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Research Article

SCIENTIFIC EVIDENCE OF *Lindernia crustacea* (L) F.MUELL, AN INDIGENOUS PLANT: A FOLKLORE MEDICINE USED TRADITIONALLY

Smriti Rekha Chanda Das [1]*, Abdul Baquee Ahmed [2], Dipankar Saha [3], Indranil Chanda [4]

[1]Department of Pharmaceutical Chemistry, Girijananda Chowdhury Institute of Pharmaceutical Science, Azara, Guwahati-17, Assam, India

[2]Department of Pharmaceutics, Girijananda Chowdhury Institute of Pharmaceutical Science, Azara, Guwahati-17, Assam, India

[3]Department of Pharmacology, Girijananda Chowdhury Institute of Pharmaceutical Science, Azara, Guwahati-17, Assam, India

[4]Assam Science and Technology University, Jalukbari, Guwahati-13, Assam, India

*Corresponding Author Email: das_smritirekha@rediffmail.com

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ABSTRACT

Lindernia crustacea (L) F.Muell is one of the commonest plants used as a medicinal plant in India, Indonesia and Malaysia. There is no scientific evidence on the pharmacological activity of this plant.The aim of this study is to determine anti-inflammatory, analgesic and antipyretic activities of various extracts of *Lindernia crustacea* using different models of rodents. The method of the study consisted of extraction, phytochemical screening, toxicity study, anti-inflammatory, analgesic and antipyretic activities. Flavonoid and phenolic compound were found to be present in all three extracts.The pharmacological screening results showed that all three extracts showed activities at the dose of 200mg/kg, but among the extracts, benzene extract showed more significant anti-inflammatory, analgesic and antipyretic activity. From the findings of the above study, it may be concluded that aerial part extracts of *Lindernia crustacea* possess significant anti-inflammatory, analgesic and antipyretic activities.

KEYWORDS: *Lindernia crustacea*; anti-inflammatory; analgesic; antipyretic; Indigenous Plant; Folklore Medicine

INTRODUCTION

The North Eastern state of India is a rich source of medicinal plants but many plants are yet to be explored scientifically. *Lindernia crustacea* (L.) F. Muell, belongs to the family Linderniaceae. This plant is an annual, diffusely branched growing 3.5-30.0 cm tall. The stem is greatly branched, the branches generally spreading. It is found throughout India in moist places like river beds, rice fields and open grassy places[1]. *Lindernia crustacea* is also a popular and useful ethnomedicinal plant has been traditionally used throughout the world. It is one of the commonest plants used as a medicinal plant in Indonesia and Malaysia. Traditionally this plant is used to treat ear ache[2], injury, fever and thrush[3], anti-inflammatory for skin disorder to relieve itching, boils, sores, dysentery, ringworm [4,5], in post-partum women[6], in navel infection and also a decoction of the leaves is used as medicine after childbirth[7]. *Lindernia crustacea* is one of such plant which is commonly available in India and used traditionally[5], but there is no scientific investigation report available in view of pharmacological screening and phytochemical studies of this plant so far to best of our knowledge.

Natural sources, the molecular architect, offer a great resource for drug research. Several plant species are traditionally used as medicine. Herbal medicines are experiencing greater importance as many people are shifting their attention from modern drugs to herbal medicine, due to the side effects of modern drugs[8]. Looking for therapeutic agents from herbal plants, there is an increased approach presently in examining herbs for their traditional claims.

Traditional claims of medicinal importance of *Lindernia crustacea* is yet to be scientifically explored. In view of the traditional importance, it was planned to evaluate anti-inflammatory, analgesic and antipyretic activities of various extracts of *Lindernia crustacea* using different models of rodents. It is expected that the results of the present research work would be beneficial in establishing scientific reason behind the traditional use of *Lindernia crustacea* in relief of pain and fever.

MATERIALS AND METHODS

Indomethacin (Sigma-Aldrich, India), aspirin(Sigma-Aldrich, India), carrageenan (Sigma-Aldrich, India), carboxy methyl cellulose (CMC, Merck, India), benzene (Merck, India), ethyl acetate (Merck, India), ethanol (Merck, India) were used during the experimental protocol.

