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Research Article

IN VIVO ANTI-INFLAMMATORY AND IN VITRO ANTIOXIDANT STUDIES ON METHANOLIC AND AQUEOUS EXTRACT OF LEUCAS INDICA LINN

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ABSTRACT

Objective: The present studies were designed to evaluate the anti-inflammatory and antioxidant activity of methanloic and aqueous extract of Leucas indica Linn. (Labiatae). **Methods:** The anti-inflammatory activity was evaluated by some in vivo models like carrageenan and formalin induced rats paw edema and cotton pellet induced granuloma formation in wistar rats using dose levels of 200 and 400 mg/kg body weight (orally) for both extract and compared to reference drugs aspirin (100 mg/kg body weight, orally). The antioxidant activity was estimated by some in vitro models like 2, 2-diphenyl-1-picryl hydrazyl hydrate (DPPH) photometric assay, superoxide anion and hydroxyl radical scavenging capacity and reducing ability measurement using a concentration of 250 and 500 µg/ml for both extract and compared to reference drug quercetin (25 and 50 µg/ml). A systematic phytochemical screening and acute toxicity study of the both extract were also performed prior to studies. **Results:** The aqueous extract of the plant showed more significant dose dependent anti-inflammatory and antioxidant properties compared to the methanloic fraction. The preliminary phytochemical screening of each fraction revealed the presence of flavonoids, total phenolic compounds, saponin and tannin. The measured LD_{50} values were 1995 and 2630 mg/kg body weight (by oral route) in wistar rats for the methanolic and aqueous extract respectively. **Conclusions:** On the basis of the current observations it was concluded that the aqueous extract of the plant possesses a more potent and safest anti-inflammatory and antioxidant properties.

Keywords: Leucas indica, Anti-inflammatory, Antioxidant, DPPH, Carrageenan, Cotton pellet, Flavonoids, Phenolic compounds.

INTRODUCTION

It is reported that the reactive oxygen species (ROS) like super oxide anion (O_2^-), hydroxyl radicals (OH^+), peroxyl radical (ROO.) and nitric oxide radical (NO.) easily react with most of the biomolecules including protein, lipoprotein, enzymes, DNA and RNA [1]. The ample generation of ROS leading to various inflammatory disorders like rheumatoid arthritis, cancer, insulin dependent diabetes mellitus (IDDM) [2][3][4]. The basic mechanism of inflammation is attributed by release of ROS from activated neutrophil, macrophages which provoke release of several secondary inflammatory cytokines like tumor necrosis factor-α (TNFα), interleukin-1 (IL_1) and interferon-γ those are further responsible for the progression of other kind of inflammatory disorders like asthma, anaphylaxis, allergic rhinitis, ulcer etc. Conventional anti-inflammatory agents are generally disease modifying agents and can't cure the diseases as well as cause greater toxic effects in organs like liver, kidney etc [5]. Therefore it is a crying need to find out new and safe antioxidants to prevent oxidative stress induced diseases.

The plant Leucas indica Linn., belonging to the family, Labiatae is commonly known as 'Dandokalos' in Bengali, is distributed in throughout the India, along with road side, waste lands, river banks, on rocky hills and abundantly present in 'Mahananda Neora Valley' in West Bengal. The herbs are almost erect, pubescent branching, leaves are linear-lanceolate, flowers are white, calyx tube slightly curved, corolla is annulated and stamens are four [6]. Traditionally, the leaves of this plant are used as vermifuge, stomachic, sedative and in sores [7]. The aerial parts of Leucas indica Linn. contain phenylethanoid glycosides, having antioxidant, Xanthene oxidsase inhibition as well as wound healing activity [8][9]. However, based on literature survey and traditional use, the present study was designed to evaluate the anti-inflammatory and antioxidant activity of methanolic and aqueous extract of Leucas indica Linn.

MATERIALS AND METHODS

Plant Materials

The aerial parts of Leucas indica Linn. were collected in August, 2011 from Duars region, Jalpaiguri District, West Bengal, subsequently identified and authenticated from Central National Herbarium, Botanical Survey of India, Howrah-711103, West Bengal (Ref No. CNH/32/2012/Tech.II/625 Dated: 06.03.2012). After proper washing, it was dried under shade at a room temperature for seven days and then grinded with a mechanical grinder. Finally, the coarse powders were separated by sieving using 40 mesh and stored in an air tight container for further use.

