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Research

Anti Analgesic Activity By Holoptelea Integrifolia

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Abstract

Holoptelea integrifolia (Ulmaceae) is a common weed occurring throughout the globe. In traditional medicine its decoction has been used for treatment of many infectious and degenerative diseases. This work was therefore designed to assess the phytochemical constitution of *Holoptelea integrifolia* dried roots extracts and to evaluate their analgesic and anti-inflammatory activity in rats. Fresh and crushed roots of *Holoptelea integrifolia* were collected and then extracted with ethanol. The ethanolic extract at the doses of 100 mg/kg, 200 mg/kg body weight was subjected to evaluation of analgesic and anti-inflammatory activities in experimental animal models. Analgesic activity was evaluated by Hot-plate and tail –flick method in albino Wistar rats; acute and chronic anti-inflammatory activity was evaluated by carrageenan-induced paw oedema and formalin-induced paw edema in Wistar albino rats. Diclofenac sodium and Indomethacin were employed as reference drugs for analgesic and anti-inflammatory studies, respectively. In the present study, the ethanolic extract of *Holoptelea integrifolia* demonstrated significant analgesic and anti-inflammatory activities in the tested models.

Keywords: *Holoptelea integrifolia*, hot-plate, tail-flick, Carragenan-induced paw edema model, Formalin-induced paw edema model, Analgesic and anti-inflammatory activity.

INTRODUCTION

INFLAMMATION

Inflammation is an important physiological reaction which occurs in response to a wide variety of injurious agents (e.g. bacterial infection, physical trauma, chemicals or any other phenomenon) ultimately aiming to perform the dual function of limiting damage and promoting tissue repair [1]. Inflammatory processes are required for immune surveillance, optimal repair, and regeneration after injury [2]. The inflammatory process protects our body from diseases by releasing cells and mediators that combat foreign substances and prevent infection [3]. However, sustained, excessive or inappropriate inflammation is the cause of numerous diseases including rheumatoid arthritis, psoriasis and inflammatory bowel disease [4].

Inflammation is a major component of the damage caused by autoimmune diseases, and is a fundamental contributor of various infectious and non-infectious diseases such as cancer, diabetes, cardiovascular disease,

rheumatoid arthritis, Alzheimer's and arteriosclerosis. Depending on the intensity of this process, mediators generated in the inflammatory site can reach the circulation and cause fever [5, 6].

Inflammation is a complex pathophysiological process mediated by a variety of signalling molecules produced by leucocytes, macrophages and mast cells undergoing various cellular responses such as phagocytic uptake, and the production of inflammatory mediators such as nitric oxide (NO), prostaglandin E2 (PGE2) and tumour necrosis factor (TNF)-α [7], that bring about edema formation as a result of extravasation of fluid and proteins and accumulation of leucocytes at the inflammatory site [8]. In addition, it is broadly accepted that cytokines, produced by either immune or central nervous system cells, might directly sensitize the peripheral nociceptors [9].

Inflammation is an important cellular response triggered by various mechanical, chemical or immunological stress factors and it is regulated by a delicate balance between local factors that finally determine the outcome of the disease process: progression or resolution. The inflammatory response is a complex and highly regulated sequence of events that start with an initial production of pro-inflammatory mediators that recruit professional inflammatory cells to the site of injury to clear the offending trigger [10]. This is followed by an anti-inflammatory phase, in which resident tissue cells may acquire the potential for protecting themselves from further activation and injury. More recently, inflammation was described as "the succession of changes which occurs in a living tissue when it is injured provided that the injury is not of such a degree as to at once destroy its structure and vitality" or "the reaction to injury of the living microcirculation and related tissues" [11].

Although, in ancient times inflammation was recognised as being part of the healing process, up to the end of the 19th century, inflammation was viewed as being an undesirable response that was harmful to the host. Based on visual observation, the ancients characterised inflammation by five cardinal signs, namely redness (rubor), swelling (tumour), heat (calor; only applicable to the body extremities), pain (dolor) and loss of function (functio laesa). The first four of these signs named by Celsus in ancient Rome (30-38 B.C.) and the last by Galen (A.D. 130-200) [12].