Plant material

The aerial parts of *Lindernia crustacea* were collected from the paddy field of Dharapur, Guwahati, Assam in the month of April and May and was authenticated by Dr P.P. Baruah, HOD, Department of Botany, Gauhati University, Guwahati, Assam, as *Lindernia crustacea* (L) F. Muell with family Linderniaceae and accession number was given for the specimen is 18063.

Preparation of extract and phytochemical analysis

Shade-dried and coarsely powdered aerial parts of *Lindernia crustacea* (L) F. Muell was subjected to successive extraction by cold maceration in Benzene, Ethyl acetate and Ethanol for 72

hours by intermittent shaking. The solvents were filtered by Whatman filter paper and concentrated by using Rotary evaporator (Buchi, Switzerland) and stored in a refrigerator at 4°C until further work. Qualitative analysis of extracts for the confirmation of the presence of various phytochemicals was carried out as per the standard methods[9].

Experimental Animals

Female (non-pregnant) Swiss Albino mice of 25-35 g weight and female (non-pregnant) Wistar albino rats of 150-200 gm weight, were obtained from animal house, Girijananda Chowdhury Institute of Pharmaceutical Science, Azara, Guwahati. All the animals were kept under standard conditions of temperature (22 ± 1°C) and 12hr day/ night cycles. Standard pellet diet supplied by Hindustan Lever Ltd., Kolkata, India and water ad libitum were fed to animals. All the experimental proposal designed as per the CPCSEA guidelines and approved by the Animal Ethics Committee of the Institute (Approval no. GIPS/IAEC/ PhD/2016/01).

Animal Group

For the study of anti-inflammatory, analgesic and antipyretic activity, the rats and mice (n = 6) were divided into eight groups. Group I and Group II served as control and reference group, respectively. Groups III to VIII served as test groups and administered Benzene (LCBE), Ethyl acetate (LCEAE) and Ethanol (LCEE) extracts, orally at the dose of 100 and 200 mg/kg of body weight.

Acute toxicity assay

OECD guidelines 425 (limit test) was followed for performing Acute toxicity assay[10, 11]. Randomly selected female Swiss albino mice were divided into groups. Each group was comprised of five animals. A single dose of 2000 mg/kg body weight of each extract (2000 mg/kg) was orally administered to their respective groups. 0.5% CMC (10 mL/kg) administered orally to the control group[12]. The mice were observed continuously for any toxic symptoms or mortality for the first 4 hours and then observed periodically up to 24 hours. The mice were kept under daily observation for 14 days.

Anti-inflammatory activity

Carrageenan-induced paw oedema in rats

The acute anti-inflammatory activity was studied using carrageenan-induced oedema in rats as per the method described by Winter et al[13]. Inflammation was produced in female Wistar rats by injecting freshly prepared 1 % w/v carrageenan solution (0.1 mL), in normal saline, into the sub-plantar region of the rat's paw. Control group orally received 0.5% CMC, reference group orally received indomethacin 10 mg/kg body weight and each test groups were orally administered LCBE, LCEAE and LCEEat the dose of 100 and 200 mg/kg body weight, 60 min prior to carrageenan injection. Paw volume was measured with the help of Mercury plethysmometer at 0, 1, 2, 3 and 4 h after injection of carrageenan solution. Initial and subsequent reading difference gave the actual volume of oedema. Percentage swelling and percentage inhibition at each time interval were calculated[14]. Paw volume reduction in comparison to the control rats was considered as anti-inflammatory response[15].