Preparation of Plant Extract

The fresh coarse powders were subjected to maceration by petroleum ether to remove fatty materials and then successively extracted with chloroform, methanol and distilled water according to ascending order of polarity of solvent using a Soxhlet apparatus. The each fraction of the extract was then filtered and concentrated to dryness in a rotary vacuum evaporator under reduced pressure and temperature and stored in desiccators. During performing the experiment, the dried methanloic and aqueous extract were dissolved in distilled water to prepare the subsequent extracts (LIME and LIAE). The preliminary phytochemical screening of LIME and LIAE done by the method mentioned by Harbone and Trease confirmed the presence of flavonoids, total phenolic compounds, saponin and tannin [10][11].

Chemicals and Reagents

2,2-diphenyl-1-picrylhydrazyl (DPPH) and Carrageenan were purchased from HiMedia (Mumbai, India). Quercetin Didydrate, Phenazine methosulfate (PMS), Nitroblue tetrazolium chloride (NBT) and Nicotinamide adenine dinucleotide (NADH) from SRL (Mumbai, India). Aspirin from Research Lab (Mumbai, India). The remaining solvents and chemicals were used for this studies are in analytical grade.

Animals

Wistar albino rats (weighing 150-200 g) of either sex were used to perform the anti-inflammatory study. The animals were randomly grouped (n=6) and housed in polyacrylic cages (38x23x10 cm) and maintained under standard laboratory conditions (Temp. 25 ± 2º C) with dark and light cycle (14/10 hr). They were allowed free access to standard dry pellet diet (Hindustan Lever, Kolkata, India) and water ad libitum. The rats were acclimatized to laboratory condition for 1 week before commencement of this experiment. Ethical

clearance was obtained from Jadavpur University Animal Ethical Committee for using animals in the present study (Vide No. 0367/01/C/CPCSEA, India).

Toxicity study

Wistar albino rats (weighing 150-200 g) of either sex were divided into several groups containing 10 animals of each. Different doses of both LIME and LIAE (200, 500, 1000, 1500, 2000, 2500, 3000, 3500 mg/kg b.w) were administered orally to the treated groups but control groups received only normal saline orally (5 ml/kg b.w) in overnight fasting condition. The sign of toxicity and mortality were recorded within 24-72 h for all groups of animals. The LD_{50} was determined using graphical representation and probit analysis [12].

In vivo Anti-inflammatory activity

Carrageenan-induced rat paw edema

The protocol for carrageenan and formalin induced rat paw edema was designed by Winter and Porter [13]. The animals were divided into six groups of six animals of each. The paw edema was induced by injecting freshly prepared 0.1 ml of 1% carrageenan dissolved in normal saline into the sub-plantar region of the right hind paw of all group of animals. Both the extracts (LIME and LIAE) at the dose level of 200 and 400 mg/kg b.w and reference drug (aspirin) at the dose level of 100 mg/kg b.w were administered orally before 1 h of carrageenan injection. Group-I served as control (normal saline, 5 ml/kg b.w, orally), Group-II, III, IV and V served as LIME and LIAE treated group respectively. Group-VI served as reference group. The paw volume was measured by plethysmometer (UGO Basile, Italy) just before and 1, 2, 3 and 4 h after administration of carrageenan. The percentage inhibition of paw edema was calculated by following formula:

% Inhibition = (1-Vt/Vc) x 100, Where, Vt and Vc represent paw volume of treated group and control group respectively.

Formalin induced rat paw edema

In this case also the same methodology (Winter and Porter) was applied for animals grouping, induction of paw edema, measurement of paw volume and percentage inhibition as mentioned in the carrageenan model but instead of carrageenan, 0.1 ml of formalin (1% w/v) solution was used to develop edema [13].

Cotton pellet-induced granuloma formation in rats

The animals were divided into six groups of six animals of each. Group-I served as control (normal saline, 5 ml/kg b.w, orally), Group-II, III, IV and V served as LIME and LIAE treated group (200 and 400 mg/kg b.w, orally for both extract) respectively. Group-VI was treated with reference drug (Aspirin, 100 mg/kg b.w, orally). The pellets (absorbent cotton wool weighing about 10 mg each) were sterilized in a hot air oven at 120°C for 2 h and implanted subcutaneously in each axilla of the animal of all groups under sterile and mild anaesthetic conditions. Following the pellets implantation, the all groups of animals were treated with respective dose for seven consecutive days and on the day of 8 the all pellets were removed surgically under anaesthesia and weighed immediately in wet condition. The all pellets were then dried at 60°C for 18 h and again weighed. Finally, the transudative weight, granuloma weight and the percentage inhibition of granuloma of all groups were noted [14].