The classical description of inflammation accounts for the visual changes seen. The sensation of heat is caused by the increased movement of blood through dilated vessels into the environmentally cooled extremities. Redness is due to the additional number of erythrocytes passing through the area. Swelling (edema) is the result of increased passage of fluid from dilated and permeable blood vessels into the surrounding tissues, infiltration of cells into the damaged area, and in prolonged inflammatory responses deposition of connective tissue. Pain is due to the direct effects of mediators, either from initial damage or that resulting of sensory nerves due to oedema. Loss of function refers to either simple loss of mobility in a joint, due tothe oedema and pain, or to the replacement of functional cells with scar tissue.

Inflammatory process has two phases: acute and chronic. Acute and chronic inflammations are known to be complicated processes induced by several different classes of chemical mediators, e.g. prostaglandins, leukotrienes and platelet-activating factor, etc. Anti inflammatory agents exert their effect through a spectrum of different modes of action. [13]

Acute inflammatory response is characterized by an increase in vascular permeability and cellular infiltration leading to oedema formation as a result of extravasation of fluid and proteins and accumulation of leukocytes at the inflammatory site for short time [14].

Chronic inflammation is the reaction arising when the acute response is insufficient to eliminate the pro-inflammatory agents. Chronic inflammation includes a proliferation of fibroblasts and infiltration of neutrophils with exudation of fluid. It occurs by means of development of proliferative cells which can either spread or form granuloma. Chronic inflammation may also occur due to the persistence of infection or antigen, recurring tissue injury, or a failure of endogenous anti-inflammatory mechanisms.

Chronic (or acute) inflammation is a multiple process mediated by activating inflammatory or immune cells[15], among which macrophages play a central role in managing many different immunopathological phenomena including the overproduction of proinflammatory cytokines and inflammatory mediators, generated by activated COX-2. Under inflammatory conditions, immune cells are also stimulated by adhesion molecule activation signals in order to enhance the migration capacity to inflamed tissue and finally to form heterotypic cell clustering between the immune cells, endothelial cells and inflamed cells.

Macrophages in the inflammatory reaction initially requires an interaction between surface receptors such as Toll-like receptors (TLR) and stimuli, and subsequent up-regulation of intracellular signalling events mediated by enzymes such as phosphoinositide 3-kinases (PI3K) and mitogen activated protein kinases (MAPKs) as well as transcription factors (e.g., nuclear factor [NF]-κB and activator protein [AP]-1) (Sekine et al., 2006). Overall, these events lead macrophages to express pro-inflammatory genes such as inducible NO synthase (iNOS) and cyclooxygenase (COX)-2. Because large amounts of macrophage-derived inflammatory mediators can cause collateral or severe damage such as septic shock, rheumatoid arthritis and arteriosclerosis, the effective blockade of these inflammatory responses is an important therapeutic target. Inflammatory diseases are a major cause of morbidity of the work force throughout the world. These have been called the "King of Human Miseries".

Inflammatory diseases

Inflammation is a physiological response of a body to stimuli, including infections and tissue injury. However, excessive or persistent inflammation causes a variety of pathological conditions. As the primary interface between the body and the external environment, the skin provides the first line of defense against traumatic injury and invasion by microbial pathogens. In addition to its properties as a physical barrier, the skin has many active defence mechanisms and regulation of these mechanisms is crucial, as inappropriate or misdirected immune activity is implicated in the pathogenesis of a large variety of inflammatory skin disorders.

Many degenerative diseases such as rheumatoid arthritis, shoulder tendonitis, gouty arthritis, polymyalgia rheumatica, heart disease, asthma, and inflammatory bowel disease are often associated with inflammatory processes.

Standard drugs for inflammation and side effects

Many steroids, specifically glucocorticoids and Mineralocorticoids reduce inflammation or swelling by binding to corticoid receptors. These drugs are often referred to as corticosteroids. Long-term corticosteroids use has several severe side effects eg. hyperglycemia, insulin resistance, diabetes mellitus, osteoporosis, anxiety effects etc.

