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Anti-inflammatory evaluation of ethanolic extract of leaves of *Holoptelea integrifolia*, Planch

Kalpana* and Aadesh Upadhyay

Guru Ram Das Institute of Management and Technology, Dehradun, (UA), India

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Abstract

The purpose of the present study was to investigate the anti-inflammatory properties of ethanolic extract of the leaves of Holoptelea integrifolia Planch. (EHI). The different anti-inflammatory models were used. The EHI at dose (250 and 500mg/kg p.o) was given to observe percentage inhibition of paw oedema which were comparable with indomethacin (10mg/kg p.o) used as a reference drug. The extract (500mgkg^{-1} b.w) exhibited maximum anti-inflammatory effect with carrageenan, dextran, histamine, serotonin and cotton pellet method respectively. A significant percentage inhibition of paw oedema by the EHI as compared to standard drug suggests its usefulness in acute and chronic anti-inflammatory models.

Keywords: Anti Anti-inflammatory activity; *Holoptelea integrifolia*; oedema; indomethacin.

INTRODUCTION

Inflammation is a local response of living mammalian tissues to injury. It is a body defence reaction in order to eliminate or limit the spread of injurious agent. There are various components to an inflammatory reaction that can contribute to the associated symptoms and tissue injury. Oedema formation, leukocyte infiltration and granuloma formation represent such components of inflammation [1].

Oedema formation in the paw is the result of a synergism between various inflammatory mediators that increase vascular permeability and/or the mediators that increase blood flow [2]. Several experimental models of paw oedema have been described. Carrageenan-induced paw oedema is widely used for determining the acute phase of inflammation. Histamine, serotonin and bradykinin are the first detectable mediators in the early phase of carrageenan-induced inflammation, [3] whereas prostaglandins are detectable in the late phase of inflammation [4].

Plants continue to be major resources for therapeutic compounds. Plants have always been an exemplary source of drugs and many of the currently available drugs have been derived directly or indirectly from them [5]. Ethnobotanical and ubiquitous plants serve as a rich resource of natural drugs for research and development. Medicinal plant-based drugs owe the advantage of being simple, effective and exhibit broad spectrum activity. Medicinal plant products when compared to their synthetic counterparts minimize the adverse effects. [6].

As a result, a search for other alternatives seems necessary and beneficial. Medicinal plants having a wide variety of chemicals from which novel anti-inflammatory agents could be discovered. Scientific studies are required to judge their efficacy.

Holoptelea integrifolia Planch. (Syn. *Ulmus integrifolia* Linn., *Chirabilva/Poothigam*) belonging to the family Ulmaceae [7] is a medium sized to large deciduous tree, whitish or yellowish grey bark in irregular flakes when freshly cut; leaves simple, alternate, elliptic, entire, glabrous with rounded (or) cordate; flowers greenish yellow racemes or fascicles; fruits sub orbicular samara (Figure 1). The tree is distributed throughout India in decidous forests [8]. The stem bark contains the triterpenoidal fatty acid esters, holoptelin-A (epi-friedelinol palmitate) and holoptelin- B (epi-friedelinol stearate), friedelin and epi-friedelinol (Figure 2) [9]. Bark has many medicinal properties and used in tuberculosis, piles, fistula, abdominal diseases, leprosy, polyuria, vomiting and rheumatism swelling [10,11]. Some recent explorations to appraise its use in traditional medicine have been reported in which antiviral, [12] antioxidant, antimicrobial and wound healing activity [13] were comprehensively emphasized. Ethno medically, the leaves and stem bark of this plant were used by tribals for skin diseases, obesity [14] and in the management of cancer [15]. The fresh material, stem bark or leaves of the plant, is applied as paste externally twice or thrice a day for wound-healing. The process of wound-healing involves inflammation, cell proliferation and contraction of collagen lattice formation [16]. Hence, the present study was under taken to evaluate the anti-inflammatory activity of ethanolic extract of *Holoptelea integrifolia,* Planch. (EHI).

MATERIALS AND METHODS

2.1 Plant material

Plants leaves of *H. integrifolia,* were collected from Biotech Green Nursery, Dehradun, in the months of October 2009 and the plant was authenticated from Botanical Survey of India, Dehradun (Voucher specimen no.112800). The leaves were dried under shade and then powdered with a mechanical grinder and stored in airtight container. 400 g of dried powder material of the leaves was defatted with petroleum ether and the marc thus obtained was then extracted with ethanol in a soxhlet extractor for 72 hr. The extracts were concentrated to dryness under reduced pressure and controlled temperature (40-50 ˚C). The ethanolic extracts yielded brown and semi-solid residue. The dried ethanolic extract of *H. integrifolia* was suspended in normal saline and used for the present study.