In vitro Antioxidant activity

2, 2 Diphenyl–1–picryl hydrazyl hydrate (DPPH) photometric assay

The measurement of DPPH scavenging activity was described by Barik et al with few modifications [15]. Different concentrations of test (250 and 500 µg/ml) and standard substances (25 and 50 µg/ml) were prepared in methanol and incubated in darkness with DPPH (0.3 mM in methanol). After 30 minutes absorbance values were measured at 518 nm. The results obtained are converted into the percentage antioxidant activity (AA) using the following formula:

$$\mathbf{AA\ \% = 100\ \{[Abs_{sample} - Abs_{control}) \times 100] / Abs_{control}\}}$$

Hydroxyl radical scavenging activity

The assay was done by Elizabeth and Rao method with few modifications [16]. It is based on quantification of the degradation product of 2-deoxyribose by condensation with TBA. Hydroxyl radical was generated by the Fe^{3+}-ascorbate-EDTA-H_2O_2 system (the Fenton reaction). The reaction mixture contained, in a final volume of 1 ml, 2-deoxy-2-ribose (2.8 mM); KH_2PO_4 -KOH buffer (20 mM, pH 7.4); $FeCl_3$ (100 µM); EDTA (100 µM); H_2O_2 (1.0 mM); ascorbic acid (100 µM) and concentrations (500 µg/ml) of the test sample or reference compound. After incubation for 1 h at 37°C, 0.5 ml of the reaction mixture was added to 1 ml 2.8% TCA, then 1 ml 1% aqueous TBA was added and the mixture was incubated at 90°C for 15 min to develop the color. After cooling, the absorbance was measured at 532 nm against an appropriate blank solution. All tests were performed for three times. Quercetin was used as a positive control. Percentage inhibition was evaluated by comparing the test and standard solutions with control.

Superoxide anion scavenging activity

Measurement of superoxide anion scavenging activity was performed with NBT-NADH-PMS system, described by Nishimiki with slight modification [17]. About 1 ml of nitroblue tetrazolium (NBT) solution, 1 ml NADH solution were given to 0.1 ml of sample solution, test extracts and the standard solution quercetin respectively. The reaction was started by adding 100 µM of phenazine methosulphate (PMS) solution in phosphate buffer, pH 7.4 to the mixture. The reaction mixture was incubated at 25°C for 5 min, and the absorbance at 560 nm was measured against blank sample. Decreased absorbance of the reaction mixture indicated increased superoxide anion scavenging activity. The percentage of inhibition was calculated by the same equation as DPPH scavenging activity assay.

Measurement of reductive ability

For the measurement of the reductive ability we investigated the $Fe^{+3} - Fe^{+2}$ transformation in the presence of the samples such as extracts of Leucas indica, Quercetin, at different dose using the method of Oyaizu [18]. Definite amount of the extract and standard drugs, 2.5 ml phosphate buffer pH – 6.6), 2.5 ml 1% $K_3Fe(CN)_6$ were incubated at 50°C for 20 minutes, 2.5 ml of 10% trichloro acetic acid (TCA) were added to the mixture and centrifuged for 10 minutes at 3000 rpm. After centrifugation 2.5 ml of the supernatant were diluted with 2.5 ml water and shaken with 0.5 ml freshly prepared 0.1% $FeCl_3$. The absorbance was measured at 700 nm. The control solution was prepared as above, but contained water instead of samples. An increase in absorbance indicated higher reductive ability.

Statistical analysis

The results were expressed as mean ± SEM. Statistical differences between the treated and control groups were determined by one way ANOVA followed by Dunnet's test using the computer software, Graph pad Prism 5. $P < 0.05$ was considered for statistically significant.

RESULTS

Acute toxicity study

In acute toxicity study the LD_{50} values were 1995 and 2630 mg/kg body weight (by oral route) for the methanolic and aqueous extract respectively. However no visual toxicity was found up to 1500 mg/kg body weight of dose for both the fractions.

