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## The Analgesic, Anti-Inflammatory and Anti-Pyretic Activities of *Tinospora cordifolia*

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A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of article

### Abstract

**Background.** *Tinospora cordifolia* (*T. cordifolia*) is a valuable resource due to its traditional uses in the treatment of pain, fever and inflammation, but no sufficient scientific literature is available online to confirm its traditional uses in these ailments.

**Objectives.** This study was carried out to validate the traditional uses of *T. cordifolia* in treating pain, inflammation and pyrexia, using albino mice as an experimental animal model.

**Material and Methods.** The analgesic effects of *T. cordifolia* extract were assessed by using the acetic acid-induced writhing test, hot plate test and tail-flick test. The carrageenan test was performed to assess anti-inflammatory potential, and anti-pyretic activity was evaluated by the brewer's yeast-induced pyrexia method.

**Results.** The results showed that the *T. cordifolia* extract exhibited significant analgesic effects in a dose-dependent manner in the three pain models tested. The extract also exhibited significant anti-inflammatory effects in the carrageenan-induced inflammation test and antipyretic effects in the brewer's yeast-induced pyrexia test in dose-dependent manner compared to the effects observed in the control group animals.

**Conclusions.** From the findings of the present study, it can be concluded that *T. cordifolia* extract has strong analgesic, anti-inflammatory and anti-pyretic effects. Further studies are required to investigate the therapeutic activities of the phytochemical constituents of *T. cordifolia* against pain, inflammation and pyrexia (*Adv Clin Exp Med* 2015, 24, 6, 957–964).

**Key words:** anti-inflammatory, anti-pyretic, experimental model, *Tinospora cordifolia*.

*Tinospora cordifolia* (*T. cordifolia*), a shady climbing shrub belongs to the family *Manispermaceae*, is found in the tropical areas of India, Pakistan, Sri Lanka, Burma, Africa, Australia and China [1]. In the Hindi language, the plant is called *giloya*, which means a divine thing that prevents aging. The phytochemical constituents of *T. cordifolia* include aliphatic compound, alkaloids, steroids, glycosides, sesquiterpenoid, polysaccharides, different types of fatty acids and essential oils [1, 2]. Panchabhai et al. have provided a detailed summary of the pharmacological properties of *T. cordifolia* [1]. Experimental studies conducted on *T. cordifolia* have shown that *T. cordifolia* has significant therapeutic effects on diabetes and

its associated complications [3–9], hepatotoxicity [10–13], different types of infections [14, 15], gastrointestinal related complications [16] and different types of cancers [17–19]. Moreover, this plant extract has traditionally been used for the treatment of fever [1].

There is a lack of scientific literature about the analgesic, anti-inflammatory and anti-pyretic activity of *T. cordifolia* and most of its traditional uses against pain, fever and inflammation. The aim of the present work was to investigate the analgesic, anti-inflammatory and antipyretic activity of *T. cordifolia* using Swiss albino mice as an experimental animal model. The results of the study have revealed that the methanolic extract of *T. cordifolia*

exhibits therapeutic effects in different models of pain, inflammation and pyrexia in a dose-dependent manner.

## Material and Methods

### Plant Material

*T. cordifolia* was collected in the month of May from the Ever Green nursery in Faisalabad, Pakistan, and was verified by the Department of Botany at the University of Agriculture Faisalabad, Pakistan. A voucher specimen (specimen# 234-2-13) was deposited in the herbarium as a record. After collection, dust, sand and damaged parts were removed from the plant. The whole plant was dried and ground to a coarse powder. An extract was prepared by cold maceration with water and methanol (30/70).

