

Antinociceptive Potential of *Terminalia Catappa* (Indian Almond) Leaves in Swiss Albino Rat

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ABSTRACT

Terminalia catappa (almond) is a combretaceous plant whose leaves are widely used as a folk medicine for treatments of dermatitis, hepatitis, inflammatory disease, diabetes and other disease. The Antinociceptive activity of the aqueous extract of *Terminalia catappa* leaves was studied using the tail flick method, glacial acetic acid induced writhing and the hot plate test in albino rats. The aqueous extract (500mg/kg) produced a significant ($p < 0.01$) dose-dependent inhibition of abdominal writhing in rat. The extract of *Terminalia catappa* (500 mg/kg) showed significant ($p < 0.05$) dose depend increase in tail flick latency in rat and the result of the hot plate test showed a dose – related and time dependant significant ($p < 0.01$) increase in pain threshold in rat 15 min after treatment at all the doses used in the study. The result indicates that the aqueous extract of *Terminalla catappa* leaves posseses analgesic activity which may mediated through both central and peripheral mechanism.

Keywords: *Terminalia catappa*, Glacial acetic acid (GAA), Hot plate, Antinociceptive activity.

INTRODUCTION

Pain is an unpleasant sensory or emotional experience associated with actual or potential tissue damage, or described in terms of such damage. The Nociception involves the activation of specific primary sensory neuron subpopulations that transmit the nociceptive information to the spinal cord from where it is relayed to supra spinal levels¹. Tissue damage occurs by activation of nociceptors through the release of several mediators, including excitatory amino acids, peptides, protons, lipids and cytokines,

which bind to receptors and activate signaling pathways². Thus, pain can be a subject to multiple levels of biochemical and pharmacological controls, involving a diversity of cell types and soluble mediators³. As a result, compounds that present antinociceptive effect are of potential therapeutic interest for the treatment of human and animal pain.

Terminalia species are native from Africa and are now widely spread out in tropical and sub-tropical regions⁴. The

leaves, bark and fruit of the tree *Terminalia catappa* L. (*Combretaceae*) have been commonly used as a folk medicine for antidiarrhea, antipyretic and haemostatic purposes⁵. The leaves of *T. catappa* have been used for the prevention and treatment of hepatitis and liver-related diseases⁶. The leaves of *T. catappa* contain many hydrolysable types of tannin, such as punicalagin, punicalin, terflavins A and B, tergalagin, tercatalin, chebulagic acid, geraniin, granatin B, and corilagin⁷. Punicalin and punicalagin showed inhibited HIV replication in infected H9 lymphocytes with little cytotoxicity and also in purified HIV reverse transcriptase⁸. A previous study has demonstrated the anti-nociceptive actions of only leaf extracts of *Terminalia catappa* in mice, when given intraperitoneally⁹. Earlier researchers have reported the nociceptive potential of aqueous juice prepared by maceration from the tender leaves of *terminalia catappa* to support its folklore use¹⁰. Hence, this study was undertaken to investigate the antinociceptive effect of *Terminalia catappa* aqueous leaf extract in rats using three experimental pain models.

MATERIALS AND METHODS

Plant material

The leaf samples were collected at periods of time: November– December in the campus of the Sagar Institute of Pharmaceutical Sciences Sagar. The plant was Plant select was identified and authenticated by Dr. Pradeep Tiwari, Department of Botany, Dr. H.S. Gour Vishwavidyalaya, Sagar (M.P.). (Herbarium no. **Bot./Her./B/2829**)

Drug

Naloxone hydrochloride (DuPont Pharmaceuticals Wilmington DE 1998), Glacial Acetic Acid (Sarabhai Chemicals,

Baroda), and *Terminalia catappa* leaf extract were used in the study.

Preparation of extract

The leaves of *Terminalia catappa* collected and shade dried. The collected drugs weighed (approx 1 kg) powdered and was extracted with deionised water by hot maceration followed by intermittent shaking and allowed to soak overnight. The suspension was centrifuged at 5000 rpm for 20 min and filtered through a Whatman filter paper No. 1. The supernatant fluid was allowed to evaporate to dryness using rotatory vacuum evaporator, yielding semisolid residue. This semi-solid residue was lyophilized to fine powder was stored¹¹.

