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Original Article

PHARMACOLOGICAL EVALUATION OF ANTI-INFLAMMATORY AND ANALGESIC POTENTIAL OF *LITCHI CHINENSIS GAERTN.*(SONN.)

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ABSTRACT

Objective: *Litchi chinensis Gaertn.* is a tropical fruit tree used traditionally for several ailments including inflammatory and painful disorders. This study was designed to investigate the potential of hydroalcohol extract of *Litchi chinensis Gaertn.* leaves (HLCL) for anti-inflammatory and analgesic activity in laboratory animals.

Methods: Hydro-alcoholic extract of leaves were sequentially prepared by Soxhlet apparatus and subjected to preliminary phytochemical screening. The anti-inflammatory activity was evaluated by employing carrageenan induced paw edema in rats and analgesic activity was evaluated using acetic acid induced writhing test and hot plate method in mice.

Results: The preliminary phytochemical analysis of extracts indicated the presence of terpenoids, flavonoids, phenols, tannins, saponins. Oral administration of 100, 200 and 400 mg/kg of HLCL exhibited a significant anti-inflammatory activity and maximum effect was observed after 4 hour of carrageenan administration. The percentage inhibition of glacial acetic acid induced writhing was found to be 11%, 26.3%, 45% at 100, 200 and 400 mg/kg dose respectively. Similar results were observed in hot plate model indicating the analgesic activity.

Conclusion: The results showed that HA extract of *Litchi chinensis Gaertn*. produced significant anti-inflammatory and analgesic activity in rats and mice respectively.

Keywords: *Litchi chinensis Gaertn.* leaves, Anti-inflammatory, Analgesic activity.

INTRODUCTION

Inflammation is the pathophysiological response of the body to tissue injury caused by physical trauma, noxious chemicals or microbiological agents which are responsible for leading accumulation of plasma fluid and blood cells locally [1]. Important components of inflammation are oedema formation, leukocyte infiltration and granuloma formation [2]. Inflammation is triggered by release of chemical mediators from the injured tissue and migrating cells. The specific chemical mediators vary with the type of inflammatory process and include amines such as histamine, serotonin, lipids such as prostaglandins and small peptides such as kinins [3]. Conventionally anti-inflammatory agents, namely glucocorticosteroids and non-steroidal anti inflammatory drugs (NSAIDs) are used in treatment of most of the acute and chronic pain and inflammatory disorders. Due to adverse side effects, like gastric lesions, caused by NSAIDs and tolerance and dependence induced by opiates, the use of these drugs has not been successful in all the cases. Therefore, alternatives to NSAIDs and opiates are being searched all over the world.

Medicinal plants have been used in folk medicine for treatment of many inflammatory diseases since ages with lesser side effects. Plants contain many useful constituents that might provide a direction for the development of novel drugs [4]. The litchi (*Litchi chinensis Gaertn.*, Sapindaceae) is a tropical fruit tree that originates from southern China and is cultivated in semi-tropical areas world-wide for the delicious taste of the fruit [5]. Litchi seeds have been analyzed and were found to possess rich amounts of polyphenols and exhibit strong antioxidant and inflammatory activities [6, 7]. Litchi pericarp contains significant amounts of flavonoids and anthocyanins, including procyanidin B_2, B_4, epicatechin, cyanidin-3-retinoside, cyanidin-3-glucoside, quercetin-3-retinoside and quercetin-3-glucoside, *etc* [8]. These compounds carry high free radical scavenging properties and could be used as anti-inflammatory, anti-oxidant or anti-cancer agents [9, 10]. Studies reported that litchi seed water extract increase insulin sensitivity and reduce the concentrations of blood fasting glucose, triglyceride, leptin and tumor-necrosis factor in a type-2 diabetes mellitus rat model [11]. Therefore, the present investigation was undertaken to demonstrate the anti-inflammatory and analgesic activities of hydro-alcoholic extract of *Litchi chinensis Gaertn.* leaves (HLCL).

MATERIALS AND METHODS

Plant Material

Leaves of *Litchi chinensis Gaertn.* were collected in the month of October 2013 from local area of Saharanpur (U.P). and authenticated by Dr. Sunita Garg, Head Raw Material Herbarium and Museum Delhi, National Institute of Science Communication and Information Resources (specimen no: NISCAIR/RHMD/Consult/2013/2352-132-3.) The leaves were dried in shade, powdered and stored for the further studies.

Preparation of Extract

The coarse powder of *Litchi chinensis* leaves was then sequentially extracted with petroleum ether, chloroform and hydro-alcohol (60%) in a Soxhlet apparatus. Finally, the hydro-alcoholic (HA) extract was filtered, concentrated and stored in a desiccator.