Egg albumin induced paw oedema in rats

This test was performed as per the method described by Winter et al. [13, 15]. For the study 24 h fasted Wistar albino female rats were used. Inflammation was induced by injecting 0.1 mL (1% in normal saline) of egg albumin solution, into the subplantar tissue of right hind paw of a rat. Each test groups orally received a dose of 100 mg/kg and 200 mg/kg body weight of LCBE, LCEAE and LCEE, one hour before inducing inflammation. The reference group received indomethacin 10 mg/kg body weight orally, and control group orally received 0.5% CMC.The degree of swelling of the paw was measured by mercury plethysmometer (in mL) at 0, 1, 2, 3 and 4 h of administration of the egg albumin and oedema rate and inhibition was calculated[16, 17].

Analgesic activity

Acetic acid induced writhing in mice

This study was conducted by as per the method described by Kosteret al[18]. Control group received 0.5% CMC, standard group was treated orally with acetylsalicylic acid (Aspirin) at the dose of 100 mg/kg. Test groups were treated orally with100 and 200 mg/kg of LCBE, LCEAE and LCEE. 0.6 % v/v acetic acid (10mL/kg body weight) was injected intraperitoneally in the mice of all groups to induce writhing. The number of writhing was counted, in each mice, over a period of 30 min and percentage of inhibition of writhing count of the treated groups was calculated[19, 20].

Hot plate method in mice

The central analgesic activity of the extracts of aerial parts of *Lindernia crustacea* was evaluated on mice by Eddy's hot plate method [21]. Controlled group orally received 0.5% CMC. Group II orally received acetylsalicylic acid (Aspirin) at the dose of 100 mg/kg, as the standard drug. Groups III-VIII administered orally LCBE, LCEAE and LCEE at the dose of 100 and 200 mg/kg of body weight respectively. The hot plate was maintained at temperature 55 ± 2 °C and each mice placed individually with the cut-off time of 15 sec to prevent damage of tissue. Paw licking or jumping as the reaction time, was measured at 0, 30, 60 and 90 mins after administration of drug or vehicle[22].

Antipyretic Activity

Yeast Induced Hyperthermia in rats

Established method [22, 23] of Brewer's yeast-induced fever in rats, with some modification, was followed for evaluation of the antipyretic activity. Digital thermometer of 3mm external diameter and 0.1°C precision was used to measure rectal temperature of rats by inserting 1.5 cm with lubricant. After measuring rectal temperature of the animals, they were injected intraperitoneally with a pyrogenic dose (10 mL/kg body weight) of baker's yeast (0.135 g/kg) suspension in distilled water for inducing pyrexia. Animals that showed an increase of 0.6°C or more in rectal temperature, after 18 hours, were selected for the antipyretic experiment. Group I was orally administered 0.5% CMC (10 mL/kg). Group II was orally given Paracetamol as standard drug at the dose of 150 mg/kg. Groups III to VIII received LCBE, LCEAE and LCEE extracts at oral dose of 100 mg/kg and 200 mg/kg respectively. After drug administration, the temperature of all the rats in each group was recorded at 0h, 1h, 2h and 3h[15].

STATISTICAL ANALYSIS

All the data were analysed using one-way ANOVA followed by Dunnett's test and the results were expressed as mean±standard error mean (n=6).$p< 0.05$ and $p<0.001$ (when compared to control) indicated significant difference between the groups.

Table 1: Effect of *Lindernia crustacea* extracts on carrageenan-induced rat paw oedema (percentage of inhibition in parenthesis)

Group	Treatment	Percentage (%) of Swelling			
		1h	2h	3h	4h
I	Control (0.5% CMC)	22.82±0.09	33.01±0.11	51.07±0.06	58.92±0.09
II	Indomethacin (10mg/kg)	14.61±0.03** (36.0)	17.91±0.06** (45.7)	21.57±0.02** (57.2)	23.32±0.07** (60.4)
III	LCBE (100 mg/kg)	20.18±0.08 (11.6)	27.52±0.11 (16.6)	40.70±0.06 (20.3)	42.73±0.08 (27.5)
IV	LCBE (200 mg/kg)	17.95±0.08 (21.3)	22.17±0.04* (32.8)	24.78±0.06** (51.5)	26.32±0.02** (55.3)
V	LCEAE (100 mg/kg)	20.48±0.08 (10.3)	28.09±0.08 (14.9)	41.27±0.02 (19.2)	44.02±0.02 (25.3)
VI	LCEAE (200 mg/kg)	18.07±0.11 (20.8)	25.12±0.06 (23.9)	29.67±0.06* (41.9)	30.28±0.05** (48.6)
VII	LCEE (100 mg/kg)	21.13±0.05 (7.4)	28.86±0.05 (12.6)	42.01±0.04 (17.7)	46.17±0.08 (21.6)
VIII	LCEE (200 mg/kg)	19.23±0.08 (15.7)	26.33±0.11 (20.2)	32.12±0.08* (37.1)	33.09±0.03** (43.8)