Abbreviations: EHI: Ethanolic extract of Holoptelea integrifolia

2.2 Animals

Studies were carried out using Wistar rats of either sex weighing 180–200 g and Swiss albino mice of either sex weighing 18–22 g. They were obtained from the animal house, Indian Veterinary Research Institute (IVRI), Izatnagar, Bareilly, India. The animals were grouped in polyacrylic cages (38 cm × 23 cm × 10 cm) with not more than six animals per cage and maintained under standard laboratory conditions (temperature 25 ± 2°C) with dark and light circle (12/12 h). They were allowed free access to standard dry pellet diet (Hindustan Lever, Mumbai, India) and water *ad libitum*. The rats were acclimatized to laboratory condition for 10 days before commencement of experiment.

2.3 Drugs

Carrageenan (S.D. Fine Chemicals Limited, Bombay), 5-hydroxytryptamine hydrochloride (Serotonin), histamine, dextran and indomethacin (Sigma, USA) were used in the study. All other chemicals were of analytical grade and purchased from Merck.

2.4 Anti-inflammatory activity:

2.4.1 Carrageenan-induced rat paw oedema

The rats were divided into four groups (n = 6). The different groups were treated orally with EHI (250 and 500 $mgkg^{-1}$ b.w.), indomethacin (10 $mgkg^{-1}$ b.w.), and vehicle control (0.9% NaCl, 5 $mlkg^{-1}$ b.w.). The administration of extract and drugs was 30 min prior to injection of 0.1 ml of 1% freshly prepared suspension of carrageenan in normal saline in the right hind paw subplantar of each rat [17,18]. The paw volume was measured initially and then at 1, 2, 3 and 4 h after the carrageenan injection by using plethysmometer.

Percentage inhibition of oedema was calculated as follows:

$$\% \text{ Inhibition} = [1 - (V_T / V_C)] \times 100$$

where V_T and V_C are the paw volume in treated rats and control group of rats respectively.

2.4.2 Dextran-induced rat paw oedema

The animals were treated in a manner similar to that of carrageenan-induced paw oedema models; dextran (0.1 ml, 1% w/v in normal saline) was used in the place of carrageenan [19].

2.4.3 Histamine-induced rat paw oedema

In this model hind paw oedema in the right foot of a rat was induced by subplantar injection of 0.1 ml of 1% freshly prepared histamine in normal saline and the paw oedema was measured as mentioned earlier [20].

2.4.4 Serotonin-induced rat paw oedema

In another model oedema of the right hind paws of the rat was induced by subplantar injection of 0.1 ml of 1% freshly prepared serotonin in normal saline. Group division and treatment of the animals were the same as the carrageenan-induced rat paw oedema model and the paw volume was measured as mentioned in Winter et al., 1962 [17].

2.4.5 Cotton pellet-induced granuloma

The rats were divided into four groups (n = 6). The different groups were treated orally with EHI (250 and 500 $mgkg^{-1}$ b.w.), indomethacin (10 $mgkg^{-1}$ b.w.), and vehicle control (0.9% NaCl, 5 $mlkg^{-1}$ b.w.). The administration of extract and drugs was 30 min prior to injection of 0.1 ml of 1% freshly prepared suspension of carrageenan in normal saline in the right hind paw subplantar of each rat. The paw volume was measured initially and then at 1, 2, 3 and 4 h after the carrageenan injection by using plethysmometer [17,21].

The percentage inhibition increase in the weight of the cotton pellet is calculated.

$$\% \text{ Inhibition} = [1 - (W_T / Wc)] \times 100$$

where W_T and W_C are difference in pellet weight of the drug treated group and control group of rats respectively.

2.5 Statistical analysis

The values were expressed as mean ± S.D. The statistical significance was determined by using the ANOVA followed by Tukey's. Values of $P < 0.05$ were considered as statistically significant.

RESULTS

In spite of tremendous development in the field of synthetic drugs during recent era, they are found to have some or other side effects, whereas plants still hold their own unique place, by the way of having no side effects. Therefore, a systematic approach should be made to find out the efficacy of plants against inflammation so as to exploit them as herbal anti-inflammatory agents.