### Animals

Young, healthy, active albino mice (25–30 g) of both sexes, aged 6–8 weeks, were selected for the present study. The animals were procured from the animal house of the University of Agriculture Faisalabad, Pakistan, and were kept in the animal house of Government College University Faisalabad. The animals were kept in a neat and clean environment in polypropylene cages. The room temperature was maintained at  $25 \pm 0.5^\circ\text{C}$  with controlled humidity. The animals were kept under a 12-h light and dark cycle. All the animals were fed in strict hygienic conditions with a rodent pellet diet and water *ad libitum*. The experiments on the animals were performed in accordance with the guidelines of the Ethics Committee of Government College University Faisalabad and were approved by the Advanced Studies and Research Board of Government College University Faisalabad.

### Drugs and Chemicals

The drugs and chemicals used in the study were methanol 95% (Merck, Germany), normal saline (Immunasol NS, A.Z. Pharmaceuticals Co., Pakistan), acetic acid (International Petrochemicals Pvt., Ltd., Pakistan), brewer's yeast and carrageenan (Bayer, Germany). Tramadol, aspirin, diclofenac sodium and paracetamol (GlaxoSmithKline).

### Phytochemical Analysis

A phytochemical analysis of the aqueous methanolic extract of *T. cordifolia* was performed following the standard procedure [20].

## Toxicity Study

A toxicity study of the plant extract under investigation was performed according to methods described by Oduola et al., with slight modifications [21]. The mice were divided into 5 groups. The first group was kept as a control group and was given 10 mL/kg p.o. of normal saline; the second, third, fourth and fifth groups were treated with aqueous methanolic extract of *T. cordifolia* at doses of 1000 mg/kg, 2000 mg/kg, 3000 mg/kg and 4000 mg/kg respectively. All the groups were closely observed for toxic effects for 24 h.

## Evaluation of the Analgesic Activity of *T. cordifolia*

Chemical tests (acetic acid-induced writhing) and thermal tests (hot plate and tail-flick tests) were used to evaluate the analgesic potential of the extract.

The acetic acid-induced writhing was performed using the procedure described by Silva et al. [22], with some modifications. In this method, pain-causing acetic acid was injected in the peritoneal cavity, which induced abdominal pain and constrictions (writhing). The animals in Group 1 (the control group) were treated with 10 mL/kg of normal saline (N/S). Groups 2, 3 and 4 were treated with aqueous methanolic extract of *T. cordifolia* at doses of 100 mg/kg (TC-100), 200 mg/kg (TC-200) and 300 mg/kg (TC-300), respectively. Group 5 was treated with 150 mg/kg of the standard analgesic drug diclofenac sodium (DS). Afterwards, the abdominal writhes were counted for 15 min and the rate of inhibition of writhing was determined. The effectiveness of the treatment was evaluated by the decrease in the number of writhes as compared with the control group.

The analgesic effect of *T. cordifolia* was also assessed by the hot plate method, using an analgesia meter according to the methods described by Nwafor and Okwuasaba [23], previously with some modifications. Briefly, Group 1 was taken as the control group and was given 10 mL/kg of N/S. Groups 2, 3 and 4 were treated with TC-100, TC-200 and TC-300, respectively. Group 5 was treated with the standard drug tramadol (30 mg/kg). After 30 min, the animals in each group were placed one by one on the hot plate of the analgesia meter and the reaction time was noted at 30-, 60- and 90-min intervals. Reaction time is the time elapsed before licking a paw or jumping, because licking and jumping are considered the end point of the pain response.

The tail-flick test was carried out following the procedure described by D'amour and Smith [24],

with some modifications. In the tail-flick test, the time it takes a mouse to take its tail out of a water bath maintained at  $55 \pm 0.2^\circ\text{C}$  was noted, after treatment was given. Group 1 was again the control group; Groups 2, 3 and 4 were treated with TC-100, TC-200 and TC-300, respectively; and Group 5 was treated with the standard drug tramadol (30 mg/kg). After predefined time intervals, the time for the each mouse to flick its tail out of the water bath was noted.