Values expressed as mean ± SEM; (n=6); *p<0.05, **p<0.001 when compared to control. Data were analysed by using One-way ANOVA followed by Dunnett's test

Table 2: Effect of *Lindernia crustacea* extracts on Egg albumin -induced rat paw oedema (percentage of inhibition in parenthesis)

Group	Treatment	Percentage (%) of Swelling			
		1h	2h	3h	4h
I	Control (0.5% CMC)	23.12±0.03	32.07±0.05	50.07±0.12	59.22±0.06
II	Indomethacin (10 mg/kg)	13.98±0.07** (39.5)	15.88±0.08** (50.5)	19.57±0.09** (60.9)	20.93±0.06** (64.7)
III	LCBE (100 mg/kg)	20.77±0.06 (10.2)	26.13±0.08 (18.5)	37.11±0.08 (25.9)	40.66±0.07 (31.3)
IV	LCBE (200 mg/kg)	16.72±0.12 (27.7)	20.37±0.04* (36.5)	23.84±0.04** (52.4)	24.72±0.04** (58.3)
V	LCEAE (100 mg/kg)	20.83±0.10 (9.9)	26.21±0.08 (18.3)	40.18±0.06 (19.8)	42.21±0.06 (28.7)
VI	LCEAE (200 mg/kg)	17.42±0.11 (24.7)	23.24±0.06 (27.5)	27.13±0.06* (45.2)	29.55±0.09** (50.01)
VII	LCEE (100 mg/kg)	21.09±0.07 (8.8)	27.15±0.08 (15.3)	41.45±0.08 (17.2)	44.70±0.11 (24.5)
VIII	LCEE (200 mg/kg)	18.19±0.07 (21.3)	24.15±0.09 (24.7)	30.26±0.04* (39.6)	32.06±0.07* (45.9)

Values expressed as mean ± SEM; (n=6); *p<0.05, **p<0.001 when compared to control. Data were analysed by using One-way ANOVA followed by Dunnett's test

Table 3: Effect of *Lindernia crustacea* extracts on acetic acid-induced writhing in mice

Group	Treatment	Number of writhing	Percentage of inhibition
I	Control (0.5% CMC)	41.58±0.33	--
II	Aspirin (100 mg/kg)	14.73±0.44**	64.57
III	LCBE (100 mg/kg)	28.10±0.23*	32.42
IV	LCBE (200 mg/kg)	17.08±0.57**	58.92
V	LCEAE (100 mg/kg)	32.22±0.62	22.51
VI	LCEAE (200 mg/kg)	21.11±0.49*	49.23
VII	LCEE (100 mg/kg)	35.48±0.77	14.67
VIII	LCEE (200 mg/kg)	24.59±0.20*	40.86

Values expressed as mean ± SEM; (n=6); *p<0.05, **p<0.001 when compared to control. Data were analysed by using One-way ANOVA followed by Dunnett's test

Table 4: Effect of *Lindernia crustacea* extracts by Eddy's hot plate method in mice