In vivo anti-inflammatory activity

Carrageenan and Formalin induced rat paw edema

Both the fraction LIME and LIAE showed significant inhibitory effect on edema formation induced by carrageenan and formalin at the dose of 200 and 400 mg/kg body weight by oral route up to four hours of observation. However LIAE showed more potential action compared to LIME in both model. Aspirin was used as reference drug at the dose of 100 mg/kg body weight by the same route which

shown about 68.24 and 68.35 % of inhibition on edema formation induced by carraggenan and formalin respectively at 3 h (Table 1, 2 and Fig 1 to 4).

Cotton pellet induced granuloma formation

The aqueous fraction of the extract showed significant dose dependent (200 and 400 mg/kg b.w, orally) inhibition of granuloma formation but no significant results were observed in case of methanolic fraction. However aspirin showed more potential action (61.42 %) than test samples (Table 3 and Fig 5, 6).

In vitro antioxidant activity

Free radicals scavenging and Reductive abilities

Both the extracts produced a concentration dependant scavenging activity on DPPH (Table 4 and Fig 7), Hydroxyl radicals (Table 5 and Fig 8) and Superoxide anion (Table 6 and Fig 9) as well as Reductive ability (Table 7 and Fig 10). The aqueous extract showed more percentage of inhibition than methanolic extract at the dose of 250 and 500 µg/ml significantly. However, Quercetin (25 and 50 µg/ml) was found to be more effective than both the extract.

Table 1: Effect of LIME and LIAE on carrageenan induced rat paw edema

Group	Initial paw volume (ml)	Increase in paw volume (ml)				
	0 h	1 h	2 h	3 h	4 h	% inhibition at 3 h
Gr-I Control	2.87 ± 0.04	0.28 ± 0.02	0.47 ± 0.02	0.85 ± 0.01	0.55 ± 0.01	-
Gr-II LIME 200	2.92 ± 0.04	0.2 ± 0.01*	0.36 ± 0.03*	0.42 ± 0.02*	0.27 ± 0.02*	50.59
Gr-III LIME 400	2.83 ± 0.05	0.18 ± 0.02*	0.32 ± 0.02*	0.38 ± 0.01*	0.24 ± 0.03*	55.29
Gr-IV LIAE 200	2.94 ± 0.06	0.19 ± 0.02*	0.33 ± 0.01*	0.35 ± 0.03*	0.25 ± 0.01*	58.82
Gr-V LIAE 400	2.96 ± 0.03	0.15 ± 0.01*	0.28 ± 0.03*	0.31 ± 0.01*	0.2 ± 0.01*	63.53
Gr-VI Aspirin 100	2.9 ± 0.04	0.13 ± 0.03**	0.19 ± 0.01**	0.27 ± 0.02**	0.12 ± 0.01**	68.24

Values are expressed as Mean ± SEM; n= 6; *P< 0.05; **P< 0.01;
LIME: Methanolic Extract of *Leucas indica;* LIAE: Aqueous Extract of *Leucas indica*

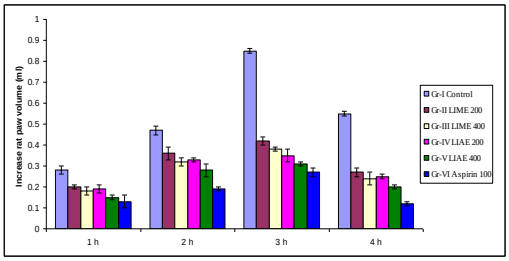

Figure 1: Effect of LIME and LIAE on carrageenan induced rat paw edema.

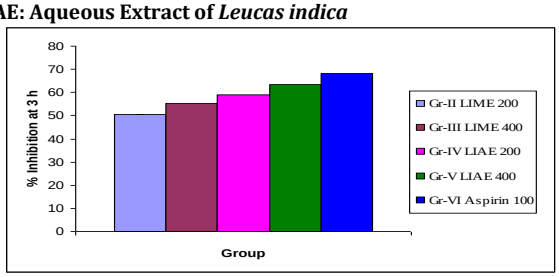

Figure 2: Percentage Inhibition of carrageenan induced inflammation in rats at 3 h.

