard procedures.[9]

## Animals

Male Swiss Albino mice, 8-10 weeks old (25-30 g) were used in the present study.The animals were procured from M/S. Mahavir Agency, Hyderabad and maintained under standard environmental conditions of temperature, relative humidity; dark/light cycles and had free access to feed and water *ad libitum* during the 7 day quarantine period. The animals were fasted for 8h before experimentation, but had been allowed free access to water. The animal experimentation was carried out according to the Committee for the Purpose of Control and Supervision of Experimentation on Animals guide lines and Institutional Animal Ethics Committee approved all the procedures for investigating experimental pain in conscious animals.[10] All the extracts and drugs were administered orally as a fine aqueous suspension of 0.5% carboxy methyl cellulose (CMC). Animals were allowed to eat feed 2 h postdose of drugs/extracts.

## Antinociceptive activity

### Acetic acid-induced writhing test

The acetic acid induced writhing test was carried out as described by Seigemond *et al.*[11] and as modified by Koster *et al.*[12] in pre-screened mice. The pre-screened animals (the animals, which show the first sign of irritation within 60 s are used for the study) were divided into nine groups of six animals each. Group 1 served as control and received vehicle CMC. Groups 2-6 received aqueous ethanolic extract (500 mg/kg), petroleum ether, chloroform, ethylacetate and butanol extracts respectively at an oral dose of 250 mg/kg. Group 7 served as a positive control and received acetyl salicylic acid at an oral dose of 100 mg/kg. In an attempt to investigate the participation of opioid system in analgesic effects of *T. plukenetii*, separate groups of mice were pre-treated with non-selective receptor antagonist, naloxone (5 mg/kg, i.p.), which was injected 15 min before the administration of chloroform extract (250 mg/kg, p.o.) and acetyl salicylic acid (100 mg/kg, p.o.) for groups 8 and 9 respectively. After 30 min of extract/drug administration, all the animals were given an i.p. injection of 0.6% acetic acid (volume of injection 0.1 ml/10 g) and the number of writhes produced in these animals was recorded for 30 min.

### Hot plate test

The method of Eddy and Leimbach[13] was employed. The test was performed using Eddy's hot plate maintained at a temperature of 55 ± 0.2°C. The basal reaction time of all animals towards thermal heat was recorded. The animals, which showed fore paw licking or jumping response within 5 s were selected for the study. The pre-screened animals were divided into nine groups of 10 animals each. Control animals were treated with CMC (group 1), groups 2-6 received aqueous ethanolic extract (500 mg/kg), petroleum ether, chloroform, ethylacetate, butanol extracts, respectively at an oral dose of 250 mg/kg. Group 7 served as a positive control and treated with morphine (5 mg/kg, p.o.). The opioid receptor antagonist naloxone (5 mg/kg) was also tested along with chloroform extract (250 mg/kg, p.o., group 8) and morphine (5 mg/kg, p.o., group 9). All the substances were administered 30 min before the beginning of the experiment. The latency time was measured before and at 30, 60, 90, 120 and 180 min after administration of extracts. The latency period of 20 s was defined as a cut-off mark and measurement was terminated if the latency exceeded this latency period to avoid injury.

## Central nervous system depressant activity

The activity of *T. plukenetii* on CNS was evaluated by performing assays of its effect on exploratory capacity (hole board test) and locomotor activity. In each experiment, the mice were divided into eight groups consisting of six animals in each group. Animals of group 1 were treated with vehicle and served as control. Ethanolic extract (500 mg/kg), petroleum ether, chloroform, ethylacetate, butanol extracts were administered to groups 2-6 animals at an oral dose of 250 mg/kg, as fine CMC suspension. Groups 7and8 served as a positive control and orally received diazepam (2 mg/kg) and morphine (10 mg/kg).

### Hole board test

Hole board test was employed as described by Boisser and Simon.[14] The animals were placed on a board (40 × 40 cm) with 16 holes symmetrically distributed in four rows. At 30 min after oral administration of test and standard substances each animal was placed carefully in the center of the board and number of head pokes (head was dipped into the hole) for 5 min were recorded.