Drugs and chemicals

All the chemicals and reagents used were of analytical grade. Petroleum ether, chloroform and ethanol were used for extraction procedure. Carrageenan (Sigma-Aldrich), diclofenac sodium, (obtained as gift sample from Hindustan Pharmaceuticals) and Pentazocine (obtained as gift sample from Ind-swift) were used as standard drugs for anti-inflammatory and analgesic activity. Other reagents such as acetic acid (Qualikems Fine Chemicals Pvt. Ltd.) were used.

Phytochemical Screening

The preliminary phytochemical investigations were performed for various phytoconstituents such as alkaloids, flavonoids, tannins, terpenoids, saponin, and steroids etc which are probably responsible for the activity of the extract [12-14].

Animals

Wistar albino rats (150-170 g) and Swiss albino mice (20-25 g) of either sex were used in the study. Normal chow diet and water *ad libitum* was provided to the animals and were kept in animal house according to CPCSEA guidelines. The protocol was duly approved by IAEC (MMCP/IAEC/13/35).

Dose preparation

Different doses 100mg/kg, 200mg/kg and 400mg/kg of HA extract were prepared by triturating with 0.25% CMC.

Anti-Inflammatory Activity

Animals were divided into six groups. Each group consists of six animals. The anti-inflammatory effect of HLCL was assessed by employing carrageenan induced paw oedema in rats [15]. The different groups of rats were pre-treated with their respective doses. After 1 hour, oedema was induced by administration of 0.1 ml of 1% carrageenan suspension into sub-plantar region of left hind paw of each rat and paw volume was measured by Plethysmometer (Medicaid Systems, Chandigarh) at 0, 1, 2, 3, 4 hr. Mean ± SEM for treated and control animals is calculated and compared for each time interval and statistically analyzed.

The edema inhibitory activity was calculated according to the following formula:

$$\% I = \frac{Ec - Et}{Ec} \times 100$$

E_c = Edema rate of control group at particular hour.

E_t = Edema rate in test group at particular hour.

Group I: Normal control

Group II: Inflammation control group received vehicle (0.25% Carboxymethylcellulose)

Group III: Standard group treated with Diclofenac sodium (100 mg/kg *p.o.*)

Group IV: Inflammation + hydroalcohol extract of LC leaves (100 mg/kg *p.o.*)

Group V: Inflammation + hydroalcohol extract of LC leaves (200 mg/kg *p.o.*)

Group VI: Inflammation + hydroalcohol extract of LC leaves (400 mg/kg *p.o.*)

Analgesic activity

Hot Plate Method

Animals were divided into five groups. Each group consists of six animals. This method was used to measure for central analgesic activity described by Eddy [16]. Different doses of extract and standard were administered to the mice. After 30, 60, 90 and 120 min animals were kept individually on hot plate at a constant temperature of 55 ± 2°C. The reaction time was recorded as the time taken by the animals to lick the fore limb or hind paw or jump out of the plate.

Percentage analgesia was calculated using the following formula:

$$\% \text{ analgesia} = MPE = (TL-BL)/(ML-BL) \times 100$$

Where MPE = Maximum possible effect

ML = Maximum latency or cut off time TL = Test latency, BL = Basal latency or control latency

Group I: Normal control (0.25% Carboxymethylcellulose).

Group II: Standard group treated with Pentazocine (2 mg/kg *i.p.*)

Group III: Stimulus + hydroalcohol extract of LC leaves (100 mg/kg *p.o.*)

Group IV: Stimulus + hydroalcohol extract of LC leaves (200 mg/kg *p.o.*)

Group V: Stimulus + hydroalcohol extract of LC leaves (400 mg/kg *p.o.*)

Acetic acid-induced writhing

Animals were divided into five groups. Each group consists of six animals. For peripheral analgesic activity, the writhing test was performed [17]. Different groups of animals were pretreated with oral dose of the HA extract, standard and normal control. After sixty minutes 0.6% acetic acid (10 ml/kg b. w.) was injected *i.p.* to each mouse and then, placed in an observation box and the number of abdominal contractions were counted for each animal for a period of 10 minutes after 15 mintutes of acetic acid injection.