Group	Treatment	Reaction time (sec, mean ± SEM)			
		0 min	30 mins	60 mins	90 mins
I	Control (acetic acid 0.6 % v/v)	8.44 ±0.12	8.62±0.22	8.48±0.09	8.56±0.18
II	Aspirin (100 mg/kg)	8.44±0.32	10.32±0.11**	14.22±0.15**	14.40±0.10**
III	LCBE (100 mg/kg)	8.88±0.11	9.04±0.30	9.44±0.32	10.11±0.23*
IV	LCBE (200 mg/kg)	8.52±0.27	9.45±0.20*	11.55±0.27*	13.06±0.25**
V	LCEAE (100 mg/kg)	8.26±0.24	8.96±0.16	9.46±0.29	9.88±0.21
VI	LCEAE (200 mg/kg)	8.05±0.39	9.12±0.19	9.68±0.19	11.28±0.11**
VII	LCEE (100 mg/kg)	8.32±0.23	8.72±0.29	8.93±0.25	9.33±0.16
VIII	LCEE (200 mg/kg)	8.50±0.17	9.02±0.24	9.29±0.30	10.52±0.21*

Values expressed as mean ± SEM; (n=6); *p<0.05, **p<0.001 when compared to control. Data were analysed by using One-way ANOVA followed by Dunnett's test

Table 5: Antipyretic effect of *Lindernia crustacea* extracts on rats

Group	Treatment	Initial Rectal Temp. in °C before Yeast Injection	Rectal Temperature in °C after 18hrs of Yeast Injection (Mean± SEM)			
			0 hr	1 hr	2 hrs	3 hrs
I	Control (0.5% CMC)	37.13 ± 0.1	38.89 ± 0.08	39.11 ± 0.11	39.19 ± 0.10	39.19 ± 0.14
II	Paracetamol (150 mg/kg)	37.12 ± 0.14	39.07 ± 0.09	38.08±0.07*	37.71 ± 0.05**	37.13 ± 0.05**
III	LCBE (100 mg/kg)	37.16 ± 0.6	39.05 ± 0.11	38.63 ± 0.05	38.27 ± 0.09	38.10 ± 0.07*
IV	LCBE (200 mg/kg)	37.16 ± 0.9	39.05 ± 0.07	38.16±0.08*	37.92 ± 0.16**	37.17 ± 0.09**
V	LCEAE (100 mg/kg)	37.18 ± 0.11	38.84 ± 0.07	38.66 ± 0.06	38.65 ± 0.10	38.45 ± 0.05
VI	LCEAE (200 mg/kg)	37.15± 0.9	38.98 ± 0.10	38.51 ± 0.11	38.02 ± 0.06*	37.20 ± 0.10**
VII	LCEE (100 mg/kg)	37.14 ± 0.07	38.82 ± 0.06	38.67 ± 0.11	38.58 ± 0.05	38.51 ± 0.09
VIII	LCEE (200 mg/kg)	37.15 ± 0.03	38.96 ± 0.08	38.66 ± 0.07	38.05 ± 0.10*	37.22 ± 0.09**

Values expressed as mean ± SEM; (n=6); *p<0.05, **p<0.001 when compared to control. Data were analyzed by using One-way ANOVA followed by Dunnett's test

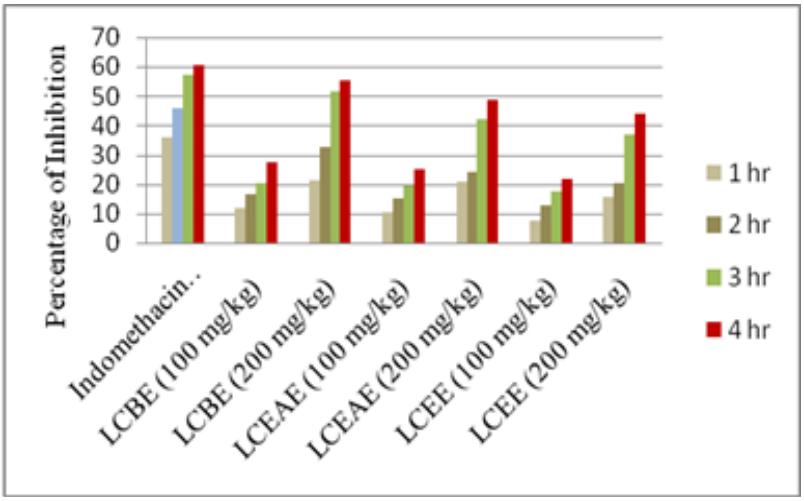

Fig. 1: Effect of *Lindernia crustacea* extracts on carrageenan-induced rat paw oedema