Table 2: Effect of LIME and LIAE on formalin induced rat paw edema

	volume (ml)	Increase in paw volume (ml)				
	0 h	1 h	2 h	3 h	4 h	% inhibition at 3 h
Gr-I Control	2.12 ± 0.05	0.25 ± 0.01	0.41 ± 0.01	0.79 ± 0.02	0.48 ± 0.03	-
Gr-II LIME 200	1.95 ± 0.05	0.18 ± 0.01*	0.32 ± 0.01*	0.39 ± 0.02*	0.24 ± 0.02*	50.63
Gr-III LIME 400	2.04 ± 0.04	0.21 ± 0.02*	0.34 ± 0.01*	0.36 ± 0.01*	0.20 ± 0.02*	54.43
Gr-IV LIAE 200	2.16 ± 0.03	0.19 ± 0.03*	0.3 ± 0.02*	0.33 ± 0.02*	0.19 ± 0.01*	58.22
Gr-V LIAE 400	1.88 ± 0.04	0.17 ± 0.02**	0.25 ± 0.02**	0.29 ± 0.01**	0.18 ± 0.02**	63.29
Gr-VI Aspirin 100	2.13 ± 0.06	0.12 ± 0.01**	0.17 ± 0.03**	0.25 ± 0.01**	0.10 ± 0.02**	68.35

Values are expressed as Mean ± SEM; n= 6; *P< 0.05; **P< 0.01;
LIME: Methanolic Extract of *Leucas indica;* LIAE: Aqueous Extract of *Leucas indica*

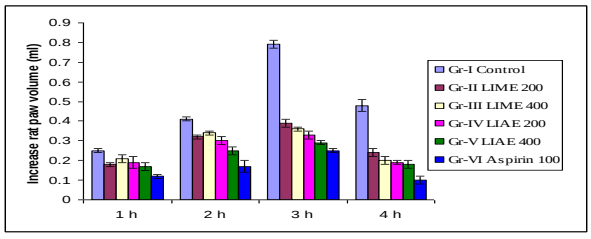

Figure3: Effect of LIME and LIAE on formalin induced rat paw edema.

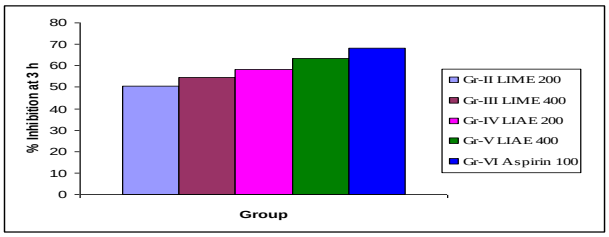

Figure 4: Percentage Inhibition of formalin induced inflammation in rats at 3 h.

Table 3: Effect of LIME and LIAE on cotton pellet induced granuloma in rats

Groups	Wet granuloma wt (mg)	Dry granuloma wt (mg)	Transudative wt (gm)	Granuloma wt (mg)	% Inhibition of granuloma
Gr-I Control	355.62 ± 1.66	55.67 ± 2.52	299.95 ± 2.64	45.67 ± 2.52	-
Gr-II LIME 200	287.53 ± 3.54ns	51.52 ± 1.82ns	236.01 ± 3.5 ns	41.52 ± 1.82ns	09.09
Gr-III LIME 400	278.84 ± 1.42ns	46.86 ± 1.45ns	231.98 ± 2.50ns	36.86 ± 1.45ns	19.29
Gr-IV LIAE 200	276.68 ± 2.87*	37.57 ± 2.30*	239.11 ± 1.55*	27.57 ± 2.30*	39.63
Gr-V LIAE 400	253.56 ± 1.68*	30.35 ± 1.76*	223.21 ± 2.15*	20.35 ± 1.76*	55.44
Gr-VI Aspirin 100	225.58 ± 1.30**	27.62 ± 2.12**	197.96 ± 3.18**	17.62 ± 2.12**	61.42

Values are expressed as Mean ± SEM; n= 6; *P< 0.05; **P< 0.01; ns: not significant;

LIME: Methanolic Extract of *Leucas indica;* LIAE: Aqueous Extract of *Leucas indica*