### Anti-Inflammatory Activity by Carrageenan Induced Inflammation Method

Inflammation is the pathophysiological response of living tissues to injuries that leads to an accumulation of plasma and blood cells at the injured site [25]. The animals in the control group (Group 1) were given 10 mL/kg p.o. of normal saline. The TC-100, TC-200 and TC-300 groups (Groups 2, 3 and 4) were treated with aqueous methanolic extract of *T. cordifolia* at doses of 100 mg/kg, 200 mg/kg and 300 mg/kg p.o., respectively. The animals in the standard group (Group 5) were treated with the standard anti-inflammatory drug aspirin (150 mg/kg p.o.). After 30 min of the treatment, carrageenan was administered in the right hind paw. After 30 min of carrageenan injection, the paw size was measured with the help of a Vernier caliper at 1, 2, 3 and 4 h intervals.

### Anti-Pyretic Activity by Brewer's Yeast Induced Pyrexia Method

The procedure used in this study to test the anti-pyretic activity of *T. cordifolia* was the method described by Chatterjee et al. in 1983 [26], with some modifications. Five groups of mice were chosen. The mice in all the groups were deprived of food for 6 h before the experiment, but were given free access to water. All the mice were treated with a suspension of 15% yeast. After 20 h of the yeast treatment, the rectal temperature of all the mice was checked. The control group was given 10 mL/kg p.o. of N/S; Groups 2, 3 and 4 were treated with aqueous methanolic extract of *T. cordifolia* at doses of 100 mg/kg, 200 mg/kg and 300 mg/kg p.o., respectively. Group 5 was treated with paracetamol (150 mg/kg p.o.). Afterwards, the rectal temperature of each mouse was noted at 1-, 2-, 3- and 4-h intervals.

### Statistical Analysis

All the results were expressed as mean  $\pm$  SEM (standard error of mean). Differences between the

control group, the TC-100, TC-200 and TC-300 groups and the standard drug group were assessed by ANOVA. A p-value of  $< 0.05$  was considered significant.

## Results

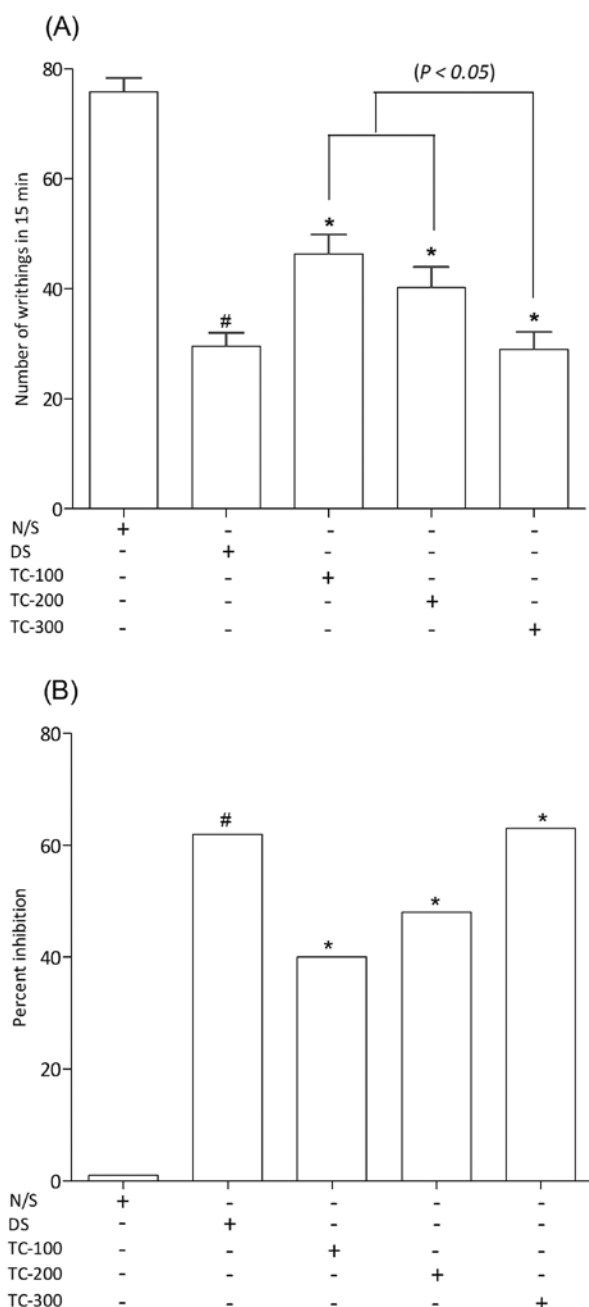
### *T. cordifolia* Phytochemical Analysis and Toxicity Studies