3.1 Carrageenan-induced rat paw oedema

The anti-inflammatory activity of EHI was measured at the dose of 250 and 500 $mgkg^{-1}$ b.w. against acute paw oedema induced by carrageenan is summarized in Figure 3. The subplantar injection of carrageenan caused a time-dependent paw oedema in the rat. In carrageenan-induced paw oedema in rats, oral administration of EHI (250 and 500 $mgkg^{-1}$ p.o.) inhibited paw swelling dose-dependently at 1, 2, 3, and 4 hr after carrageenan injection ($P < 0.05$) which was comparable with the indomethacin treated group. EHI exhibited 22.62 and 30.01% of inhibition at the dose of 250 and 500 $mgkg^{-1}$ b.w. respectively in carrageenan-induced rat paw oedema (Table 1).

3.2 Dextran-induced paw oedema

The differences in the paw volume after the administration of EHI and standard drug indomethacin were presented in Figure 4. The extract produced significant anti-inflammatory activity and the results were comparable to that of the standard drug indomethacin. EHI exhibited 18.62 and 21.72% of inhibition at the dose of 250 and 500 $mgkg^{-1}$ b.w. respectively in dextran-induced paw oedema in rats.

3.3 Histamine and serotonin-induced paw oedema

The anti-inflammatory effect of EHI against acute pedal oedema induced by phlogistic agent's histamine and serotonin has been shown in Figure 5 and Figure 6. The extract (500 $mgkg^{-1}$) showed a maximum 32.34% inhibition in histamine and 29.62% inhibition in serotonin-induced rat paw oedema (Table 1).

3.4 Cotton pellet-induced granuloma

In the cotton pellet induced inflammation studies in rats, higher dose of EHI i.e., 500$mgkg^{-1}$ and the standard drug indomethacin showed significant decrease in wet weight of granuloma tissue formation ($P<0.05$) (Table 2). Further, EHI in the higher dose of 500$mgkg^{-1}$ also significantly decreased the dry weight of granuloma tissue formation, which was comparable than indomethacin ($P<0.05$).

DISCUSSION

EHI showed significant anti-inflammatory effects in various animal models. Our results revealed that administration of ethanolic extract inhibited the oedema starting from the first hour and during all phases of inflammation, which is probably inhibition of different aspects and chemical mediators of inflammation.

For evaluating the anti-inflammatory activity most effective and widely used model for inflammation is carrageenan-induced paw edema. Carrageenan is a mixture of polysaccharides composed of sulfated galactose units and is derived from Irish Sea moss, *Chondrous crispus*. Its use as an endemogen was introduced by Winter et.al. [17]. Carrageenin-induced oedema falls in the category of acute inflammation, which involves the synthesis or release of inflammatory mediators at the injured site which further cause pain and fever [22,23]. On the other hand, the proliferative phase or chronic inflammation is measured by methods for testing granuloma formation such as cotton pellet granuloma [24–25]. EHI was effective in both cotton pellet granuloma as well as carrageenin-induced paw oedema.

In the first phase (during the first 2 h after carrageenan injection), chemical mediators such as histamine and serotonin play role, while in second phase (3–4 h after carrageenan injection) kinin and prostaglandins. It can be assumed that EHI is effective in all the phases of inflammation i.e., acute, subacute and proliferative phases.

EHI showed 18.61 and 21.72 % inhibition against dextran induced oedema. In dextran induced aseptic arthritis test in rats (Fig. 5), EHI showed significant inhibition of oedema within 60 min.
In Histamine induced paw edema, histamine causes vasodialation and increase in vascular permeability followed by edema which is one of the phases of inflammation. Histamine induced paw edema is said to occur in earlier stage in mounting of vascular of the vascular reaction in the chemically induced inflammation. In this, swelling occurs primarily due to action of histamine. Generally histamine is released following the mast cell degranulation by number of inflammatory mediators including substances P interleukin-1. This is likely to evoke the release of neuropeptide as well as release of prostaglandins and monohydroxy eicosatetranoic-acid from endothelial cell leading to hyperalgesia and other pro-inflammatory effects. Both doses i.e., 250

and 500 $mgkg^{-1}$ of EHI and 10 $mgkg^{-1}$ doses of indomethacin showed 20.71, 32.34 and 41.46 % inhibition, respectively against histamine produced oedema (Table 1).