There are over 50 different NSAIDs available and they can be divided into different groups based on their chemical structure, pharmacokinetics and selectivity towards Cox-1 or Cox-2. NSAIDs can be classified broadly into two types based on their chemical structure. Most NSAIDs are carboxylic acids; but a few, most noticeably phenylbutazones, are enolic acids. Carboxylic acid containing drugs include salicylate derivatives (eg. aspirin), carbocyclic and hetrocyclic acid derivatives (eg. indomethacin), fenamic acid derivatives (eg. Ibuprofen, ketoprofen, fenbufen, flurbiprofen, suprofen and naproxen) and phenyl acetic acid derivatives (eg. diclofenac, aceclofenac, etc.). Enolic acid containing drugs include oxicam derivatives (eg. piroxicam, tenoxicam and meloxicam) and pyrazoles (eg. phenylbutazone and oxyphenbutazone). Non acidic group compounds include nabumenton.

Most of the NSAIDs have three major types of action:

1) Anti-inflammatory action for treating several conditions including rheumatoid arthritis, osteoarthritis, musculoskeletal disorders and pericarditis.

2) Analgesia for treating pain of mild to moderate intensity. Their maximum therapeutic efficiency is much lower than that of the opioids, but they do not cause dependence.

3) Antipyretic action, which mediates by the release of endogenous pyrogen from monocytes and macrophages in the presence of infection or inflammation.

Non-steroidal anti-inflammatory drugs (NSAIDs) typically relieve inflammation and associated pain by inhibiting cyclooxygenase enzymes involved in the production of prostaglandins. These enzymes exist in two isoforms (COX-1 and COX-2) coded by distinct genes on different chromosomes.

NSAIDs can cause liver damage, renal failure, aseptic meningitis and can interfere with bone fracture healing. NSAID use is associated with a high risk of upper gastrointestinal symptoms and lesions such as oesophagitis, gastritis, peptic ulcers, and their severe complications including bleeding and perforation and results mostly from inhibition of Cox-1 in the gastric mucosa.

Diclofenac reduces inflammation, swelling and arthritic pain by inhibiting prostaglandins synthesis and/or production [29]. Diclofenac has also been reported to suppress inflammation induced by various phlogistic agents in experimental animal models. However, it may cause side effects including gastrointestinal disorders when administered by oral route and cutaneous lesions by intramuscular injection. There are several published reports of cases of diclofenac-associated hepatotoxicity.

MATERIALS AND METHODS

The designing of methodology involves a series of steps taken in a systematic way in order to achieve the set goal(s) under the prescribed guidelines and recommendations. It includes in it all the steps from field trip to the observation including selection and collection of the medicinal plant, selection of dose value, standardization of protocol, usage of instruments, preparation of reagents, selection of specific solvents for extraction, formation of protocols and final execution of the standardized protocol. All this requires good build of mind and a good and soft technical hand to handle the materials and procedure in a true scientific manner.

DRUGS AND CHEMICALS

Drugs and Chemicals used in this study were of analytical grade and of highest purity procured from standard commercial sources in India.

EXPERIMENTAL ANIMALS

Healthy adult albino wistar rats weighing 200-250grams of either sex were selected for the study. Animals were housed in appropriate cages in uniform hygienic conditions and fed with standard pellet diet (Amrul Laboratory Animal Diet) and water ad libitum. They were fasted overnight before the day of experiment, after 72hours of fasting from the day of Alloxan introduction. Animals were housed within the departmental animal house and the room temperature was maintained at 27° C. Animal studies had approval of IAEC.

PLANT MATERIAL COLLECTION

The roots of *Holoptelea integrifolia* was collected from the local market. The plant root material was cleaned, reduced to small fragments, air dried under shade at room temperature and coarsely powdered in a mixer. The powdered material was stored or taken up for extraction process.

PREPARATION OF PLANT EXTRACTS

Preparation of ethanolic Extract

Fresh leaves of *Holoptelea integrifolia* were collected and washed under tap water. The leaf extract used was prepared by taking 50gms of finely cut roots into 250ml beaker containing 200ml of ethanol. The contents were mixed well and then the mixture was boiled up to 50-60^{0}C for 4-5hrs. Further the extract was filtered with whatmann filter paper. The filtrate was boiled until the concentrated residue is formed. The concentrated product was sealed in sample covers and stored under room temperature and used for further experiment to check the activities.