The preliminary phytochemical analysis showed that *T. cordifolia* contains saponins, tannins, terpenoids, flavonoids, alkaloids and glycosides, which conformed with descriptions of the plant's phytochemical constituents as described in the literature [1]. The purpose of the toxicity study was to evaluate whether the plant extract was toxic in nature or not. In the present study, application of the plant extract led to no weight changes, no mortality after 24 h and no behavioral changes, even at the maximum dose of 4000 mg/kg.

### Analgesic Activity of *T. cordifolia*

At first, pain was induced using the acetic acid-induced method; three different doses of *T. cordifolia* extract were then administered to evaluate its analgesic activity. For comparison, the standard drug diclofenac sodium (DS) was administered to one group. At doses of 100 mg/kg, 200 mg/kg and 300 mg/kg, *T. cordifolia* significantly inhibited the number of writhes, and the rate of inhibition of these writhes was observed to be dose-dependent (Fig. 1A). The standard drug (DS) also resulted in a low number of writhes (29.6) as compared to the N/S-treated mice group (Fig. 1A). Moreover, the maximum degree of inhibition was observed in the TC-300 treated mice; a non-significant difference was observed between the TC-300 treated mice and the DS treated mice (Fig. 1B).

In the hot plate-induced analgesia test, mice were pretreated with aqueous methanolic extract of *T. cordifolia* at doses of 100 mg/kg, 200 mg/kg and 300 mg/kg p.o.; one group was administered the standard drug tramadol at a dose of 30 mg/kg. The time elapsed before licking the paw in different groups was noted at 30-, 60- and 90-min intervals. For the TC-100 group, the reaction time (in s) was found to be 5.9, 6.7 and 6.7 at 30-, 60- and 90-min intervals respectively, while for the TC-200 and TC-300 mice, the reaction time was almost the same at all the time intervals. For the mice treated with tramadol the reaction time (in s) was 8.8, 8.6 and 8.7 at 30-, 60- and 90-min intervals respectively (Fig. 2).



**Fig. 1.** Analgesic effects of *T. cordifolia* on acetic acid-induced writhing. A – the number of writhes and B – the percent of inhibition. Values are expressed as mean  $\pm$  SEM and  $n = 6$ . N/S – normal saline 10 mL/kg; DS – 150 mg/kg of diclofenac sodium; TC-100 – 100 mg/kg of *T. cordifolia* extract; TC-200 – 200 mg/kg of *T. cordifolia* extract; TC-300 – 300 mg/kg of *T. cordifolia* extract. When the results were compared with the N/S-treated group, all the groups showed significant ( $p < 0.05$ ) analgesic potential in dose-dependent manner

In the tail-flick test, the mice's tails were placed in a water bath maintained at  $55 \pm 0.2^\circ\text{C}$ . After treatment, the time elapsed before flicking the tail was noted at 30-, 60- and 90-min intervals. For the TC-100 group, reaction times were 2.03 s, 3.94 s and 4.06 s at 30-, 60- and 90-min intervals

respectively. The TC-200 and TC-300 groups had reaction times of 3.06 s, 5.04 s, 5.08 s and 5.96 s, 6.82 s, 7 s at 30-, 60- and 90-min intervals respectively. The flick tail time with the standard drug tramadol was 5.2, 6.08, and 6.12 s at 30-, 60- and 90-min intervals respectively (Fig. 3).

