Original Article

Piper pedicellatum C. DC. Leaf Methanol Extract Inhibits Edema and Granuloma and Modulates Mediators and Cytokines in Rats

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ABSTRACT

Background: *Piper pedicellatum* is an ethnomedicinal plant used in various inflammatory conditions by many ethnic groups and exploration of its therapeutic potentials is imperative. Objective: This study was carried out to assess anti-inflammatory potential of the methanol extract of the plant leaves using *in vivo* experiments to validate the traditional uses. **Materials and Methods:** Carrageenan-induced paw edema and Cotton pellet-induced granuloma models were used to investigate into the anti-inflammatory potential of the plant extract in rats at 200 mg/kg and 400 mg/kg dose. Indomethacin (10 mg/kg) is used as reference standard. Serum levels of inflammatory markers and cytokines, hematological and serum hepatic enzyme indices were analyzed in granuloma-induced model. **Results:** The methanol extract suppressed Carrageenan-induced paw edema significantly at different time intervals. The highest percentage inhibition was observed at 3rd hr after Carrageenan injection, which was 42.67% and 49.33% for 200 mg/kg and 400 mg/kg dose of the extract respectively. In granuloma-induced model, the methanol extract at 200 mg/kg and 400 mg/kg dose inhibited exudate formation by 28.58% and 41.27%, respectively and inhibition of granuloma tissue was 32.45% and 43.71%, respectively along with significant decrement in serum C-reactive protein, Prostaglandin E2, Tumor necrosis factor-α, Interleukin-6 while increases in Interleukin-10 levels. Also, the results showed hematological and serum hepatic enzyme levels were rectified to near normal levels in treated rats. **Conclusion:** The outcome of the investigation reveals the potential of extract as possible anti-inflammatory agent, which can be develop into therapeutic agent or supplement and also necessitates further pharmacological studies on the plant.

Keywords: Cytokines, Edema, Granuloma, Interleukins, *Piper pedicellatum* C. DC.

INTRODUCTION

Piper pedicellatum C. DC. (Family Piperaceae) is a shrub grows in foothills and mostly distributed across India (Arunachal Pradesh, Assam, North Bengal and Sikkim), Bhutan, China and Bangladesh. The leaves and young shoots of the plant are used in inflammatory conditions like internal body pains, fever, gastrointestinal ailments, abscesses and allergy.[1,2] Varying levels of antioxidant activities by some *in vitro* methods along with phenolic, flavonoid and flavonol content were reported in different extracts of the plant.[3] The plant reported to contain different types of sesquiterpenes and monoterpenes and phytosterols.[4,5] However there is lack of sufficient scientific evidence about the bioactivity of the plant. This study was designed to investigate the methanol extract of the plant leaves to evaluate its anti-inflammatory property by *in vivo* methods in inflammatory conditions for the pharmacological exploration of this plant.

MATERIALS AND METHODS

Reagents

Carrageenan was obtained from Sigma-Aldrich (St. Louis, Missouri, USA). Indomethacin was purchased from Merck Life Sciences (Bangalore, India). Rat CRP ELISA kits were procured from Sigma-Aldrich (St. Louis, Missouri, USA). Rat PGE2 ELISA kits were purchased from Cayman Chemical Company (Michigan, USA). Rat TNF-α ELISA kits were acquired from BD Biosciences (San Jose, CA, USA). Rat IL-6 and IL-10 ELISA kits were purchased from R to D Systems (Minneapolis, USA). ALT, AST and ALP kits were procured from Sigma-Aldrich (St.

Louis, Missouri, USA). Other chemicals and solvents used were of analytical grade.

Collection of plant materials and extraction

The leaves of *Piper pedicellatum* C. DC. was collected from the regions of Papum Pare district of Arunachal Pradesh, India. The plant material was authenticated by taxonomist at Department of Forestry, North Eastern Regional Institute of Science and Technology, Nirjuli, Arunachal Pradesh, India, and a herbarium specimen (No. DU-Ph.Sc. 05) is preserved. The collected plant material was cleaned, shade dried, pulverized and further subjected to successive extraction with petroleum ether, chloroform and methanol respectively in a Soxhlet apparatus and solvents were evaporated *in vacuo* (Rotavapour RII, Buchi Labortechnik AG, Switzerland). In this study, the Methanol Extract of the *Piper pedicellatum* C. DC. Leaves (PPLME) was used to evaluate *in vivo* anti-inflammatory potential in rats.

Experimental animals

Adult Swiss albino mice (20-30g) and Wistar rats (140-180g) of either sex were used in the study. The animals were housed in polypropylene cages, at temperature of 27±2°C, 12 hr light/dark cycles and controlled humidity conditions. The animals were fed with standard pellet diet and water *ad libitum*. The food was withdrawn 18 hr before and during the experiment, except water *ad libitum*. The procedures and protocols performed during the experiments were approved by Institutional Animal Ethical Committee (approval No. IAEC/DU/189, Regd. No 1576/GO/ERe/S/11/CPCSEA) under the Department of Pharmaceutical Sciences, Dibrugarh University, Dibrugarh, Assam, India.

Acute toxicity study

The acute oral toxicity study was conducted on Swiss albino mice and carried out as per the guideline set by the Organization for Economic Cooperation and Development (OECD guidelines 425).[6] The test procedure requires less number of animals for assessment of acute oral toxicity. Albino Mice (female, *n*=5) were administered PPLME at limit dose of 2000 mg/kg body weight by orogastric intubation. Signs of toxicity (like tremors, convulsions, salivation, diarrhea, lethargy, etc.) behavioral pattern and mortality were closely observed at 1, 4 and 24 hr after the dose and monitored carefully each day for up to 14 days for any signs of delayed toxicity and mortality. No signs of toxicity or mortality was noticed. Therefore, the LD_{50} is considered as > 2000 mg/kg.