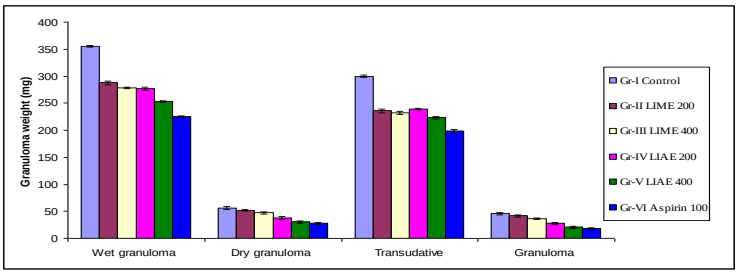

Figure 5: Effect of LIME and LIAE on cotton pellet induced granuloma in rats

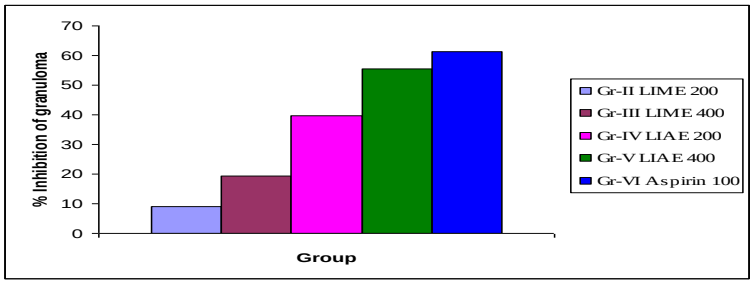

Figure 6: Percentage Inhibition of granuloma formation

Table 4: Antioxidant activity estimated by DPPH assay

Group	Absorbance	% Inhibition
Gr-I Control	0.800 ± 0.01	
Gr-II LIME 250	0.697 ± 0.12	12.18
Gr-III LIME 500	0.630 ± 0.06	23.53
Gr-IV LIAE 250	0.730 ± 0.01	52.39
Gr-V LIAE 500	0.277 ± 0.01	65.13
Gr-VI Quercetin 25	0.310 ± 0.01	60.92
Gr-VII Quercetin 50	0.231 ± 0.01	73.11

Values are expressed as Mean ± SEM; n= 3; *P< 0.05; **P< 0.01; ns: not significant;
LIME: Methanolic Extract of *Leucas indica;* LIAE: Aqueous Extract of *Leucas indica*

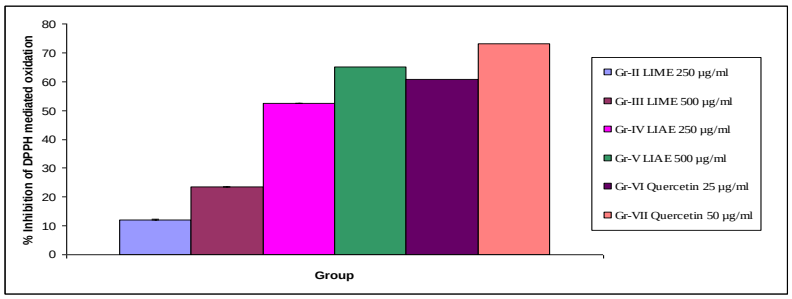

Figure7: Percentage inhibition by LIME and LIAE on DPPH assay

Table 5: Antioxidant activity estimated by hydroxyl radical assay

Group	Absorbances	% Inhibition
Gr-I Control	1.54 ± 0.02	-
Gr-II LIME 250	1.33 ± 0.11^{ns}	13.23
Gr-III LIME 500	1.00 ± 0.07^{ns}	34.92
Gr-IV LIAE 250	0.73 ± 0.01*	52.39
Gr-V LIAE 500	0.56 ± 0.01*	63.67
Gr-VI Quercetin 25	0.67 ± 0.01*	56.40
Gr-VII Quercetin 50	0.43 ± 0.43*	72.02

Values are expressed as Mean ± SEM; n= 3; *P< 0.05; ns: not significant;
LIME: Methanolic Extract of *Leucas indica;* LIAE: Aqueous Extract of *Leucas indica*

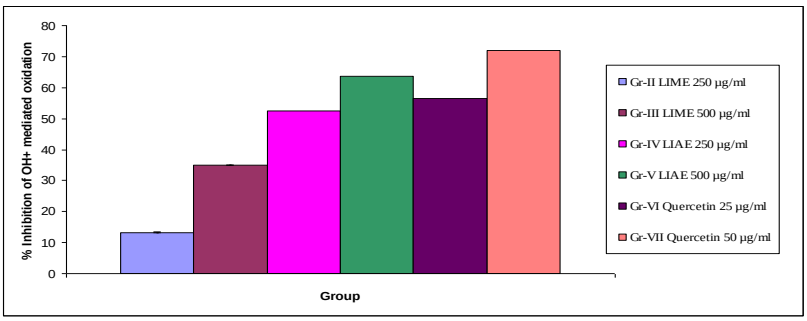

Figure 8: Percentage inhibition by LIME and LIAE on hydroxyl radical assay

Table 6: Antioxidant activity estimated by super oxide anion scavenging assay

Group	Absorbance	% Inhibition
Gr-I Control	0.833 ± 0.01^{ns}	-
Gr-II LIME 250	0.563 ± 0.07^{ns}	32.40
Gr-III LIME 500	$0.526 \pm 0.07^{*}$	36.80
Gr-IV LIAE 250	$0.367 \pm 0.01^{*}$	56.00
Gr-V LIAE 500	$0.297 \pm 0.01^{*}$	64.40
Gr-VI Quercetin	25 $0.393 \pm 0.01^{*}$	52.80
Gr-VII Quercetin	50 $0.367 \pm 0.01^{*}$	56.00