## Locomotor activity

The methods described by Kulkarni[15] and Yadav[16] were employed. The locomotor activity was measured using Actophotometer (IncoPhotoactometer, Ambala, India). A count is recorded when the beam of light falling on the photocell of the actophotometer is cut-off by animal. The basal activity score was obtained by placing each animal individually in actophotometer for 10 min. Thirty minutes after administration, each animal was placed carefully in the center of actophotometer for recording the activity score.

### Statistical analysis

All the values were expressed as a mean ± SEM. Results were analyzed statistically by using analysis of variance (ANOVA) followed by Dunnett multiple comparisons test. Values of $P < 0.05$ were considered to be significant.[17]

## Results

The results of qualitative phytochemical analysis of *T. plukenetii* are presented in Table 1. All the extracts have shown negative reactions for alkaloids, cardiac glycosides, anthraquinone glycosides and coumarins.

### Effect on acetic acid induced writhing test

The inhibitory effect of ethanolic extract and its fractions in writhing test is shown in Table 2. All the extracts have significantly inhibited the writhings in mice. Among all the extracts, chloroform extract (250 mg/kg) produced maximum effect with 68.83% inhibition, which is ($P < 0.01$) comparable with aspirin (100 mg/kg) activity (72.09% inhibition). The inhibitory activity produced by other extracts is less compared with chloroform extract. The administration of naloxone (5 mg/kg) along with chloroform extract and aspirin demonstrated significant ($P < 0.01$) analgesic effects with percentage inhibition of 65.11 and 69.2 respectively. These results indicate that naloxone has no effect on antinociceptive activity of *T. plukenetii* and acetyl salicylic acid.

### Effect on the hot plate test

The results are presented in Table 3, shows the time course of antinociception produced by various extracts of *T. plukenetii*. Oral administration of all the extracts resulted in significant prolongation of latency time in hot plate test. Among all the extracts, ethanolic extract and chloroform extract have produced higher latency time at 120 and 90 min, respectively. Antinociceptive effect produced by chloroform extract is constantly and significantly maintained throughout the experiment from 30 to 180 min after drug administration. At 90 min the mean latency of chloroform extract was 6.3 ± 0.31 s compared with 3.4 ± 0.34 s and 19.0 ± 0.85 s for control and morphine treated groups, respectively. The analgesic effect of chloroform extract is not comparable with morphine during any course of time. Administration of naloxone (5 mg/kg) has not reversed the antinociception induced by chloroform extract. However, the effect produced by morphine (5 mg/kg) is significantly blocked by administration of naloxone.

### Hole board test

The results of hole board test are presented in Table 4. The head dip responses were significantly reduced in groups treated with chloroform, ethanolic extract when compared with control.

**Table 1: Qualitative phytochemical analysis of *Tragia plukenetii***

| Extracts | Chemical class |
|---|---|
| Ethanolic | Steroids and/or triterpenoids, flavonoids and their glycosides, tannins, carbohydrates, proteins and amino acids |
| Petroleum ether | Steroids and/or triterpenoids |
| Chloroform | Steroids and/or triterpenoids and flavonoids |
| Ethyl acetate | Steroids and/or triterpenoids, flavonoids and their glycosides, tannins and carbohydrates |
| Butanolic | Steroids and/or triterpenoids, flavonoids and their glycosides, tannins and carbohydrates |

**Table 2: Effect of *T. plukenetii* on acetic acid induced abdominal writhing in mice**

| Groups | Treatment (mg/kg) | Writhings | % inhibition |
|---|---|---|---|
| 1 | Control | 71.66±0.76 | - |
| 2 | Ethanolic extract (500) | 56.33±1.40[a] | 21.39 |
| 3 | Petroleum ether extract (250) | 64.0±1.52[a] | 10.60 |
| 4 | Chloroform extract (250) | 22.33±0.55[a] | 68.83 |
| 5 | Ethyl acetate extract (250) | 48.33±1.38[a] | 32.55 |
| 6 | Butanolic extract (250) | 54.83±1.40[a] | 23.48 |
| 7 | Aspirin (100) | 20.00±0.57[a] | 72.09 |
| 8 | Chloroform extract (250)+ naloxone (5) | 25.00±0.63[a] | 65.11 |
| 9 | Aspirin (100)+ naloxone (5) | 22.00±0.68[a] | 69.2 |