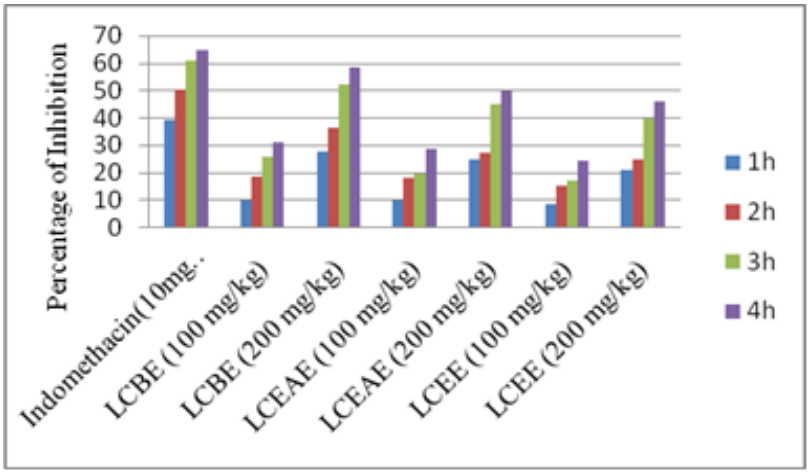

Fig. 2: Effect of *Lindernia crustacea* extracts on Egg albumin -induced rat paw oedema

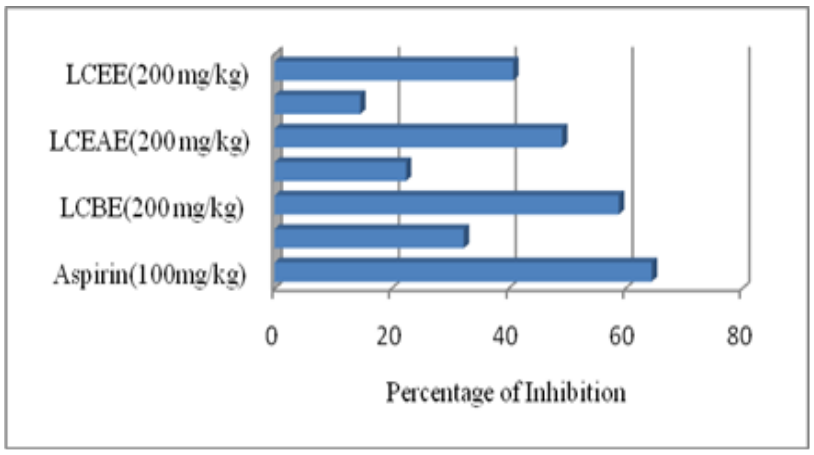

Fig. 3: Effect of *Lindernia crustacea* extracts on acetic acid-induced writhing in mice