SELECTION OF DOSE FOR ANIMAL STUDY

The dose considered for the experiment on rats was obtained from conversion of human dose of *Holoptelea integrifolia* (3-5 g/kg). The conversion factor of human dose (per 200 g body weight) is 0.018 for rats. Hence the calculated dose for the rats (considering human dose 5 g/kg) is 100 and 200 mg/kg. Acute toxicity was done at dose of 2000 mg/kg body weight.

PHARMACOLOGICAL EVALUATION

Preparation of extracts

The ethanolic extracts of *Holoptelea integrifolia* suspended in water in presence of 3%v/v Tween-20 solution. All the drugs were administered orally for experimental purpose. Each time preparations of the extracts were prepared when required. The drugs were administered at a constant volume of 10ml/kg for each animal.

ACUTE ORAL TOXICITY

The acute oral toxicity of ethanolic extracts of *Holoptelea integrifolia* was determined by using Albino Wistar rats (200-250g) which were maintained under standard conditions. The animals were fasted 12 hour prior to the experiment, up and down procedure OECD guideline no. 425 were adopted for toxicity studies. Animals were administered with single dose of individual extract up to 2000mg/kg and observed for its mortality during 2days and 7days study period (short term) toxicity and observed up to 7days for their mortality, behavioral and neurological profiles.

ASSESSMENT OF ANALGESIC ACTIVITY

Table 1: Group Classification

Group	Treatment	Dose(mg/kg)
Group 1	Normal control received distilled water	------
Group 2	Standard group received Diclofenac	10ml/kg
Group 3	Ethanolic extract of *Holoptelea integrifolia*	100
Group 4	Ethanolic extract of *Holoptelea integrifolia*	200

- **HOT-PLATE METHOD**

Animals were grouped and divided randomly into four groups of four and each was fasted to overnight. Take the basal reaction-time by observing hind paw licking or jumping response in animals when placed on the hot-plate maintained at constant temperature (55^{0}C). Normally animals showed such response in 6-8 sec. a cut-off period of 15sec i.e observed to avoid damage to the paws. Inject the standard drug to group-2 and test drugs to group-3&4 and noted the reaction time of animals on the hot plate at 15, 30, 60 and 120min after the drug administration. Calculated the percentage increase in reaction time at each time interval.

➢ **TAIL-FLICK METHOD**

Animals were grouped and divided randomly into four groups of four and each was fasted to overnight. The animals were screened for sensitivity test by placing the tip of the tail on the radiant heat source. Any animals that held to withdraw its tail in 5 second was rejected from the study. Analgesia was assessed with a tail flick apparatus (Analgesiometer). The basal reaction time was measured initially and another set of four measures were taken as 15, 30, 45 and 60 minutes interval and the reaction of the animals considered as the post – drug reaction time. A cut-off period of 10sec. was observed to prevents tissue damage of the tail of the animals.

ASSESSMENT OF ANTI-INFLAMMATORY ACTIVITY

Table 2: Group Classification

Group	Treatment	Dose(mg/kg)
Group 1	Normal control received distilled water	------
Group 2	Standard group received Indomethacin	10ml/kg
Group 3	Ethanolic extract of *Holoptelea integrifolia*	100
Group 4	Ethanolic extract of *Holoptelea integrifolia*	200

➢ **CARRAGEENAN-INDUCED PAW EDEMA METHOD**

Carrageenan-induced paw edema is a suitable experimental animal model for evaluating an anti edematous effect. Edema developed following injection of carrageenan serves as an index of acute inflammatory changes, was and can be determined from differences in the paw volume measured immediately after carrageenan injection and then every hour for 6 hours. Edema induced by carrageenan is believed to be biphasic: the first phase (1 h) involves the release of serotonin and histamine and the second phase (over 1 h) is mediated by prostaglandins, cyclooxygenase products. Continuity between the two phases is provided by kinins.

The anti-inflammatory activity was determined using a carrageenan-induced paw edema model, Sixty Sprague-Dawley rats (200-240 g) either sex, were randomly divided into 4 groups and fasted overnight before the experiment with free access to water. Treatments administered at their body weight to rats for one hour before subcutaneous injection of carrageenan (1% in NSS) into the plantar surface of the left hind paw.