### Anti-Inflammatory Activities of *T. cordifolia*

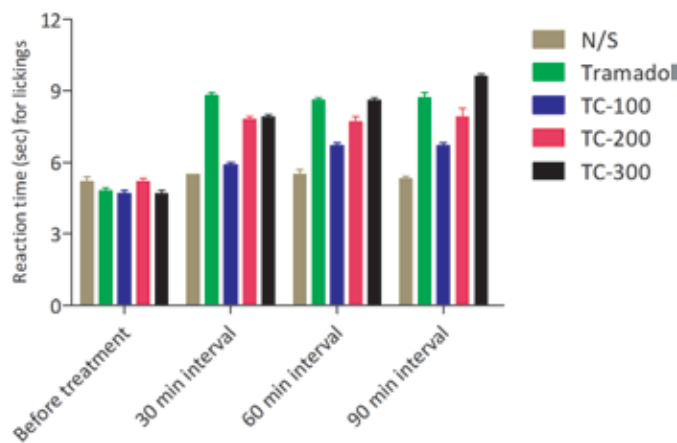
Carrageenan was used as an inflammation inducing agent in the present study and the paw size was increased due to inflammation. Aspirin was used as the standard drug at a dose of 150 mg/kg. Paw size was measured at 1-, 2-, 3- and 4-h intervals after treatment with *T. cordifolia* at doses of 100 mg/kg, 200 mg/kg and 300 mg/kg after inducing inflammation. The mice treated with TC-100 showed the following average values of paw size: 0.695 mm, 0.5825 mm, 0.585 mm and 0.56 mm at 1-, 2-, 3- and 4-h intervals respectively. The TC-200 group showed average paw sizes of 0.69, 0.56, 0.51 and 0.457 mm at 1-, 2-, 3- and 4-h intervals respectively. In the TC-300 group the average paw size measurements were observed to be 0.73, 0.5025, 0.4050 and 0.3175 mm at 1-, 2-, 3- and 4-h intervals respectively (Fig. 4).

### Anti-Pyretic Activities of *T. cordifolia*

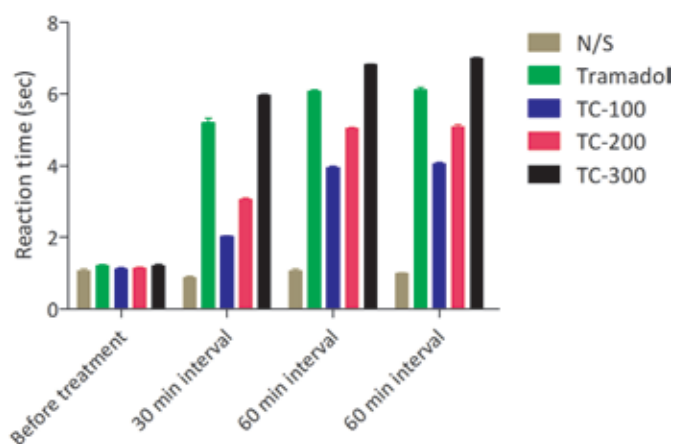
After inducing pyrexia, reduction of pyrexia by different doses of *T. cordifolia* extract and paracetamol were noted at 1-, 2-, 3- and 4-h intervals. In TC-100 treated mice group, the rectal temperature readings obtained were  $102.9^\circ\text{F}$ ,  $102.75^\circ\text{F}$ ,  $100.9^\circ\text{F}$  and  $99.8^\circ\text{F}$  at 1-, 2-, 3- and 4-h intervals respectively. In TC-200 treated mice the readings were  $102.25^\circ\text{F}$ ,  $101.95^\circ\text{F}$ ,  $100.05^\circ\text{F}$ ,  $99.4^\circ\text{F}$  at 1-, 2-, 3- and 4-h intervals respectively, and in the TC-300 group the readings were  $100.85^\circ\text{F}$ ,  $101.65^\circ\text{F}$ ,  $98.85^\circ\text{F}$ ,  $98.575^\circ\text{F}$  at 1-, 2-, 3- and 4-h intervals respectively. The group treated with paracetamol had the following readings:  $98.875^\circ\text{F}$ ,  $98.575^\circ\text{F}$ ,  $98.625^\circ\text{F}$ ,  $98.675^\circ\text{F}$  at 1-, 2-, 3- and 4-h intervals respectively (Fig. 5). *T. cordifolia* was found to reduce the rectal temperature in a dose-dependent manner.