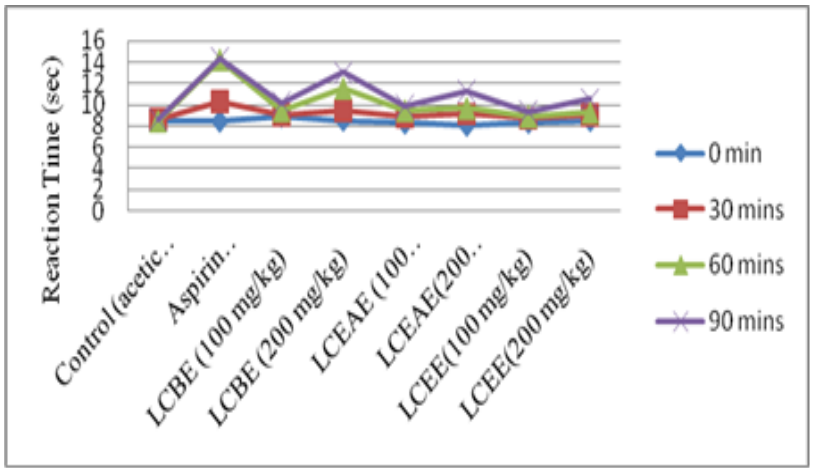

Fig. 4: Effect of *Lindernia crustacea* extracts by Eddy's hot plate method

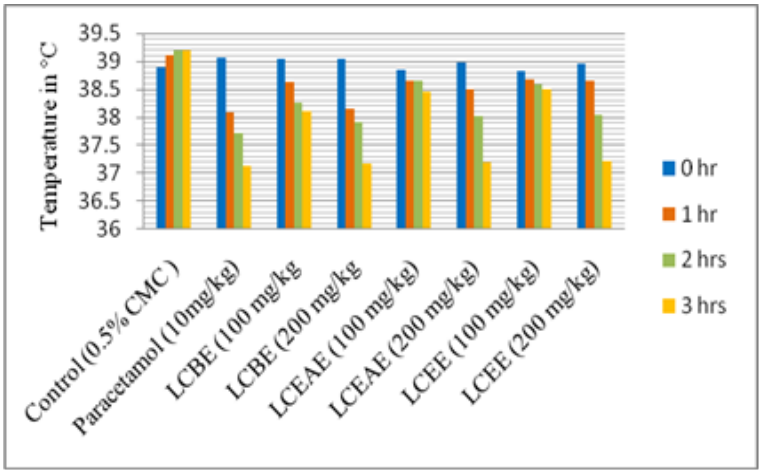

Fig. 5: Antipyretic effect of *Lindernia crustacea* extracts on albino rats

RESULTS

Phytochemical Screening

The result showed that the plant secondary metabolites-flavonoid, phenolic compound and tannins are present in benzene extract, ethyl acetate extract and ethanol extract. Carbohydrate is present in ethyl acetate and ethanolic extract and amino acid is present in ethanolic extract only.

Acute oral toxicity study

At the given dose of 2000 mg/kg of extracts, no adverse effect or mortality was observed. All the animals were alive, active and healthy during the observational period of 14 days. So the LD50 was considered as more than 2000 mg/kg for all extracts.

Anti-inflammatory activity

Carrageenan-induced paw oedema in rats

The results of the carrageenan-induced paw oedema study of *Lindernia crustacea* are presented in Table 1 and Fig.1. Maximum inhibition was showed by LCBE at 200 mg/kg dose at 2h, 3h and 4hr. While LCEAE and LCEE showed anti-inflammatory activity at doses of 200 mg/kg after 3 h and 4h of drug administration. 100 mg/kg dose of any extract did not show any significant inhibition of paw oedema. Indomethacin at 10 mg/kg dose inhibited paw oedema at 1h, 2h, 3h and 4h.

Egg albumin induced paw oedema in rats

Table 2 and Fig.2 represent the results of Egg albumin induced paw oedema study in rats. It was observed that LCBE at dose of 200 mg/kg produced inhibition at 1h, 2h, 3h and 4h. Whereas the reference drug showed significant inhibition of paw oedema at dose of 10mg/kg. LCEAE and LCEE at dose of 200 mg/kg showed inhibition at 3h and 4h. Though all extracts at dose of 100 mg/kg showed some inhibition but significant inhibition compared to control group was not found at this dose.

Analgesic activity

Acetic acid induced writhing in mice

When compared with control group, LCBE significantly reduced acetic acid-induced writhing count by 58.92% ($p<0.001$) and 32.42 % ($p<0.05$) at 100 mg/kg and 200 mg/kg dose respectively. LCEAE and LCEE also showed significant ($p < 0.05$) analgesic activity at 200 mg/kg dose while aspirin (100mg/kg) showed highest inhibition of 64.57% (Table 3 and Fig. 3).