After the carrageenan injection, the paw volumes were measured at 15, 30, 60&120min using a Plethysmometer (Dolphin, India). The difference between the intial and subsequent readings gave the actual edema volume Edema was expressed as the mean increase in paw volume relative to control animals. The percentage inhibition of edema was calculated by the following equation:

% inhibition of edema = 100 (1-Vt/Vc),

Where
Vc is the edema volume in the control group and
Vt is the edema volume in tested group.

➢ **FORMALDEHYDE INDUCED PAW EDEMA METHOD**

Inflammation was induced by injection of 0.1ml of freshly prepared Formaldehyde (3%) underneath the plantar tissue of right hind paw. the test drug was administered consecutively for seven days to all groups. on seventh day, after 1h of drug administration, paw edema of the rat was induced by subplantar injection of formaldehyde solution. The paw volume was determined at 0h and at 3, 6, 24 and 48h after formaldehyde injection.

STATISTICAL ANALYSIS

The values were expressed as mean ± SEM data was analyzed using one-way ANOVA followed by T-test. Two sets of comparison had made. i.e. 1. Normal control Vs All treated groups. Differences between groups were considered significant at $P<0.001$ and $P < 0.05$ levels.

RESULTS

a ACUTE TOXICITY STUDIES

Acute toxicity studies revealed that the ethanolic extracts of *Holoptelea integrifolia* were safe up to 1000 mg/kg of body weight and approximate LD 50 is more than 2000 mg/kg. No lethality or any toxic reactions was observed up to the end of the study period.

EVALUATION OF ANALGESIC ACTIVITY
HOT PLATE METHOD

The analgesic activity of EEHI was assessed using Hot plate method in Swiss albino rats were illustrated in Table. EEHI showed significant analgesic activity at 100 and 200 mg/kg, i.p. Analgesic activity was comparable with the standard drug Diclofenac. Both doses has showed maximum analgesic activity at reaction time is 12 and 5 sec respectively and the standard drug Diclofenac reaction time is 10 sec.

Table 3: Effect of extracts of *Holoptelea integrifolia* on Analgesic activity.

Groups	Dose	Basal reaction time(sec)		After drug administration(sec)							
		0min		15min		30min		60min		120min	
		Paw licking(P)	Jumping(J)	P	J	P	J	P	J	P	J
Control	10	-	9	4	6	5	3	3	-	5	-
Standard	40	9	-	10	-	6	-	14	-	-	10
EEHI	100	14	-	5	-	12	-	8	-	12	-
EEHI	200	12	10	4	10	-	10	-	12	-	5

The results are expressed as means ± S.E.M Differences in mean values between groups were analyzed by a one-way analysis of variance (ANOVA). Statistical significance was assessed as $p < 0.05$.

TAIL-FLICK METHOD

The analgesic activity of EEHI was assessed using Tail-Flick method in Swiss albino rats were illustrated in Table. EEHI showed significant analgesic activity at 100 and 200 mg/kg, i.p. Analgesic activity was comparable with the standard drug Diclofenac. Both two doses showed maximum analgesic activity at reaction time is 14 and 12 sec respectively and the standard drug Diclofenac reaction time is 13 sec.

S.No	Treatment	Dose (mg/kg)	Reaction Time (sec)				
			30min	45min	60min	75min	90min
1.	control	-	6.43±0.13	6.35±0.10	6.20±0.09	6.32±0.09	6.25±0.06
2.	standard	10	9.78±0.14	11.62±0.08	13.52±0.13	13.42±0.05	11.72±0.08
3.	EEHI	100	9.45±0.10	9.64±0.15	12.65±0.07	14.25±0.05	12.75±0.04
4.	EEHI	200	9.64±0.06	9.86±0.12	10.04±0.14	12.67±0.03	10.75±013

The results are expressed as means ± S.E.M Differences in mean values between groups were analyzed by a one-way analysis of variance (ANOVA). Statistical significance was assessed as $p < 0.05$.

EVALUATION OF ANTI INFLAMMATORY ACTIVITY
CARRAGENAN INDUCED PAW OEDEMA IN RATS

In carragenan induced paw oedema activity, the paw volumes and percentage of inhibition of the control, standard and test compounds are shown in Table No: ---. The tests compounds are compared with Diclofenac as a standard at a dose of 40mg/kg for anti-inflammatory activity. Presently Diclofenac showed 20% inhibition of inflammation at 2 hours when compared to control.

