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Full Length Research Paper

Anti-nociceptive and anti-inflammatory activities of *Holoptelea integrifolia* (Roxb.) planch fruit extract on laboratory animals

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The methanolic extract of the fruit of *Holoptelea integrifolia* (Roxb) Planch was investigated for its anti-inflammatory and analgesic activities in animal models. The extract at 100, 150 and 200 mg/kg body weight reduced significantly the formation of edema induced by carrageenan. In the acetic acid-induced writhing model, the extract showed a good analgesic effect characterized by reduction in the number of writhes when compared to the control. The analgesic effect of extract of *H. integrifolia* was also significant ($P < 0.05$) in tail immersion test and was dose dependent. These results were also comparable to those of diclofenac sodium (50 mg/kg), the reference drugs used in this study. Though the study has provided some justification for the folkloric use of the plant in several communities for conditions such as pain and inflammations but caution should be exercised in its use for medicinal purpose.

Key words: *Holoptelea integrifolia,* anti-nociceptive activity, tail flick test, hot plate test, anti-inflammatory activity.

INTRODUCTION

The world has changed so much so has the need of human beings changed for the treatment of different disease. Researchers are trying to find out alternative medicines through natural sources; there are so many plants that have potential medicinal properties which have to be discovered, and this process of exploring the natural sources must go on. Herbal medicines are cost effective and have fewer side effects (Samsam and Moatar, 1991). According to World Health Organization (WHO), 80% of the people worldwide rely on herbal medicines for some aspects. It is estimated that there are 250,000 to 500, 000 species of plants on earth, out of which 75,000 plants species are of medicinal value and only 800 plants species are used in the preparation of herbal drugs, so there is comprehensive scope of finding new molecule (Borris, 1996).

In continuation of this exploring of the natural sources, the plant *Holoptelea integrifolia* (Roxb) Planch plays a vital role. *H. integrifolia* (Ulmaceae) is distributed over tropical and temperate regions on Northern hemisphere

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including Pakistan, India peninsula to indo china, and Srilanka. The common vernacular names of the plant are Papri, Magzi (Urdu), Chilbil, Dhamma, Begana (Hindi) (Mahmud et al., 2010; Anonymous, 2001). It is traditionally used in the treatment and prevention of several ailments like leprosy, inflammation, rickets, leucoderma, scabies, rheumatism, ringworm, eczema, malaria, intestinal cancer, and chronic wounds. *In vitro* and *in vivo* pharmacological investigations on crude extracts and isolated compounds showed antibacterial, antifungal (Vinod et al., 2010a, b), antioxidant (Saraswathy et al., 2008), anti-inflammatory (Srinivas et al., 2009), anthelmintic (Durga and Paarakh, 2010), antidiabetic (Sharma et al., 2010), antidiarrhoeal (Sharma and Lakshami, 2009), adaptogenic (Puri et al., 2011), anticancer (Lakshmi et al., 2010), wound healing (Srinivas et al., 2008a), hepatoprotective (Hemamalini and Sathya, 2013), larvicidal (Singha et al., 2012), antiemetic (Srinivas et al., 2008b) and central nervous system (CNS) depressant (Hemamalini et al., 2011) and analgesic activities (Rizwani et al., 2012). Phytochemical analysis showed the presence of terpenoids, sterols, saponins, tannins, proteins, carbohydrates, alkaloids, phenols, flavonoids, glycosides, and quinines. Numerous compounds including holoptelin-A, holoptelin-B, friedlin, epifriedlin, β-amyrin, stigmasterol, β-sitosterol, 1, 4-napthalenedione, betulin, betulinic acid, hexacosanol, and octacosanol have been identified and isolated from the plant species (Harleen et al., 2011).

The basic aim of our study is to determine the anti-nociceptive and anti-inflammatory activity of *H. integrifolia* fruit extract which is not explored yet.