Hot plate method in mice

The results of the analgesic effect of *Lindernia crustacea* extracts using hot plate method are presented in Table 4 and Fig. 4. LCBE at the dose of 100 mg/ kg showed significant ($p<0.05$) reaction time at 90 min and at 200mg/kg dose it increase reaction time significantly at 30 min, 60 min ($p<0.05$)and at 90 min maximum reaction time was observed ($p<0.001$). At the dose of 200 mg/kg, maximum reaction time was showed at 90 min by LCEAE ($p<0.001$) and LCEE ($p<0.05$). Aspirin at the dose of 100mg/kg increased the reaction time significantly ($p<0.001$) during the study period.

Antipyretic activity

Yeast Induced Hyperthermia in rats

The results of antipyretic activity in brewer's yeast-induced pyrexia model are summarised in Table 5 and Fig. 5. Significant antipyretic effect was observed with LCBE at dose of 100 mg/kg at 3 h ($p<0.05$) and at the dose of 200 mg/kg at 1hr ($p<0.05$), 2h and 3h ($p<0.001$). Whereas LCEAE and LCEE only showed significant antipyretic activity at the dose of 200 mg/kg at 2 hrs ($p<0.05$) and 3 hrs ($p<0.001$).

DISCUSSION

Established synthetic drugs for inflammation, pain and fever, like NSAID, opioids and corticosteroids, are reported to have many clinically important adverse effects like gastrointestinal tract dyspepsia, haemorrhage, peptic ulceration, renal disorder etc.[24, 25]. Research on global level is focused to establish novel potent compounds which will be cost-effective and have minimum adverse effects. Medicinal plants are already proved to be important sources of number of compound with variety of therapeutic effects[26]. The study reveals that the aerial parts extracts of *Lindernia crustacea* exhibited significant anti-inflammatory, analgesic and antipyretic activity. Among the all extracts benzene extract showed most significant anti-inflammatory, analgesic and antipyretic activity. Phytochemical profile showed the presence of flavonoid and phenolic compound in all three extracts.

Carrageenan and Egg albumin induced paw oedema is mostly used experimental model for the study of acute inflammatory response. Inflammation involves a complex mechanism starting from enzyme activation to repair[27]. Acetic acid induced writhing

model commonly used to test peripheral analgesic effects whereas the hot plate model is commonly used to study central analgesic effects[28]. Flavonoids from various plants are found to be important constituents, which exhibits many beneficial health effects on human. Various flavonoids have been studied for the anti-inflammatory and analgesic properties in order to characterise their potentiality as anti-inflammatory agents[29-32]. Flavonoids inhibit biosynthesis of prostaglandins, which acts as secondary messengers and are involved in various immunological responses[33]. Secondary messenger inhibition provides the basis of mechanism by which flavonoids inhibit inflammation[34]. As, prostaglandins are also involved in the pain mechanism[35], inhibition of their synthesis might be the possible reason behind the analgesic activity of the extracts. So, anti-inflammatory effect of the extracts is assumed to be contributed to its analgesic effect.

The extracts of *Lindernia crustacea* showed significant antipyretic effect against the pyrexia induced by Brewer's yeast. The possible mechanism for the antipyretic activity of extracts may be due to the inhibition of PGE2 synthesis[36,37]. The antipyretic activity may be due to flavonoids or phenolic compounds found in the extracts. Flavonoids have antipyretic activity by suppressing TNF-α[38]and reducing fever and pain by reduction of prostaglandin levels by its related compounds is due to inhibition of arachidonic acid peroxidation[39].

The presence of flavonoid and phenolic compound identified might be responsible for the anti-inflammatory, analgesic as well as antipyretic activities of the extracts. However, the proposed mechanisms require further study.

CONCLUSION

From the findings of the above study, it may be concluded that aerial part extracts of *Lindernia crustacea* showed significant anti-inflammatory, analgesic and antipyretic activities, where benzene extract showed more significant activity among all three extracts. Since this is a pioneer work on this plant to study the traditionally claimed medicinal importance, so, further studies are necessary to validate the results. Also, detailed study on isolation and identification of the active compound is essential to promote the compound for clinical use.

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