Ethanolic extracts of *Holoptelea integrifolia* roots (100 and 200 mg/kg) shown significant inhibition of inflammation with 30% and 10% respectively at 2 hours when compared with control. The results of test compounds were found to be statistically significant at value $P<0.05$.

Table 4: Effect of extracts of *Holoptelea integrifolia* on paw edema volume

GROUPS	Dose (mg/kg)	Change in paw volume (ml) mean±SEM & Percentage inhibition									
		0min		15min		30 min		60 min		120min	
		R	L	R	L	R	L	R	L	R	L
Control	--	0.2±0.1	0.3±0.2	0.2±0.1	0.5±0.3	0.2±0.1	0.3±0.2	0.2±0.1	0.3±0.1	0.2±0.1	0.3±0.2
Std (Diclofenac Sodium)	10	0.2±0.1	0.3±0.2	0.2±0.1	0.4±0.2	0.2±0.1	0.3±0.1	0.2±0.1	0.3±0.1	0.2±0.1	0.2±0.1
EEHI	100	0.3±0.1	0.5±0.2	0.3±0.2	0.5±0.2	0.2±0.1	0.4±0.2	0.1±0.2	0.3±0.1	0.2±0.1	0.2±0.1
EEHI	200	0.2±0.1	0.3±0.1	0.2±0.1	0.3±0.2	-	0.2±0.1	0.2±0.1	0.2±0.1	0.1±0.2	0.2±0.1

The results are expressed as means ± S.E.M Differences in mean values between groups were analyzed by a one-way analysis of variance (ANOVA). Statistical significance was assessed as $p < 0.05$.

FORMALIN INDUCED PAW EDEMA

The results of anti-inflammatory activity of ethanolic extract of *Holoptelea integrifolia* Leaves in formaldehyde induced paw edema is shown in table. Injection of formaldehyde subcutaneously into hind paw of rats produces localized inflammation. The administration of EEAR-100 and 200mg and Indomethacin-10mg for 7days successfully inhibited edema induced by formaldehyde. EEAR-100 and 200 group showed maximum decrease in paw volume at 3h ($P<0.05$ and $P<0.01$) and decreased in paw volume at 48h.

Table 5: Anti-inflammatory activity of ethanolic extract of *Holoptelea integrifolia* roots in formaldehyde *induced rat paw edema.*

S.No	Treatment	Dose Mg/kg	% increase in paw volume					
			After 3h		After 24h		After 48h	
			Vol increase	% change	Vol increase	% change	Vol increase	% change
1.	Control	--	4.12±0.06	-	4.4±0.04	-	5.0±0.02	-
2.	Standard	10	2.59±0.03	56.4%	2.43	55.2%	2.12	42.4%
3.	EEHI	100	2.01±0.03	48.7%	2.21	50.2%	2.13	42.6%
4.	EEHI	200	2.33±0.03	56.5%	2.24	50.9%	2.02	40.4%

DISCUSSION

ANALGESIC ACTIVITY

HOTPLATE METHOD

The extracts increased reaction latency to thermal pain induced by the hot plate method in rats, which is a specific central antinociceptive test. Inhibition of histamine or kinin pathway may reduce pain. The results of the present study also showed that extract exhibited a comparable magnitude of antinociceptive activity in hot plate method of pain which suggested that the phytochemical constituents are responsible for the analgesic effect. The results of the present study indicated that the ethanolic extracts of *Holoptelea integrifolia* might contain constituents capable of relieving or modifying responses to pain caused by either thermal or chemical stimulation of the nociceptors mediated by both central and peripheral mechanisms.

TAIL FLICKMETHOD

The extracts showed significant analgesic activity at all tested dose levels. In tail flick method, the ethanolic extracts of *Holoptelea integrifolia* leaves at a dose of 100 and 200 mg/kg showed significant activity. The results showed significant analgesic activity against thermal stimuli. The analgesic studies revealed that the methanolic extract of *Holoptelea integrifolia* leaves exhibited potent analgesic (central analgesic activity) effect against thermal noxious stimuli and also revealed that the extract shows dose dependent analgesic effect.