MATERIALS AND METHODS

Collection and extract preparation

The fresh fruit of *H. integrifolia* was collected from the premises of University of Karachi. The plant was taken to the harbium of Department of Pharmacognosy, Faculty of Pharmacy, University of Karachi where the plant was identified by Prof. Dr. Ghazala H. Riwani. It was dried under shade and macerated in methanol at room temperature for 15 days. This was then filtrated using filter paper (Whatmann size no. 1) and filterate was evaporated using rotary evaporator (Buchi, Switzerland). The dried residue of fruit extract of *H. integrifolia* was kept in air tight bottle until it was reconstituted for administration.

Management of experimental animals

Strain of albino Wister rats (150 to 160 g) and mice (20 to 25 g) of both sexes were procured from the Animal House of Department of Pharmacology, Dow University of Health Sciences, Pakistan. The animals were kept in well aerated laboratory cages in the animal house of Department of Pharmacology, Faculty of Pharmacy, University of Karachi. They were allowed to acclimatize to the laboratory environment for a period of one week before the commencement of the experiment. The animals were given access to standard feed and drinking water during the acclimatization period.

Acetic acid induced writhing

Acetic acid was used for assay of analgesic potential of extract of *H. integrifolia*. Mice weighing 25 g were used for the activity. Group I received normal saline while Group II standard drug diclofenac sodium 50 mg/kg orally. While the remaining groups III, IV and V were orally administered with 100, 150 and 200 mg/kg of the extract of *H. integrifolia,* respectively. After 30 min of drug administration, pain was induced by intraperitonial administration of 0.6% acetic acid. The number of abdominal constrictions (writhes) was counted after 5 min of acetic acid injection for the period of 10 min (Koster et al., 1959).

Hot plate test

For hot plate analgesic activity, mice were placed on a hot plate (50 ± 2°C) and their reaction to heat was observed. Five groups of 6 mice each were used. Group I received normal saline while Group II standard drug diclofenac sodium 50 mg/kg orally. While the remaining groups III, IV and V were orally administered with 100, 150 and 200 mg/kg of the extract of *H. integrifolia,* respectively. When the animal raised and licked the front paws they were quickly removed from the hot plate and the time period observed. The observations were recorded after 30 min of drug administration (Dharmasiri et al., 2003). The cut-off time, that is time of no response was put at 30 s. Percent analgesia was calculated using the following formula:

% Analgesia = (Test latency – control latency) / (Cut – off time – control latency) × 100

Tail flick test

Tail flick method was used for analgesic activity. Mice were divided into four groups of five animals each. Group I received normal saline while Group II standard drug diclofenac sodium 50 mg/kg orally. While the remaining groups III, IV and V were orally administered with 100, 150 and 200 mg/kg of the extract of *H. integrifolia*, respectively. In this method, tail was immersed in water heated at 50 ± 2°C in water bath. The time period in which tail flicked out from water bath was recorded. The observations were made for 180 min at every 30 min interval (Owoyele et al., 2004). The reaction time was determined at 30, 60, 90, 120,150 and 180 min after treatment. Baseline was considered as reaction time before administration of extracts or reference drug. The cut-off time was taken as 10 s to prevent tissue damage. Tail flick antinociceptive index (TFAI) was calculated from the expression:

TFAI = reaction time-baseline/cut-off baseline

Carrageenan-induced rat paw edema

Thirty rats were used in this study and they were divided into five groups of six per group. Group I received normal saline while Group II standard drug diclofenac sodium 50 mg/kg orally. While the remaining groups III, IV and V were orally administered with 100, 150 and 200 mg/kg of the extract of *H. integrifolia*, respectively. Acute inflammation was produced by the sub-plantar administration of 0.1 ml of 1% carrageenan in normal saline in the right paw of rats. The paw volume was measured at 0, 1, 2, 3, 4 and 5 h after carrageenan injection using a micrometer screw gauge. Increases in the linear diameter of the right hind paws were taken as an indication of paw edema. The percentage inhibition of the inflammation was calculated from the formula:

Table 1. Effect of extract of *H. integrifolia* on acetic acid induced writhing in mice.