Animals

Swiss albino rat were acclimatized to animal house prior to experimentation; they were divided in ten groups with ten animals per group (n=10). The rat were kept at controlled light condition (light: dark, 12:12 hr) and temperature $22 \pm 1^\circ \text{C}$ and were given standard mouse pellet and water *ad libitum*. . The experimental pain models i.e. Tail flick response to thermal stimulation by analgesiometer, Hot plate analgesimeter (**Harvard apparatus Ltd., U.K.**) and writhing induced by Glacial Acetic Acid (GAA) was used for assessing central and peripheral origin of analgesic effects, respectively. (**Animal ethical commity no. SIPS/EC/2013/32**)

Tail flick method

The Tail flick method was carried out as described by Janssen *et al.* (1963) & (Davies *et al.*, 1946). The heat intensity of thermal stimulation (Techno Analgesiometer) was adjusted such that rats had control tail flick latency of 3-4 sec and a 10 sec cut off latency was used to prevent thermal injury. The initial reaction time was recorded in thirty animals and then they were divided into

3 groups of 10 rats each. Drugs were given to the various groups as mentioned in **Table 1**. Dose of extract was selected by performing acute toxicity method in OECD Guidelines 423¹⁴. Tail flick latency in seconds was recorded every 30 min for a duration of 3 hours after drug administration.

Glacial acetic acid (GAA) writhing method

Forty rats were divided into four groups of ten each. Writhing was induced by injecting 1% v/v solution of glacial acetic acid (300 mg/kg, ip)¹⁵. Drugs were given to the various groups as described in **Table 2**. *Terminalia catappa* leaf extract given 30 min before the administration of GAA¹⁶.

Hot plate method

Rats were placed on aluminum hot plate kept at 55 ± 0.5 °C for a maximum time of 30 sec.¹⁷. Reaction time was recorded when the animals licked their fore- and hind paws and jumped; at before (0) and 15, 30, 45, and 60 min after intraperitoneal administration of *Terminalia catappa* extract (500mg/kg) to different groups of ten animals each. Naloxone 1mg/kg was used as the reference drugs. (**Table 3**)

Statistical analysis

Data were expressed as the mean + SEM and Three parallel experiments was performed and analyzed using the Students 't' test. Results were considered significant when $p < 0.01$ or $p < 0.05$.

RESULTS

The tail flick reaction time was significantly ($p < 0.001$) increased in rats after *Terminalia catappa* leaf extract (TCLE). Peak analgesic effect of extract was observed after 60 min of oral administration (**Table 1**). The increase in tail flick reaction time at 60 min in TCLE treated group was 102%. Naloxone pretreatment significantly reduced the antinociceptive effect of TCLE (**Table**

1).GAA produced writhing in all the control animals. The mean writhing count in control rats was 5.2 ± 0.12 . The number of writhing within 90 seconds was decreased after pretreatment of rats with *Terminalia catappa* leaf extract (**Table 2**). Pretreatment of rats with the opioid antagonist, naloxone, partially reversed the inhibitory effects of leaf extract on Glacial acetic acid induced writhing count. The results of hot plate test presented in **Table 3** showed that the administration of *Terminalia catappa* leaf extract at the doses of 500 mg/kg and Naloxone (1mg/kg) a reference drug significantly raised the pain threshold at observation time of 45 min in comparison with control ($P < 0.001$).

DISCUSSION

The results of the present study revealed the antinociceptive effect of *Terminalia catappa* leaf extract in aforesaid experimental pain models. Acetic acid induced writhing and tail flick test to thermal stimulation are models of pain that mainly involve peripheral and central mechanisms respectively^{13,15}. Antinociceptive effect observed in these experiments with TCLE indicates the involvement of both peripheral and central mechanisms. Also pretreatment with the opioid antagonist, naloxone partially reduced the antinociceptive effect of *Terminalia catappa*. This indicates the involvement of endogenous opioid peptides in mediation of antinociceptive response of *Terminalia catappa* leaf extract. As the analgesic effect is reduced partially after naloxone, some other nonopioid mechanisms may also be involved. It may be possible that TCLE may modulate some other neurotransmitters /neuromodulators involved in the regulation of pain sensitivity.

The data presented in our study suggests that *Terminalia catappa* have analgesic property which might be potentially useful as such or also when it is employed for its other actions like anti-inflammatory¹⁸.

However further studies are required at different dose level or by modifying protocol to justify the exact chemical entity present or responsible for antinociceptive action.

ACKNOWLEDGEMENT

The author acknowledges & sincerely thanks Prof. M. D. Kharya, Department of Pharmaceutical Sciences, Sagar for guiding our research work.