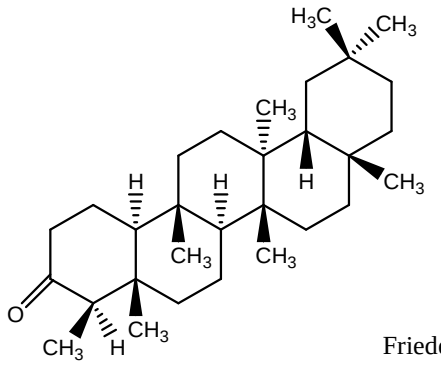
It was reported that the capillary permeability increased in serotonin induced inflammation in rats [26]. EHI inhibited inflammation is almost such as indomethacin. EHI showed 24.28 and 29.62 % inhibition of the inflammation produced by serotonin with doses of 250 and 500 $mgkg^{-1}$ respectively.

The cotton-pellet model is based on the foreign body granuloma which is provoked in rats by subcutaneous implantation of pellets of compressed cotton. The cotton-pellet granuloma is widely used to evaluate the transudative and proliferative components of the chronic inflammation. The moist weight of the pellets correlates with transuda, the dry weight of the pellet correlates with the amount of granulumatous tissues. Chronic inflammation occurs by means of the development of proliferate cells. These cells can be either spread or in granuloma form. Non-steroidal anti-inflammatory drugs decrease the size of granuloma which results from cellular reaction by inhibiting granulocyte infiltration, preventing generation of collagen fibers and suppressing mucopolysaccharides.

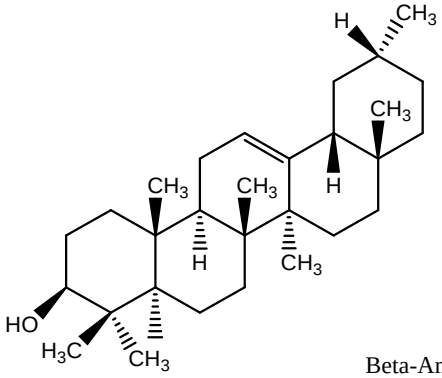
The inhibition of subacute cotton-pellet induced granuloma by EHI was similar to the effect of actual anti-inflammatory agents. In the developing arthritis test the extract showed significant inhibitory activity of the paw oedema in rats.

The use of friedelin or friedelin-type compounds has been considered for treatment of convulsions, inflammation, topical ulcers, rheumatic inflammation, fever and dysentery [27, 28]. Therefore it can be expected that anti-inflammatory activity of the plant extract is due to its active triterpenes like friedelin, epifriedelinol and triterpene fatty acid esters (like, holoptelin A and B) content.

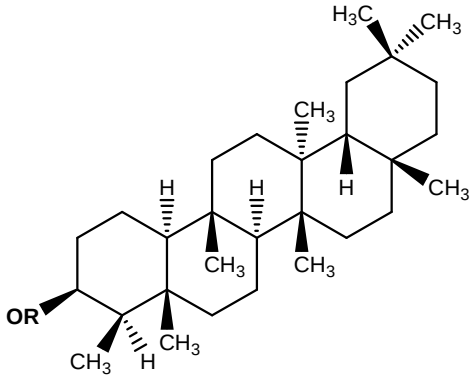
Figure 1: Plant of *Holopetea Integrifolia*



Friedelin



Beta-Amyrin



R-H	3-Epifriedelinol
R-Palmitic Acid	Holoptelin-A
R-Stearic Acid	Holoptelin-B

Figure 2: Triterpenoids and triterpenoids fatty acid esters of *Holoptelea integrifolia*

Table 1: Percentage inhibition of inflammation by standard Indomethacin, ethanolic extract of *Holoptelea integrifolia* (250, 500 mgkg^{-1} b.w.) in different inflammation models

	Percentage Inhibition (%)			
	Carageenan	**Dextran**	**Histamin**	**Serotonin**
Indomethnacin	40.95	29.85	41.46	46.86
EHI 250	22.62	18.61	20.71	24.28
EHI 500	30.01	21.72	32.34	29.62

EHI 250: Ethanolic extract of Holoptelea integrifolia at a dose of 250mgkg^{-1}
EHI 500: Ethanolic extract of Holoptelea integrifolia at a dose of 500mgkg^{-1}