Treatment	Dose (mg/kg)	Mean No. of writhes±S.E.M
Control	-	55±1.86
Diclofenac sodium	50	16±1.382**
Extract of *H. integrifolia*	100	38.80±2.50**
	150	28.00±1.50**
	200	20.00±1.14**

Values are expressed as mean ± S.E.M (n=6). Statistical significance were calculated by ANOVA followed by Tukey post hoc test when compare to the control group *P < 0.05, ** P< 0.01

Table 2. Effect of extract of *H. integrifolia* on hot plate method.

Treatment group	Dose (mg/kg)	Reaction time						
		0 min	30 min	60 min	90 min	120 min	150 min	180 min
Control	-	5.17±0.04	5.24±0.03	6.39±0.14	5.55±0.14	6.60±0.02	5.88±0.2	5.98±0.25
Diclofenac sodium	50	8.33±0.6	10.10±0.7	12.34±0.7	13.7±0.7	14.45±1.1	11.54±0.7	10.23±0.8
Extract of *H. integrifolia*	100	8.81±0.7	16.40±3.7**	13.40±4.7	9.20±4.0	7.00±3.3	7.60±3.2	12.40±4.7
	150	9.20±0.8	21.80±7.5**	20.60±12.1*	14.20±8.8	21.20±14.5*	14.4±10.3	15.20±4.7
	200	7.80±0.8	13.80±4.4*	13.20±5.6	11.20±4.0	9.60±4.0	12.0±6.5	12.40±5.31

Values are expressed as mean ± S.E.M (n=6). Statistical significance were calculated by ANOVA followed by Tukey post hoc test when compare to the control group *P < 0.05, ** P< 0.01

% Inhibition of inflammation = D0 – Dt / D0 × 100

Where D0 was the average inflammation (hind paw edema) of the control group of rats at a given time; and Dt was the average inflammation of the drug treated (that is, extracts or reference diclofenac sodium) rats at the same time (Sawadogo et al., 2006; Moody et al., 2006).

Statistical analysis

Data were expressed as mean ± standard error of mean (SEM). The data obtained were statistically analyzed using one-way analysis of variance (ANOVA) with Tukey's multiple comparison post hoc test to determine the level of significance between control and experimental group. Values of P < 0.05 were considered as significant.

RESULTS

Acetic acid-induced writhes

Peripheral analgesic activity of extract of *H. integrifolia* was assessed by acetic acid induced writhing method. The results showed that the pain relief was achieved in a dose dependent manner, at all test doses (100, 200 and 300 mg/kg, o.p.) when compared to the diclofenac sodium (50 mg/kg) as shown in Table 1.

Hot plate test

The results of the hot plat test revealed that the latency time was significantly (P < 0.05) increased from 8.81 ± 0.7 to 12.40 ± 4.7 at the dose of 100 mg/kg after 180 min as shown in Table 2. The most significant (P < 0.01) increase in latency time noticed against 150 mg/kg of extract of *H. integrifolia* was 21.80 ± 7.5 whereas, the percent analgesia of the standard diclofenac sodium and extract of *H. integrifolia* (HIE) shown in Figure 1.

Tail flick Test

The analgesic effect of extract of *H. integrifolia* was also significant (P < 0.05) in tail immersion test and was dose dependent. The reaction time of all doses and diclofenac sodium is given in Table 3. The maximum analgesic effect was noticed at 60 min after the dose administration. Tail flick antinociceptive index (TFAI) of *H. integrifolia* (HIE) at 100, 150 and 200 mg/kg, respectively is presented in Figure 2.