## Discussion

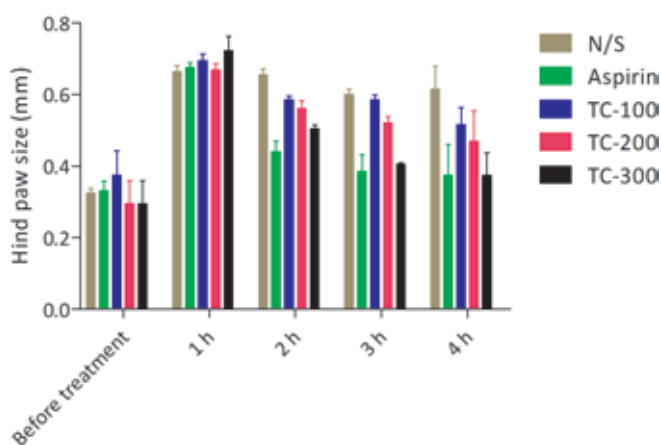
It has been reported that inflammation is a response to many physiological conditions, such as infection, thermal and/or physical injuries [27]. The inflammatory response is necessary for survival against environmental pathogens and injuries.



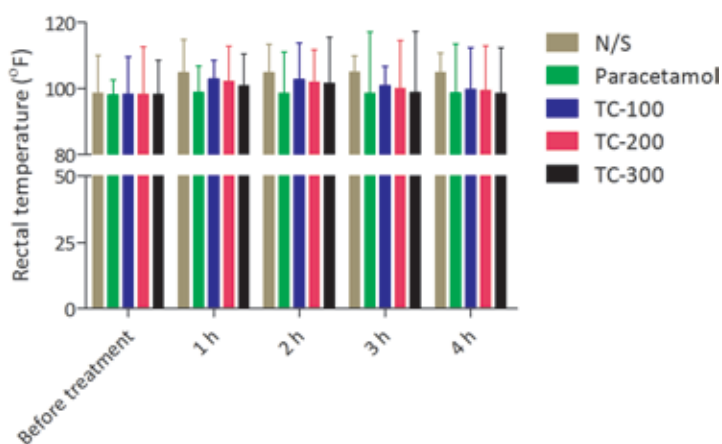
**Fig. 2.** Analgesic effects of *T. cordifolia* on hot plate-induced pain. Values are expressed as mean  $\pm$  SEM and  $n = 6$ . N/S – normal saline 10 mL/kg; tramadol – 30 mg/kg; TC-100 – 100 mg/kg of *T. cordifolia* extract; TC-200 – 200 mg/kg of *T. cordifolia* extract; TC-300 – 300 mg/kg of *T. cordifolia* extract. When the results were compared with the N/S-treated group, all the groups showed significant ( $p < 0.05$ ) analgesic potential



**Fig. 3.** Analgesic effects of *T. cordifolia* on the tail-flick test. Values are expressed as mean  $\pm$  SEM and  $n = 6$ . N/S – normal saline 10 mL/kg; tramadol – 30 mg/kg; TC-100 – 100 mg/kg of *T. cordifolia* extract; TC-200 – 200 mg/kg of *T. cordifolia* extract; TC-300 – 300 mg/kg of *T. cordifolia* extract. When the results were compared with the N/S-treated group, all the groups showed significant ( $p < 0.05$ ) analgesic potential