Table 2: Effect of oral administration of ethanolic extract of *Holoptelea integrifolia* on cotton pellet induced granuloma in rats

	Dose ($mgml^{-1}$)	Wt. of cotton pellet mg (wet)	% Inhibition	Wt. of cotton pellet mg (dry)	% inhibition
Control	5ml (0.9% NaCl)	183.75±10.56	-	46.23±2.52	-
Indomethacin	10	77.38±9.17*	57.88	22.35±1.90*	51.65
EHI 250	250	106.66±10.13*#	41.95	32.18±2.39*#	30.38
EHI 500	500	81.1±5.35*	55.86	25.51±3.53*	44.81

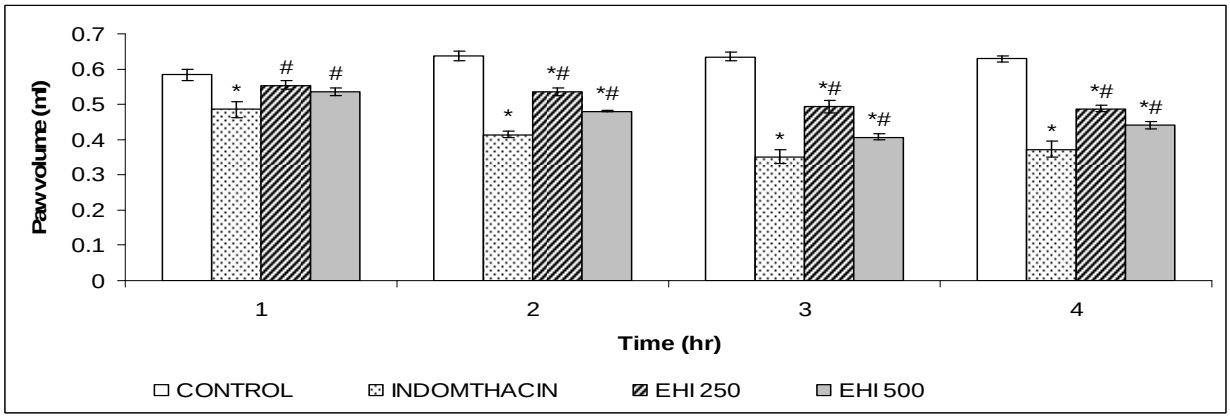


Figure 3: Effect of oral administration of ethanolic extract of *Holoptelea integrifolia* on carrageenan induced paw oedema in rats

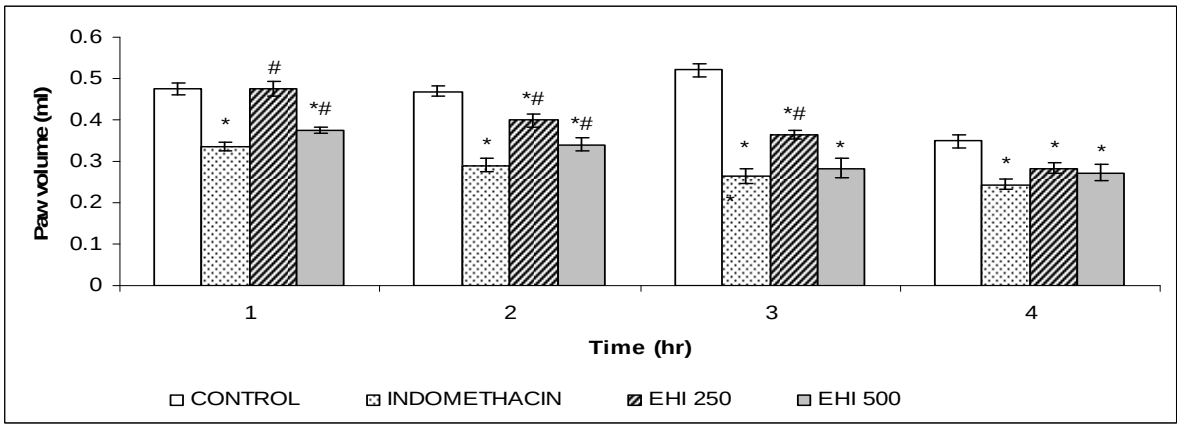


Figure 4: Effect of oral administration of ethanolic extract of *Holoptelea integrifolia* on dextran induced paw oedema in rats