Carrageenan-induced rat paw edema

The anti-inflammatory activity at test doses (100, 150 and

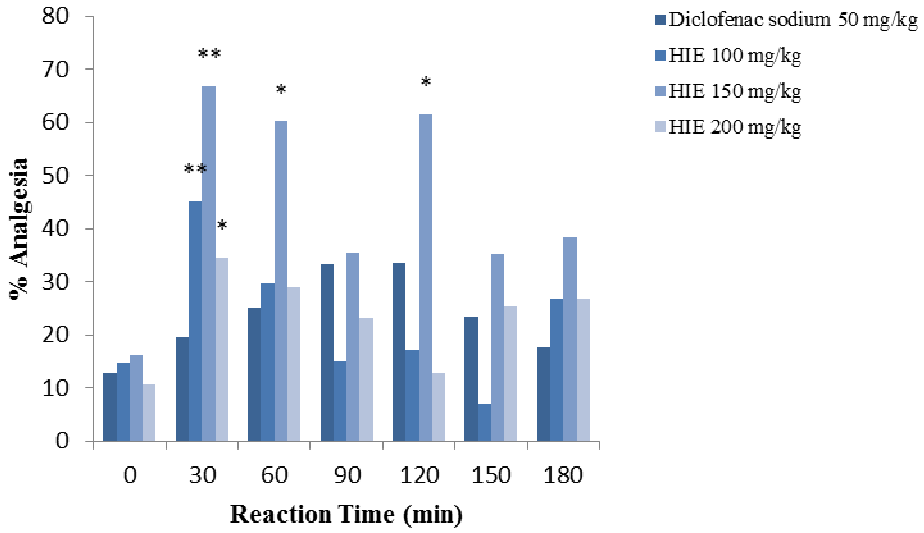

Figure 1. Percent effect of extract of *H. integrifolia* (100, 150 and 200mg/kg) and diclofenac sodium (50mg/kg) on hot plat pain model in mice. Each point represent the mean ± SEM of six animals. The data was analyzed by ANOVA followed by Tukey's test. Asterisks indicated statistically significant values from control. *P < 0.05, **P < 0.01.

Table 3. Effect of extract of *H. integrifolia* on Tail- flick response in mice

Treatment group	Dose (mg/kg)	Reaction time						
		0 min	30 min	60 min	90 min	120 min	150 min	180 min
Control	-	1.30±0.39	1.03±0.17	0.99±.008	1.10±1.4	1.03±0.4	1.03±0.4	1.01±0.4
Diclofenac sodium	50	1.33±0.2	3.25±0.2*	5.59±0.71**	6.55±0.54**	6.18±0.6**	5.6±0.53**	4.49±0.41**
	100	1.37±0.47	3.87±0.84**	6.30±0.78*	6.54±1.18**	5.90±2.1**	5.45±1.1**	2.75±1.1**
Extract of *H. integrifolia*	150	1.32±0.21	4.23±1.49**	7.58±5.0**	9.18±3.5**	6.70±1.7**	4.73±1.0**	3.24±0.6*
	200	1.38±0.19	4.75±1.6**	9.86±3.2**	9.13±1.2**	8.40±1.3**	6.98±0.5**	5.02±0.9**

Values are expressed as mean ± S.E.M (n=6). Statistical significance were calculated by ANOVA followed by Tukey's post hoc test when compare to the control group *$P < 0.05$, **$P < 0.01$