Then percentage inhibition of writhing was calculated.

$$\% \text{ Inhibition} = \frac{\text{Number of writhes in control group - number of writhes in drug treated group}}{\text{Number of writhes in control group}} \times 100$$

Group I: Normal control (0.25% Carboxymethylcellulose).
Group II: Standard group treated with Diclofenac sodium (100 mg/kg *p.o.*).
Group III: Acetic acid + hydroalcohol extract of LC leaves (100 mg/kg *p.o.*)
Group IV: Acetic acid + hydroalcohol extract of LC leaves (200 mg/kg *p.o.*)
Group V: Acetic acid + hydroalcohol extract of LC leaves (400 mg/kg *p.o.*)

Statistical analysis

The results were expressed as mean ± S.E.M. The data obtained from various groups were statistically analyzed using one-way ANOVA followed by Tukey's test. The *p*-value < 0.05 was considered to be statistically significant.

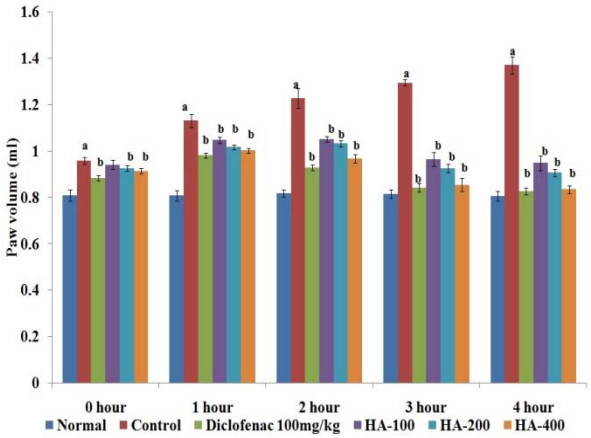
RESULTS

Phytochemical Screening

The preliminary phytochemical investigations with the extract revealed the presence of flavonoids, terpenoids, tannins, phenolic compounds and saponins.

Acute anti-inflammatory activity

Different doses of 100, 200 and 400 mg/kg significantly inhibited the oedema to the extent of 25.58, 28.68 and 33.7 % at 3 h and 31.38, 34.30 and 39.05 % at 4 h respectively. Diclofenac sodium reduced the inflammation by 34.08% at 3 h and 40.14 % at 4 h. HLCL significantly reduced carrageenan induced paw oedema in a dose dependant manner **(Fig. 1)**.

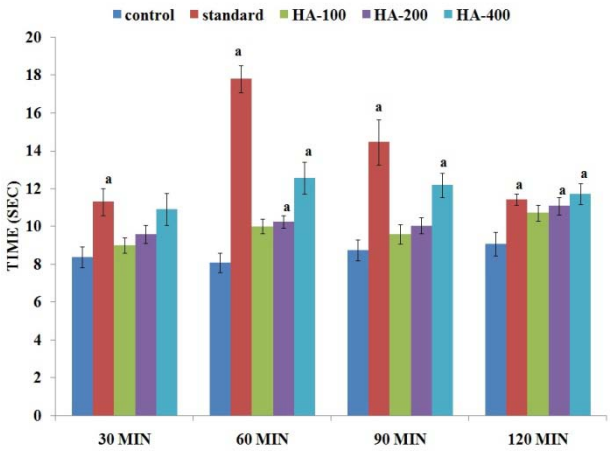


Standard – Diclofenac sodium, HA – Hydro-alcoholic extract

Fig.1: It shows the effect of HA extract of *Litchi chinensis Gaertn. leaves* on carrageenan induced paw edema in rats. The data obtained from various groups were statistically analyzed using one-way ANOVA followed by Tukey's Multiple Range Test. *p*<0.05, is considered statistically significant. a: vs normal; b: vs control

Analgesic Activity

In hot plate method, the different doses of HLCL and pentazocine significantly increases the reaction time *i.e.* paw licking and jumping response as compared to the control at different time intervals. The maximum analgesic activity was shown after 60 minutes of dose administration. In this model, higher dose 400 mg/kg of the extract exhibited better analgesic activity after 60 min. of dose administration. **(Fig. 2)**

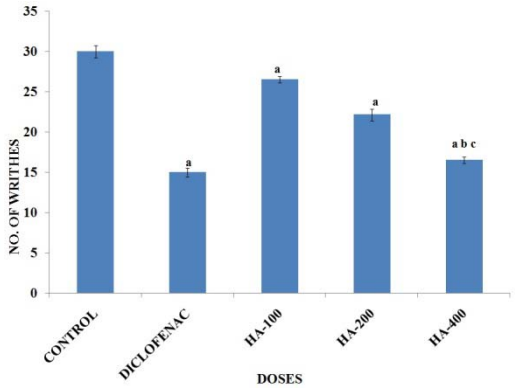


Standard- Pentazocine, HA – Hydroalcohol extract

Fig. 2: It shows the effect of HA extract of *Litchi chinensis Gaertn. leaves* in Hot Plate Test. The data obtained from various groups were statistically analyzed using one-way ANOVA followed by Tukey's Multiple Range Test. $p<0.05$, is considered statistically significant. a: vs control.