**Fig. 4.** Anti-inflammatory effects of *T. cordifolia* on carrageenan-induced inflammation. Values are expressed as mean  $\pm$  SEM and  $n = 6$ . N/S – normal saline 10 mL/kg; aspirin – 150 mg/kg; TC-100 – 100 mg/kg of *T. cordifolia* extract; TC-200 – 200 mg/kg of *T. cordifolia* extract; TC-300 – 300 mg/kg of *T. cordifolia* extract. When the results were compared with the N/S-treated group, all the groups showed significant ( $p < 0.05$ ) analgesic potential



**Fig. 5.** Anti-pyretic activity of *T. cordifolia* on brewer's yeast-induced pyrexia. Values are expressed as mean  $\pm$  SEM and  $n = 6$ . N/S – normal saline 10 mL/kg; paracetamol – 150 mg/kg; TC-100 – 100 mg/kg of *T. cordifolia* extract; TC-200 – 200 mg/kg of *T. cordifolia* extract; TC-300 – 300 mg/kg of *T. cordifolia* extract. When the results were compared with the N/S-treated group, all the groups showed significant ( $p < 0.05$ ) analgesic potential

Pain, fever and inflammation are not considered major diseases, but are common clinical manifestations associated with many fatal diseases [27]. Inflammation plays a crucial role in the pathogenesis of many auto-immune diseases [28–33]. Naturally occurring anti-inflammatory agents have shown anti-inflammatory activities against various auto-immune diseases [34–42]. Plant-based medicines are often considered to have minimal side effects and have the potential to treat many life-threatening diseases [43–45]. Recently, many plants have been reported to have analgesic, anti-inflammatory and antipyretic activities, including *Fragaria vesca* [27], *Mimulus elengi* linn [46], *Melanthera scandens* [47] and *Caesalpinia decapetala* [48].

Recently, the therapeutic properties of various medicinal plants have been evaluated [48–53]. The present study has focused on evaluating the analgesic, anti-inflammatory and anti-pyretic activities of *T. cordifolia*, using acetic acid, hot plate and tail flick tests, as well as yeast-induced and carrageenan-induced methods, as described above.

Acetic acid causes a sensation of pain due to the release of certain endogenous substances such as serotonin, histamine, bradykinin, prostaglandins and substance P. These endogenous chemicals stimulate nerve endings and peritoneal receptors, producing abdominal constrictions in response. All the doses of *T. cordifolia* extract were found to decrease the abdominal constrictions produced by acetic acid in a dose-dependent manner (Fig. 1). Similar findings have also been observed in the authors' previous study using different doses of *Caesalpinia decapetala* [48]. Similarly, the increase in the reaction time in the tail-flick test and hot

plate tests reflected the presence of central analgesic activity.

Carrageenan is the preferred phlogistic agent for use in studies of anti-inflammatory activity, because it is non-antigenic and has no systemic side effects. Carrageenan-induced inflammation is a significant test for assessing a substance's anti-inflammatory activity by evaluating the degree to which it inhibits the inflammatory mediators of acute inflammation [54]. Flavonoids have anti-inflammatory activity, as they cause inhibition of chemical mediators of inflammation [55]. The current study revealed that *T. cordifolia* extract possesses good anti-inflammatory activity (Fig. 4). This anti-inflammatory activity was observed to be dose dependent and might be due to the flavonoid content of the plant extract [55]. It was clearly evident from this study that *T. cordifolia* extract produced the maximum anti-pyretic effect at a dose of 300 mg/kg. It has been reported that alkaloids and flavonoids have anti-pyretic potential [56], and the phytochemical analysis of *T. cordifolia* showed the presence of both alkaloids and flavonoids.

The present study revealed that aqueous methanolic extract of *T. cordifolia* has significant potential to (i) inhibit pain, (ii) suppress inflammation and (iii) decrease pyrexia, probably due to the presence of flavonoids and alkaloids. The lack of toxicity and the potential benefits of the plant extract in treating pain, pyrexia and inflammation suggest that it may be used in the future for the reduction of these conditions. Further investigations are required to analyze the phytochemical constituents of *T. cordifolia* and to explore the possible mechanisms of therapeutic potential against various conditions.

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