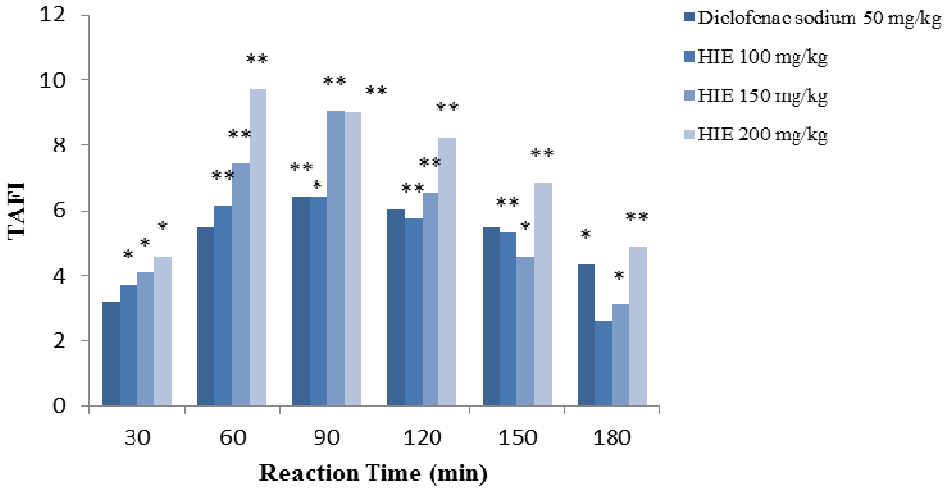

Figure 2. Tail flick antinociceptive index (TFAI) of extract of *H. integrifolia* (100, 150 and 200mg/kg) and diclofenac sodium (50mg/kg) on tail immersion pain in mice. Each point represents the mean ± SEM of 6 animals. The data was analyzed by ANOVA followed by Tukey's test. Asterisks indicated statistically significant values from control. *P < 0.05, **P < 0.01.

Table 4. Effect of extract of *H. integrifolia* on paw edema in rats

Treatment group	Dose (mg/kg)	Reaction time					
		0 h	1 h	2 h	3 h	4 h	5 h
Control	-	2.17±0.35	3.17±0.6	4.15±0.299	4.83±0.2	5.32±0.16	5.35±0.1
Diclofenac sodium	50	2.08±0.2	2.35±0.3	2.5±0.30**	2.68±0.31**	3.04±0.29**	2.7±0.2**
Extract of *H. integrifolia*	100	2.08±0.33	3.16±0.35	3.42±0.3*	3.87±0.42**	3.89±0.53**	3.96±0.45**
	150	2.08±0.32	3.03±0.47	3.36±0.30**	3.49±0.15**	3.37±0.28**	3.46±0.2**
	200	2.18±0.43	2.82±0.40	2.96±0.4*	3.15±0.40**	3.18±0.29**	3.05±.026**

Values are expressed as mean ± S.E.M (n=6). Statistical significance were calculated by ANOVA followed by Tukey's post hoc test when compare to the control group *$P < 0.05$, ** $P < 0.0$

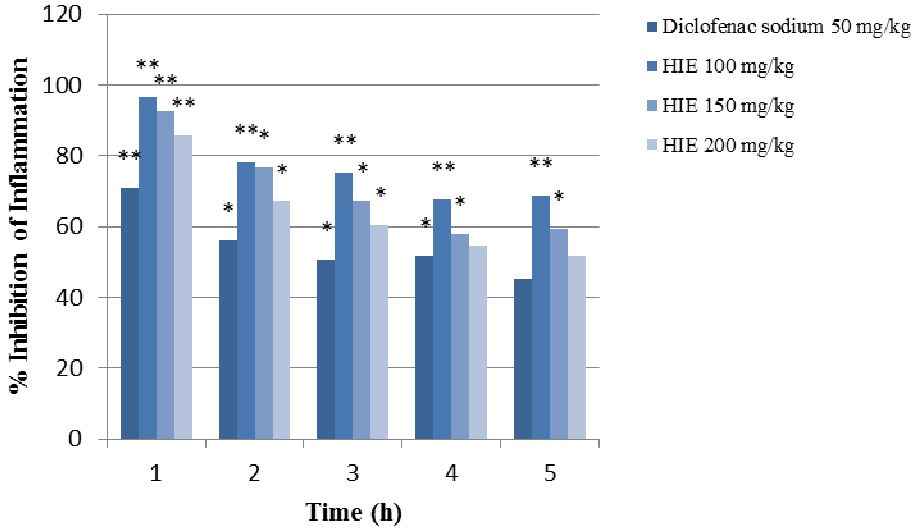

Figure 3. Percent inhibition of inflammatory of *H. integrifolia* (100, 150 and 200 mg/kg) in carrageenan induced paw edema in rats. The data was analyzed by ANOVA followed by Tukey's test. Asterisks indicated statistically significant values from control. *P < 0.05, **P < 0.01.