Values are expressed as Mean ± SEM; n= 3; *P< 0.05; ns: not significant;

LIME: Methanolic Extract of *Leucas indica;* LIAE: Aqueous Extract of *Leucas indica*

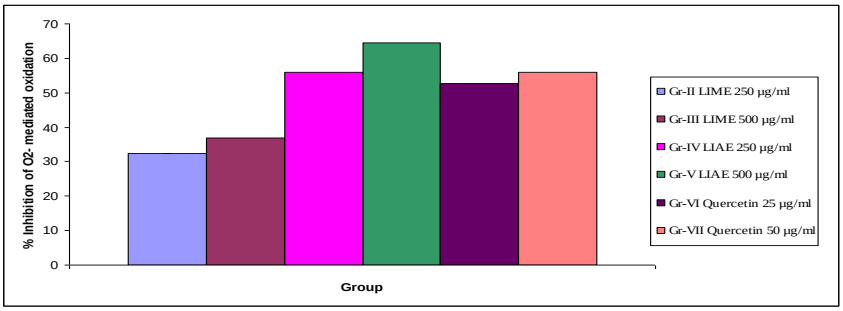

Figure 9: Percentage inhibition by LIME and LIAE on super oxide anion scavenging assay

Table 7: Antioxidant activity estimated by reductive ability assay

Group	Absorbances	% Inhibition
Gr-I Control	0.437± 0.02	-
Gr-II LIME 250	0.483 ± 0.11^{ns}	10.68
Gr-III LIME 500	0.563 ± 0.06^{ns}	29.00
Gr-IV LIAE 250	$0.695 \pm 0.01^{*}$	59.16
Gr-V LIAE 500	$0.785 \pm 0.01^{*}$	79.77
Gr-VI Quercetin 25	$0.763 \pm 0.01^{*}$	74.80
Gr-VII Quercetin 50	$0.823 \pm 0.01^{*}$	88.54

Values are expressed as Mean ± SEM; n= 3; *P< 0.05; ns: not significant

LIME: Methanolic Extract of *Leucas indica;* LIAE: Aqueous Extract of *Leucas indica*