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Table 1. Antinociceptive effect of *Terminalia cattapa* leaves extract (TCLE) on reaction time by tail flick method

Sr. No.	Drug	Control	30min	60min	90min	120min	150min	180min
1	TCLE	3.7±0.10	5.9±0.29**	7.5±0.12**	6.1±0.31**	4.9±0.23**	4.5±0.22*	3.8±0.23
2	Naloxone alone	3.0±0.14	2.3±0.12*	2.3±0.15*	2.5±0.15*	2.6±0.13	2.5±0.12	2.6±0.15
3	Naloxone +TCLE	3.6±0.15	4.1±0.21	4.6±0.21**	4.2±0.22*	4.3±0.31	4.0±0.21	3.7±0.21

Naloxone alone was given 10. Min prior to TCLE, Dose of TCLE 500mg/kg, *p.o*; naloxone 1mg/kg, *p.o*. **p*<0.05, ***p*<0.01 when compared with control. Data represented as mean ±SEM, n =10 in each groups.

Table 2. Effect of *Terminalia cattapa* leaves extract (TCLE) by Glacial acetic acid (GAA) induced writhing in rat

Sr.No.	Drug	Writhing count	% inhibition of writhing
1	Control (GAA)	5.2±0.12	-
2	TCLE + GAA	3.3±0.21**	40
3	Naloxone +GAA	5.0±0.16	37
4	Naloxone +GAA+TCLE	4.4±0.22*	15

p*<0.05, *p*<0.01, when compared with control. Values are mean ±SEM, n=10 in each group.

Table 3. Effect of *Terminalia cattapa* leaves extract (TCLE) on the latency time by hot plate method

Sr. No.	Drug	Dose	0min	15min	30min	45min	60min
1	---		6.52±0.26	6.22±0.21	6.38±0.31	7.25±0.38	5.0±0.16
2	Naloxone	1mg/kg	6.56±0.23*	11.45±0.38*	14.65±0.36**	14.79±0.81**	5.0±0.16
3	TCLA	500mg/kg	6.45±0.27**	10.10±0.30**	12.56±0.24*	12.86±0.72**	13.67±0.18**

p*<0.05, *p*<0.01, when compared with control. Values are mean ±SEM, n=10 in each group.

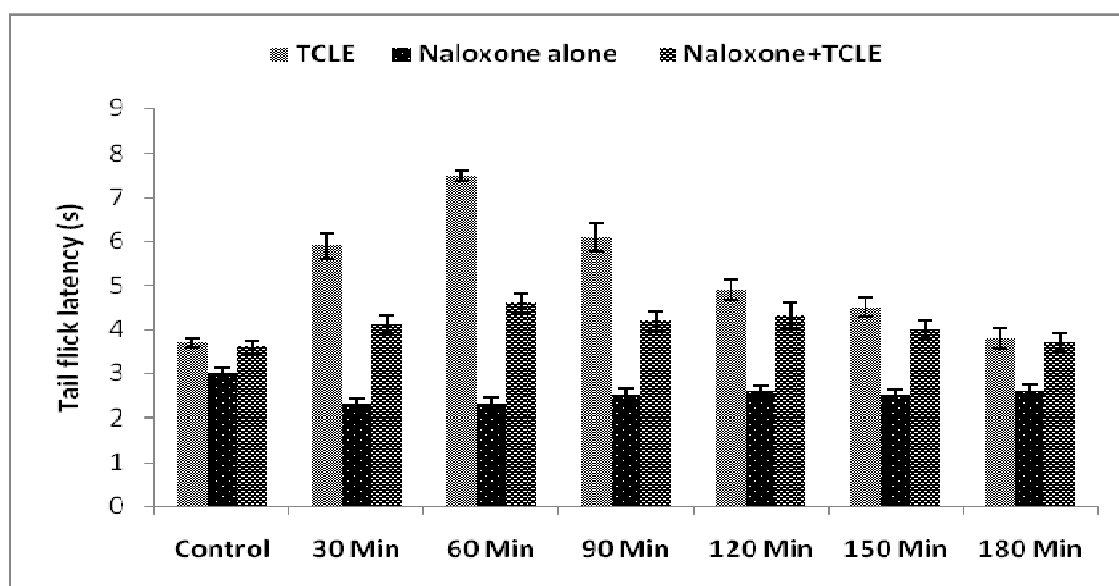


Figure.1. Graphical presentation of antinociceptive effect of *Terminalia cattapa* leaves extract(TCLE) on reaction time by tail flick method