ANTI-INFLAMMATORY ACTIVITY

CARRAGEENAN INDUCED RAT PAW EDEMA MODEL

It is believed that current anti-inflammatory drugs such as opioids and non-steroidal anti-inflammatory drugs are not useful in all cases because of their side effects and low potency. As a result, search for other alternatives became necessary and imperative. Therefore, the present study was aimed at evaluating the scientific basis for the traditional use of *Holoptelea integrifolia* leaves using carragenan induced rat paw edema for anti-inflammatory models.

Carrageenan has been widely used as a harmful agent able to induce experimental inflammation for the screening of compounds possessing anti-inflammatory activity. Carrageenan induced rat paw edema is a suitable model to predict the value of anti-inflammatory agents, which act by inhibiting the mediators of acute inflammation. Carrageenan-induced hind paw edema in rat is a biphasic event. The early phase (90 - 180 min) of the inflammation is due to the release of histamine, serotonin and similar substances; and the later phase (270–360 min) is associated with the activation of kinin-like substances, i.e., prostaglandins, proteases and lysosome. The aqueous and alcoholic extracts of *Holoptelea integrifolia* leaves inhibited the carrageenan induced rat paw edema formation, at both early and later phase. This result tends to suggest that the inhibitory effect of the extract on edema formation is probably due to the inhibition of the synthesis and/or release of the inflammatory mediators, especially the cyclooxygenase products.

FORMALIN INDUCED RAT PAW EDEMA MODEL

Formalin induced paw edema in rats is one of the most suitable test procedure to screen the acute inflammation and it is believed to be a biphasic event. The anti inflammatory effects of triterpenes have been attributed to various mechanisms including inhibition of lipoxygenase and cycloxygenase activities.

Lipid peroxidation has been implicated in the pathogenesis of various diseases including arthritis. It is well established that bioenzymes are very much susceptible to LPO, which is considered to be the starting point of many toxic as well as degenerative processes. LPO level was increased during inflammation. Administration of formalin produced an elevated level of LPO, which may due to the free radicals and is responsible for damaging cell membranes there by further intensifying inflammatory damage. The inflammatory tissue damages could be due to the liberation of reactive oxygen species from phagocytes invading the inflammation sites. Hence, in the present study, the concentration of LPO was found to be higher in formalin induced rats. On treatment with the *Holoptelea integrifolia* at the dose level of 100, 200 mg/kg bw, the LPO level was significantly decreased.

Formalin induction causes the changes in connective tissue metabolism, is one of the major biochemical events during the process of inflammation. These changes are effected in the alteration of relative composition of various constituents of connective tissue such as muco polysaccharides, glyco protein, hexosamine and hydroxy proline, sialic acid. Hence the levels of hexosamine and hydroxyproline were found to be higher in formalin induced rats. Pretreatment of *Holoptelea integrifolia* inhibited the accmulation of hydyoxy proline and hexosamine in edematous tissue of formalin induced rats.

ANTI-INFLAMMATORY ACTIVITY

In the present study it has concluded that the ethanolic extracts of *Holoptelea integrifolia* have anti-inflammatory activity in carragenan-induced and Formalin induced paw edema in rats. This extracts has showed that decrease in paw edema volume when compared to control and standard drugs. Therefore the anti-inflammatory effects observed in this study may be due to the activity of one or a combination of some of the identified constituents. It may suggest that the inhibitory effect of the constituents in the extract on edema formation is probably due to inhibition of the synthesis and/or release of the inflammatory mediators, especially the cyclooxygenase products. The carragenen induced paw edema test is effectively controlled with the arachidonate COX inhibitors due to its COX-dependent mechanism.

ANALGESIC ACTIVITY

In the Present study it has concluded that ethanolic extracts of *Holoptelea integrifolia* have an analgesic activity in both Hotplate and Tail-flick method in rats. This extracts has showed that increase mean latency to thermal pain. The presence of some chemical constituents contains a capable of relieving or modifying responses to pain caused by inhibition of histamine or kinnin pathway.

CONCLUSION

Holoptelea integrifolia is a plant and it has anti-diabetic, anti-bacterial, anti-microbial, anti-fungal, anti-oxidant and CNS activities. Among these studies it could be concluded that roots of *Holoptelea integrifolia* have shown great potential of anti-inflammatory and Analgesic activity. Awareness of local community should be enhanced incorporating the traditional knowledge with scientific drugs.

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