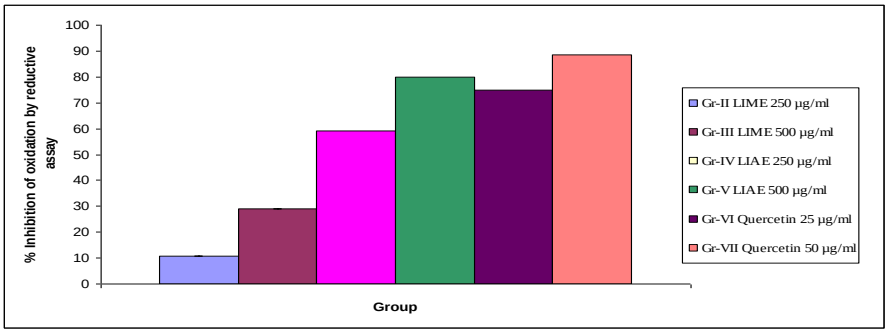

Figure 10: Percentage inhibition by LIME and LIAE on reductive ability assay

DISCUSSION

Presently NSAIDs are the most commonly prescribed agents for the management of inflammatory disorders like fever, pain, arthritis, gout etc. Recently, the adverse drug reactions associated with these agents are great matter of concern. Therefore, several new approaches are now considered for the development of superior anti-inflammatory agents, to avoid the toxic manifestations associated with current anti-inflammatory medications on gastrointestinal tract or kidney [5]. It is already reported by Charlier and Michaux that anti-inflammatory agents like phenidone, BW755C posses potent antioxidant activity due to their cyclooxygenase (COX)- lipooxygenase (LOX) dual inhibitory action [19]. Based on this evaluation and the traditional uses of the plant, the free radical scavenging activity, reductive ability and the anti-inflammatory activity of aqueous and methanolic extracts of aerial parts of *Leucas indica* were done. Carrageenan and formalin induced rat paw edema models were used for the evaluation of anti-inflammatory activity of the fractions. Carrageenan is known to produce a biphasic response where the early phase is associated with the production of histamine, leukotrienes, platelet activating factors and COX products and the late phase is related to the neutrophils infiltration, release of ecosanoid, free radicals and other neutrophils derived mediators [20][21]. The present study showed the inhibitory effect of LIME and LIAE against carrageenan induced rat paw edema (Table 1 and Fig 1, 2) on both early and delayed phase, that suggests it's possible inhibitory action on the release of inflammatory mediators like histamine, leukotrienes, prostaglandins as well as free radicals. According to report, NSAIDs does not have inhibitory action on delayed phase of carrageenan induced edema [22]. But our aqueous fraction is found to possess similar action like BW755C. Moreover, the fractions did not show any remarkable toxic reaction up to 1500 mg/kg b.w orally in acute toxicity study. Therefore the adverse drug reactions of NSAIDs by LOX pathways like renal toxicity, asthma, cancer could be avoided by the use of *Leucas indica* methanolic and aqueous extract in inflammatory disorders. Formalin is also able to induce inflammation in two phases. In the first phase the release of histamine and bradykinin occur followed by the production of pro-inflammatory factors including prostaglandins [23]. Therefore, the long term inhibitory effect of aqueous fraction of *Leucas indica* on formalin induced paw edema (Table 2 and Fig 3, 4) in rats indicates it's inhibitory action on histamine as well as prostaglandin release [24]. Further it is revealed that fibroblast proliferation is the characteristic feature of repair phase of inflammation [25]. The inhibitory action of LIME and LIAE on cotton-pellet induced granuloma formation suggests their significant action (Table 3 and Fig 5, 6) in proliferative phase of inflammation. Several researchers around the world believe that the numbers of phytochemicals present in the plant source having good antioxidant property by free radical scavenging activity [26]. It has been also observed that many plant derived anti-inflammatory compounds possess both anti-inflammatory and free radical scavenging activity. In our *in vitro* antioxidant studies, the reducing power and free radical scavenging effects of *Leucas indica* against DPPH, superoxide, hydroxyl radical was found on a dose dependant manner. It is strongly suggested that the DPPH free radical abstracts the phenolic hydrogen of the electron-donating molecule. From the present study with DPPH it could be inferred that both fractions LIME and LIAE possibly donate proton from the phenolic groups (present in the compounds in the extract) thereby reducing DPPH free radical (Table 4 and Fig 7). However, LIAE shown more potential action at same dose probably due to it's more total phenolic compound contain found in phytochemical screening. The reducing capacity of the fractions further suggests their potential antioxidant activity [27]. Superoxide anion (O_2^-) and hydroxyl radical (OH^+) are produced in many aerobic cells by the mitochondrial respiratory chain, may damage the mitochondrial proteins, membrane lipids and structure of DNA base [28]. Superoxide scavenging activity of LIME and LIAE at the dose of 250 and 500 µg/ml indicates that it will be able to cure such damages and associated diseases (Table 6 and Fig 9). It is known that reactive oxygen species are capable of the activation of a number of intracellular signaling pathways like NF-κβ that further activates the transcription factors for the production of pro-inflammatory cytokines like TNF-α, interleukins, cell adhesion molecules and COX_2 [29]. The significant free radical scavenging activity of aqueous fraction revealed it's probable inhibitory action on the production of pro-inflammatory cytokines by intracellular signaling by NF-κβ pathway. Finally, on the basis of current finding it can be suggested that the anti-inflammatory effects of *Leucas indica* crude extracts are probably due to the free radical scavenging action as well as the dual inhibitory action on COX-LOX enzyme. More percentage of inhibition of LIAE compared to LIME is probably due to more flavonoids and total phenolic contents of the extract found in phytochemical screening.

CONCLUSIONS

From the present studies it can be concluded that the aqueous extract of the plant *Leucas indica* Linn. having more potent anti-inflammatory and antioxidant activity due to its significant content of flavonoids and total phenolic compounds compared to methanolic fraction. So it can be widely used as a potent and safe medication for various types of inflammatory disorders and oxidative stress mediated degenerative diseases.

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