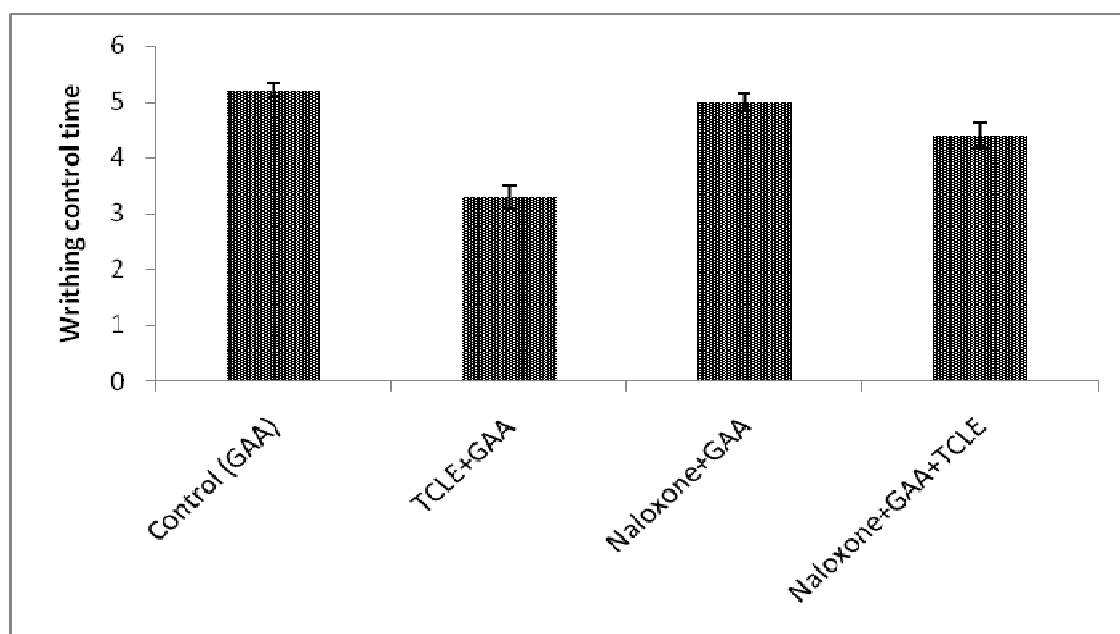


Figure.2. Graphical presentation of antinociceptive effect of *Terminalia cattapa* leaves extract (TCLE) by Glacial acetic acid (GAA) induced writhing in rat

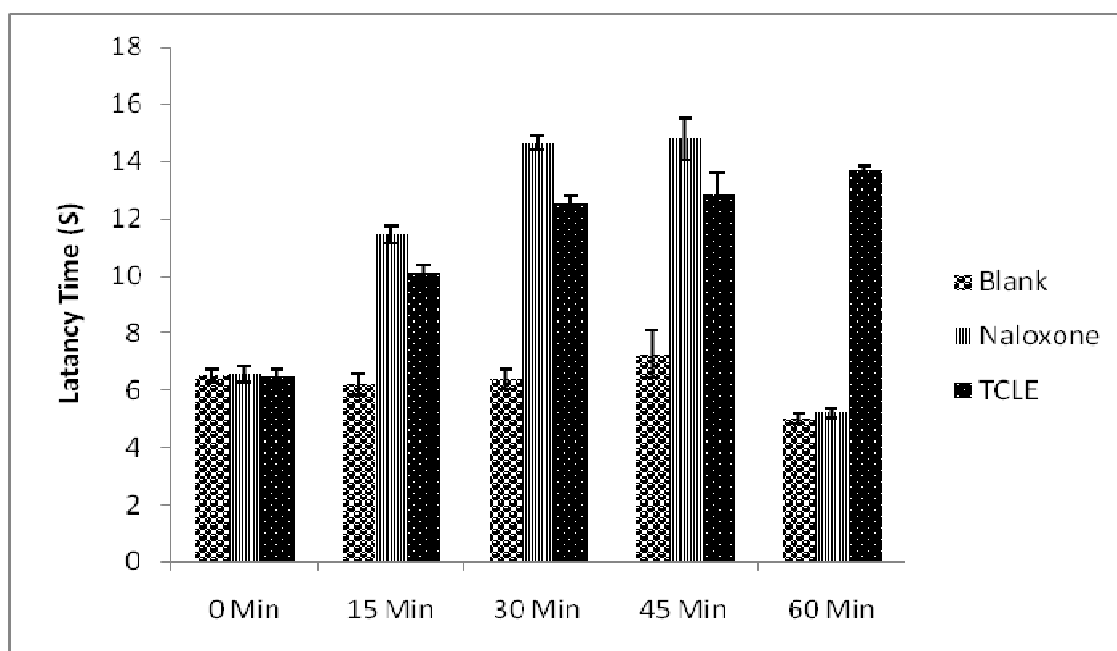


Figure.3. Graphical presentation of antinociceptive effect of *Terminalia cattapa* leaves extract (TCLE) on the latency time by hot plate method