Values are mean±SEM, $n$=6, [a]$P$<0.01 versus control, SEM: Standard error of the mean

**Table 3: Effect of *T. plukenetii* extracts in hot plate test**

| Groups | Treatment (mg/kg, p.o.) | Latency time (s) | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Pre-treatment | After treatment | | | | | |
| | | 0 min | 30 min | 60 min | 90 min | 120 min | 150 min | 180 min |
| 1 | Control | 3.3±0.21 | 3.6±0.31 | 2.8±0.20 | 3.4±0.34 | 3.2±0.36 | 3.1±0.23 | 3.0±0.21 |
| 2 | Ethanolic extract (500) | 2.6±0.22 | 4.6±0.26 | 4.0±0.25 | 5.6±0.30[b] | 7.6±0.34[b] | 7.8±0.49[b] | 9.0±0.29[b] |
| 3 | Pet ether extract (250) | 3.0±0.29 | 4.0±0.44 | 4.2±0.45 | 3.5±0.42 | 3.8±0.41 | 3.6±0.48 | 5.0±0.47 |
| 4 | Chloroform extract (250) | 3.8±0.24 | 5.9±0.62[a] | 6.1±0.23[b] | 6.3±0.31[b] | 5.5±0.34[b] | 5.4±0.52[a] | 5.2±0.48[a] |
| 5 | Ethyl acetate extract (250) | 4.0±0.25 | 5.5±0.34 | 5.1±0.27[a] | 5.0±0.25[b] | 4.1±0.31 | 3.8±0.24 | 4.3±0.37 |
| 6 | Butanolic extract (250) | 3.2±0.25 | 3.5±0.27 | 4.7±0.26 | 6.8±0.38[b] | 4.5±0.43 | 4.8±0.32 | 4.2±0.55 |
| 7 | Morphine (5) | 3.3±0.33 | 15.3±1.37[b] | 18.0±1.29[b] | 19.0±0.85[b] | 19.2±0.61[b] | 16.0±1.02[b] | 16.4±0.90[b] |
| 8 | Chloroform extract (250)+naloxone (5) | 2.8±0.24 | 5.8±0.59[a] | 6.5±0.30[b] | 7.0±0.25[b] | 5.1±0.31[b] | 5.3±0.47[a] | 5.2±0.61[a] |
| 9 | Morphine (5)+naloxone (5) | 3.2±0.39 | 6.0±0.56[a] | 6.4±0.58[b] | 6.2±0.49[b] | 5.1±0.48[b] | 5.8±0.47[b] | 5.3±0.46 |

Values are mean±SEM, $n$=10, [a]$P$<0.05, [b]$P$<0.01 versus control, SEM: Standard error of the mean