200 mg/kg) of extract of *H. integrifolia* is presented in Table 4 with the average volume of the paw edema. The percent inhibition of inflammation is presented in Figure 3. The injection of the carrageenan in paw created an inflammatory edema which increased gradually. The extract of *H. integrifolia* at the dose of 200 mg/kg exhibited an anti-inflammatory activity that became significant ($P < 0.01$) 2 h after the injection of carrageenan with a maximum effect of 67.17%.

DISCUSSION

Acetic acid-induced writhing is a well recommended protocol in evaluating medicinal agents for their analgesic property. The pain induction caused by liberating endogenous substances as well as some other pain mediators such as arachidonic acid via cyclooxygenase, and prostaglandin biosynthesis (Khan et al., 2010). This pain paradigm is widely used for the assessment of peripheral analgesic activity due to its sensitivity and response to the compounds at a dose which is not effective in other methods. The local peritoneal receptor could be the cause of abdominal writhings (Khan et al., 2009). Pain sensation in acetic acid induced writhing paradigm is elicited by producing localized inflammatory response due to release of free arachidonic acid from tissue phospholipids via cyclo-oxygenase (COX), and producing prostaglandin specifically PGE2 and PGF2α, the level of lipoxygenase products may also increase in peritoneal fluids (Duarte et al., 1988). Regarding the results of extract in acetic acid-induced abdominal constriction assay, a prominent inhibition of writhing reflux was observed. These findings strongly recommend

that extract of *H. integrifolia* has peripheral analgesic activity and their mechanisms of action may be mediated through inhibition of local peritoneal receptors which may be the involvement of cyclooxygenase inhibition potential.

Thermal nociception models such as hot plat and the tail immersion tests were used to evaluate central analgesic activity. The extract of *H. integrifolia* showed significant ($P < 0.01$) analgesic effect in both the hot plat and tail immersion tests, implicating both spinal and supraspinal analgesic pathways. Carrageenan edema is a multimediated phenomenon that liberates diversity of mediators. It is believed to be biphasic; the first phase (1 h) involves the release of serotonin and histamine while the second phase (over 1 h) is mediated by prostaglandins, the cyclooxygenase products, and the continuity between the two phases is provided by kinins (Asongalem et al., 2004; Silva et al., 2005). Development of edema induced by carrageenan is commonly correlated with early exudative stage of inflammation (Vinegar et al., 2000). This study has shown that the methanolic extract of the fruit of *H. integrifolia* possessed a significant anti-edematogenic effect on paw edema induced by carrageenan. Since carrageenan-induced inflammation model is a significant predictive test for anti-inflammatory agents acting by the mediators of acute inflammation, the results of this study are an indication that *H. integrifolia* can be effective in acute inflammatory disorders.

Conclusion

The methanolic extract of fruit of *H. integrifolia* was proved as a natural safe remedy for the treatment of analgesia and inflammation. Our current findings demonstrated scientific rationale for the folk use of the plant as analgesic and anti-inflammatory. Interestingly the extract of *H. integrifolia* exhibited both peripheral as well as central analgesic effect which might have been attributed to the presence of betulinic acid, betulin and other active principles. Nevertheless, the isolation of pure secondary metabolites from the plant will help us further in understanding the mechanism of these activities and identification of lead compounds of clinical utility.

Conflict of interest

The authors declare there are no conflict of interest concerning the publication of the manuscript.

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