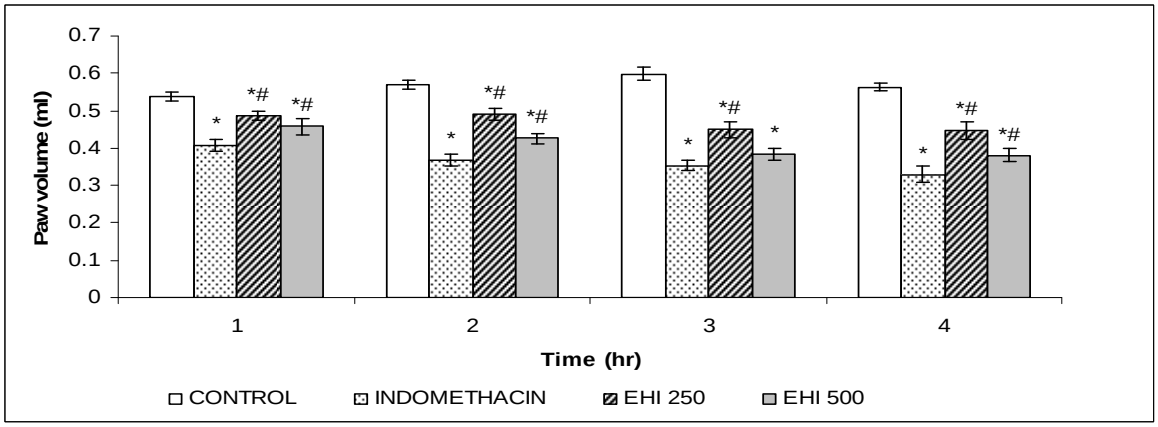


Figure 5: Effect of oral administration of ethanolic extract of *Holoptelea integrifolia* on histamine induced paw oedema in rats

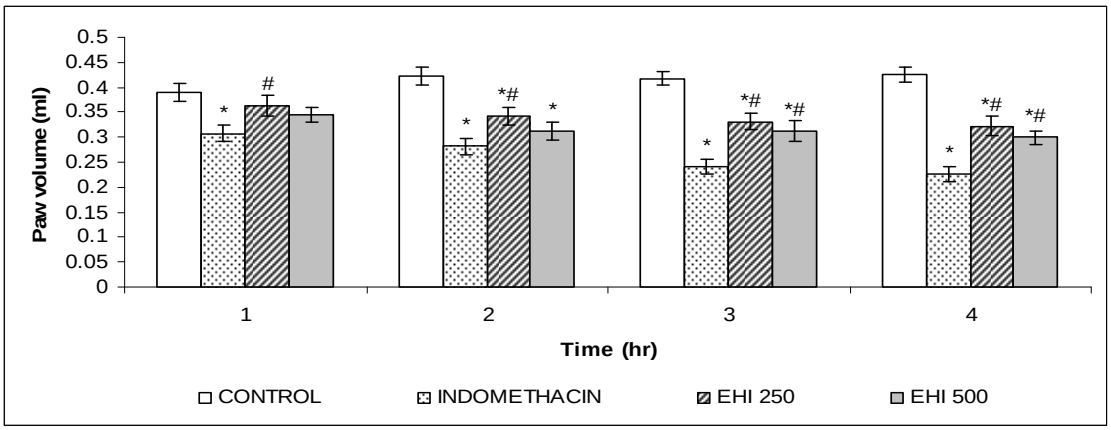


Figure 6: Effect of oral administration of ethanolic extract of *Holoptelea integrifolia* on serotonin induced paw oedema in rats

EHI 250: Ethanolic extract of *Holoptelea integrifolia* at a dose of 250mgkg^{-1}
EHI 500: Ethanolic extract of *Holoptelea integrifolia* at a dose of 500mgkg^{-1}
P<0.001 *, P<0.01 **, P<0.05 † significantly different from control.
P<0.001 $^{\#}$, P<0.01 $^{\#\#}$, P<0.05 ¶ significantly different from standard (Indomethacin).

CONCLUSION

Thus, in the present investigation, ethanolic extract of *Holoptelea Intigrifolea* showed that it possesses potent anti-inflammatory activities. Further studies involving the purification of the chemical constituents of the plant and the investigations in the biochemical pathways may result in the development of a potent anti-inflammatory agent with low toxicity and better therapeutic index.

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