**Table 4: Effect of *T.plukenetii* extracts on hole board test in mice**

| Groups | Treatment (mg/kg, p.o.) | Head pokes/dips | | | | |
|---|---|---|---|---|---|---|
| | | 1 min | 2 min | 3 min | 4 min | 5 min |
| 1 | Control | 11.16±1.10 | 10.0±1.15 | 8.83±1.19 | 7.83±0.65 | 8.33±1.08 |
| 2 | Ethanolic extract (500) | 8.00±0.36[a] | 10.0±0.68 | 5.17±0.48 | 5.50±0.43 | 5.0±0.44[a] |
| 3 | Petroleum ether extract (250) | 8.67±0.49 | 8.33±0.57 | 7.0±0.85 | 7.67±0.67 | 7.83±0.70 |
| 4 | Chloroform extract (250) | 6.66±0.49[b] | 6.16±0.60[b] | 3.17±0.48[b] | 3.08±0.32[b] | 4.0±0.57[b] |
| 5 | Ethyl acetate extract (250) | 10.5±0.61 | 8.33±0.42 | 10.83±0.94 | 11.66±1.52[a] | 8.66±1.30 |
| 6 | Butanolic extract (250) | 9.33±0.71 | 7.66±0.66 | 5.0±1.36[a] | 4.33±0.91[a] | 4.0±0.73[b] |
| 7 | Diazepam (2) | 7.16±0.30[b] | 6.0±0.36[b] | 5.16±0.87[a] | 4.0±0.57[a] | 4.16±0.87[b] |
| 8 | Morphine (10) | 5.0±1.09[b] | 3.83±0.87[b] | 3.33±0.55[b] | 3.08±0.66[b] | 3.05±0.66[b] |

Values are mean±SEM, $n$=6, [a]$P$<0.05, [b]$P$<0.01 versus control, SEM: Standard error of the mean

**Table 5: Effect of *T. plukenetii* extracts on locomotor activity**

| Groups | Treatment (mg/kg) | Locomotor activity (score) in10 min | | % reduction in activity |
|---|---|---|---|---|
| | | Before treatment | After treatment | |
| 1 | Control | 448.33±44.23 | 434.16±26.91 | - |
| 2 | Ethanolic extract (500) | 511.66±53.49 | 252.0±23.73[a] | 41.95 |
| 3 | Petroleum ether extract (250) | 493.0±10.09 | 465.0±11.02 | - |
| 4 | Chloroform extract (250) | 462.33±53.79 | 207.0±31.03[a] | 52.32 |
| 5 | Ethyl acetate extract (250) | 478.0±18.66 | 360.0±28.69 | 17.08 |
| 6 | Butanolic extract (250) | 396.0±20.23 | 377.0±26.75 | 13.16 |
| 7 | Diazepam (2) | 403.16±27.46 | 173.83±33.98[a] | 59.96 |
| 8 | Morphine (10) | 454.0±36.284 | 169±10.12[a] | 61.07 |

Values are mean±SEM, $n$=6, [a]$P$<0.01 versus control, SEM: Standard error of the mean

However, the action produced by chloroform is significantly high when compared to diazepam at third and fourth min and the activity is comparable with morphine.

### Locomotor activity

Table 5 represents the locomotor activity of *T. plukenetii*. Among all the extracts, chloroform and ethanolic extracts have produced significant ($P < 0.01$) reduction in locomotor activity with percentage reduction of 52.32 and 41.95 respectively. Petroleum ether extract did not produce any action compared with control. The effect of diazepam and morphine is more marked when compared to other test substances with 59.96% and 61.07% reduction in activity.

## Discussion

The result of the present study indicate that oral administration of chloroform and ethanolic extract of *T. plukenetii* produces significant central analgesic (hot plate test) and protective effect on chemical (acetic acid injection) stimuli at the respective dose of 500 and 250 mg/kg, respectively. Such an efficacy on these two stimuli is a characteristic of central analgesic like morphine, while peripheral analgesics (acetyl salicylic acid) are known to be inactive on thermal pain stimuli.

The chloroform and ethanolic extract significantly inhibited the acetic acid induced writhing in mice with percentage protection of 68.83 and 21.39, respectively. The analgesic effect of chloroform extract is comparable with standard aspirin (72.09% inhibition). This test is widely used for evaluation of peripheral analgesics and involves the release of prostaglandins and phlogistic mediators like $PGE_2$ and $PGE_2$ alpha and these levels were increased in peritoneal fluid of the acetic acid induced mice.[18] Despite the significant results observed, the abdominal writhing test is reported to be less selective model. Collier *et al.* postulated that acetic acid acts indirectly by inducing the release of endogenous mediators, which stimulates the nociceptive neurons that are sensitive to NSAIDs and narcotics.[19]