Acetic acid induced writhing model

Different doses 100, 200 and 400 mg/kg of HLCL significantly reduced the number of writhing produced by the administration of acetic acid in the peritoneal cavity, i.e. 11.00, 26.13 and 45.00 % respectively, when compared to control. Diclofenac sodium shows the maximum 50 % inhibition. (**Fig. 3**).



HA – Hydroalcohol extract

Fig. 3: It shows the effect of HA extract of *Litchi chinensis Gaertn. leaves* in acetic acid induced writhing method.

The data obtained from various groups were statistically analyzed using one-way ANOVA followed by Tukey's Multiple Range Test. $p<0.05$, is considered statistically significant. a: vs control, b: vs HA-100 mg/kg, c: vs HA-200 mg/kg

DISCUSSION

Carrageenan induced rat paw oedema model is widely used animal model used for evaluation of novel anti-inflammatory compounds [18]. Inflammation induced by carrageenan is biphasic response. The early phase of the inflammation is due to the release of histamine, serotonin and similar substances; and the later phase is associated with the activation of kinin-like substances, i.e., prostaglandins, proteases and lysosome [19, 20]. *Litchi chinensis* fruit has a sweet odor and a delicious taste, and has been used in traditional medicine as a tonic for the liver, heart and brain [21]. *Litchi chinensis* has been reported to have anti-inflammatory [22], antioxidant [23] and antidiabetic [24] activities. Administration of different doses of HLCL significantly attenuated carrageenan induced paw oedema at different interval of time and maximum percentage of inhibition was observed at 4th hr. Preliminary phytochemical studies show the presence of flavonoids, saponins and terpenoids. It has one of the highest polyphenol contents of any fruit [25]. Flavonoids can normalize cellular activities of inflammation related cells i.e. mast cells, macrophages, neutrophils and lymphocytes. Studies showed that triterpenes can inhibit synthesis of several cytokines, prostaglandin E2 and nitric oxide in LPS-stimulated macrophages [26]. Other studies also suggest that saponins have a role in attenuating the expression of tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), and IL-6 pro-inflammatory cytokines [27].

The inflammatory cascade that usually precedes, and accompanies pain is transcriptional regulated by Nuclear factor (NF)-κB which is involved with mediating the release of inflammatory mediators [28-30], responsible for pain, hyperalgesia and nociception [31]. Pain is the most common symptom of injuries and diseases [32]. Nerve cell endings or receptors, commonly known as nociceptors, are central to pain sensation. Nociceptors relay information to the CNS thus indicating the location, nature and intensity of the ensuing pain.

Acetic acid induced writhing method is a widely employed inflammatory pain model for screening of peripherally acting analgesic drugs. Administration of glacial acetic acid (0.6%; 0.1 ml) into the peritoneal cavity of mice release various mediators like cytokine, histamine, serotonin, bradykinin etc. These mediators are responsible for the activation of peripheral nociceptors to induce writhing in animals [33-35]. Furthermore, acetic acid also induces prostaglandin biosynthesis which may be possibly involved in the mechanism of the writhing [36]. The doses of 100, 200, 400 mg/kg HLCL extract significantly reduced the number of writhing with percentage inhibition of 11.00, 26.13 and 45.00 % respectively and the possible mechanism for the activity is the inhibition of the biosynthesis of prostaglandin and other inflammatory mediators. Central analgesic activity through hot plate test possesses several advantages such as particularly the sensitivity to strong antinociceptive and limited tissue damage [37]. The HLCL extract also significantly delayed the latency time of animals to the heat stimulus in hot plate test at the higher dose of 400 mg/kg. Moreover, a number of flavonoids and tannins have been reported to produce analgesic activity mediated by central system [38]. So, the presence of phenolic compounds and tannins may be associated with analgesic activity of leaves of *Litchi chinensis Gaertn.* [39, 40].

CONCLUSION

The present study concludes that Hydro-alcoholic extract of leaves of *Litchi chinensis Gaertn.* possess potential anti-inflammatory and analgesic activity. Further studies could be undertaken to elucidate the exact mechanism of action by which extract exert their analgesic and anti-inflammatory effect. Bioactive constituents responsible for this activity need to be elucidated. Furthermore, pharmacological exploration is required to evaluate other activities of this potential plant.

CONFLICT OF INTERESTS

Declared None

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