The hot plate test is employed to verify if *T. plukenetii* ethanolic and chloroform extract could show central antinociceptive effects, as the test is specific for central antinociceptive drugs.[20] Among all the extracts the ethanolic and chloroform extract have significantly increased the latency time in hot plate test. Apparently, the effect is largely depends on endogenous opioids. Although the hot plate test is commonly used for assay of narcotic analgesics, other drugs such as sedative, muscle relaxants and psychometric drugs acts centrally.[21]

To determine the possible mechanism of action of the chloroform extract of *T. plukenetii* to produce analgesia, we used naloxone, as an selective antagonist of opioid receptors. Naloxone apparently acts by antagonizing the action of endogenous opioid involved in pain and stress.[22] The results indicate that naloxone markedly reversed the antinociceptive effect of morphine (10 mg/kg) in heat induced pain (hotplate). The effect of chloroform extract in both analgesics models is unaffected by naloxone or the analgesic effects were not antagonized.

The results of antinociceptive study led us to carry out further investigations of *T. plukenetii* extracts on CNS hole board test and locomotor activity in mice. The chloroform and ethanolic extract produced significant depressant activity

**Figure 1: *Tragia plukenetti* R. Smith**

on CNS. The mechanism underlined this effect is unknown, however, from the point of CNS depressant and good protective effect on chemical and thermal pain stimuli indicates that *T. plukenetii* extract may have morphinomimetic properties. However, naloxone is not able to alter the *T. plukenetii* induced antinociceptive effect in writhing and hot plate test. Thus, the observed antinociceptive activity of *T. plukenetii* might have resulted from the activation of peripheral receptors.

## Conclusion

Phytochemical analysis of chloroform and ethanolic extract indicates that their major constituents are steroids and/or triterpenoids and flavonoids, which may be responsible for the observed pharmacological activity. The present investigation concludes that *T. plukenetii* chloroform extract is potent analgesic agent, but the exact mechanism of action is not known at this stage and has to be established in various models.

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# हिन्दी सारांश

## *Tragia plukenetii* के antinociceptive प्रभावा का सम्भावित कार्यकारिता

समा व्यंकटेश, साबा फातिमा

एक सीधा, प्रोस्टेट जड़ी बूटी है, जिसके रोमिल छोटे-छोटे और कड़े चुभने वाले बाल है । वर्तमान अध्ययन में इथानोलिक सत्त्व और भिन्न भागों में Antinociceptive और CNS अवसाद (खिन्न या म्लान करनेवाली) प्रभावो का मूल्यांकन किया गया है । सभी निष्कर्षों के बीच, क्लोरोफॉर्म सत 250 Mg/Kg जाँच मात्रा में acetic acid induced writhing and eddy's hot plate परीक्षणों के द्वारा महत्वपूर्ण एनाल्जेसिक गतिविधि का उत्पादन किया है । क्लोरोफॉर्म सत्त (६८.८३ % निषेध) का एनाल्जेसिक प्रभाव acetic acid induced writhing परीक्षण के द्वारा, एस्पिरिन (७२.०९ %) के साथ काफी तुलनीय है । क्लोरोफॉर्म सत्त ने hot plate परीक्षण द्वारा विलंबित वृधि दिखाई है । CNS अवसाद प्रभाव अध्ययन में क्लोरोफॉर्म सत्त ने गतिविधि (locomotor activity) और खोजपूर्ण क्षमता (exploratory capacity) में महत्वपूर्ण कमी का उत्पादन किया ($P$<0.01). CNS अवसाद और chemical और thermal दर्द उत्तेजनाओं पर अच्छे सुरक्षात्मक प्रभावो की दृष्टि से, ये संकेत मिलता है के *T. plukenetti* क्लोरोफॉर्म सत्त में morphinomimetics गुण हो सकते है । Naloxone, writhing और hotplate परीक्षण, *T. plukenetti* प्रेरित antinociceptive प्रभाव को बदलने में असक्षम रहा । इस प्रकार प्रेरित गतिविधि परिधीय रिसेप्टर्स की सक्रियता की वजह से हो सकता है ।