Study of *in vivo* anti-inflammatory activity

Carrageenan-induced paw edema model in rats

The rats were divided into four groups (*n*=6) each receiving vehicle (negative control), Indomethacin 10 mg/kg *p.o.* (reference standard), and PPLME 200 mg/kg *p.o.* and 400 mg/kg *p.o.* dose respectively one hour before Carrageenan injection. Carrageenan

(0.1 mL of 1%w/v suspension) was injected into the subplantar tissue of the right hind paw of each rat.[7,8] The paw volume of the carrageenan injected foot was measured at 1st, 2nd, 3rd, 5th and 24th hr by using digital plethysmometer (PLM-01 plus, Orchid Scientific, India). The Percentage Inhibition (PI) of paw edema at different time interval was calculated as:

$$PI = \frac{Vc - Vt}{Vc} \times 100$$

Where, Vc is mean increase in paw volume of negative control group and

Vt is mean increase in paw volume of treated group

Cotton pellet-induced granuloma model in rats

The rats were divided into five groups (*n*=6). Group I (normal control) and Group-II (negative control) receives vehicle. Group-III (reference standard) receives Indomethacin 10 mg/kg *p.o* and Group IV and Group V receives PPLME at dose of 200 mg/kg and 400 mg/kg *p.o.*, respectively. The rats were anesthetized, aseptically implanted two cotton pellets (sterilized, 10±1.0 mg each) bilaterally in the axilla region of the rats, under anesthesia (except group I). Drugs/vehicle were dosed once daily for the 7 days.[7,8] The rats were sacrificed on 8th day by euthanasia and blood samples was collected for the estimation of hematological and serum parameters. Cotton pellets were removed surgically, extraneous tissues were emancipated, then pellets weighed and dried at 60°C in a hot-air oven for constant weight. The wet weight and dry weight of the pellets were recorded to estimate exudate and granuloma formation respectively. The Percentage Inhibition (PI) of exudates/granuloma tissue was calculated as:

$$PI = \frac{\text{Pellet weight (negative control)} - \text{Pellet weight (treated)}}{\text{Pellet weight (negative control)}} \times 100$$

Further, the concentrations of CRP, PGE2, TNF-α, IL-6, IL-10, ALT, ALP and AST in serum of rats were estimated by using commercially available Kits (ELISA/Colorimetric) by conforming to the manufacturer's instructions.

Statistical Analysis

The results were expressed as mean ± SEM. The data were analyzed by one way Analysis of Variance (ANOVA) followed by Dunnett's multiple comparison test for statistical significance. The difference was considered to be significant at $p<0.05$ when compared to the negative control group.

RESULTS

PPLME suppresses rat paw edema

Rats injected with Carrageenan showed increase in paw volume exhibited by marked peripheral edema in the injected

paw. In PPLME administered rats, paw volume is decreased significantly in dose-dependent manner at different time intervals as compared to negative control rats. The significant reduction also reflects in standard group (Indomethacin treated) rats. The highest percent inhibition was observed at 3rd hr after Carrageenan injection. PPLME at 200mg/Kg and 400 mg/kg dose reduced the Carrageenan induced edema by 42.67% ($p<0.01$) and 49.33% ($p<0.01$) respectively and Indomethacin inhibited 53.33% ($p<0.01$) as compared to the negative control group at 3rd hr (Table 1).

PPLME suppresses exudates and granuloma formation

In Cotton pellet-induced granuloma model, PPLME has effectively inhibited both the exudatory and granulatory phases of inflammation compared to negative control group. At the doses of 200 mg/kg and 400 mg/kg, PPLME inhibited exudate formation by 28.58% ($p<0.01$) and 41.27% ($p<0.01$) respectively and inhibition of granuloma tissue was found to be 32.45% ($p<0.05$) and 43.71% ($p<0.01$), respectively. The standard drug Indomethacin (10mg/kg) showed 43.45% ($p<0.01$) and 46.19% ($p<0.01$) reduction in the weight of exudate and granuloma tissues, respectively (Table 2).

PPLME suppresses inflammatory marker CRP

Inflammation induction in cotton pellet induced rats caused elevated levels of serum CRP. Administration of Indomethacin and PPLME at dose 200mg/kg and 400mg/kg significantly decreases ($p<0.01$) serum CRP levels to 12.34±0.65 µg/mL, 21.23±1.27 µg/mL and 14.31±1.15 µg/mL respectively in treated rats as compared to negative control rats (31.72±1.14 µg/mL) in cotton pellet-induced granuloma model (Figure 1).

PPLME suppresses pro inflammatory mediator PGE2

The increase in serum PGE2 levels in cotton pellet implanted rats suggested that obvious inflammation response was induced in the rats and PPLME could regulate the release of inflammatory mediators. PPLME significantly decreases serum PGE2 levels in dose dependent manner at 200mg/kg (2.43±0.15 pg/mL, $p<0.01$) and 400mg/kg (1.75±0.11 pg/mL, $p<0.01$) as compared with negative control rats (3.12±0.13 pg/mL). Significant decrease (1.31±0.14 pg/mL, $p<0.01$) was also in observed in Indomethacin treated rats (Figure 2).

PPLME suppresses pro-inflammatory cytokines TNF-α and IL-6

Induction with cotton pellet granuloma caused increase release of pro-inflammatory cytokines TNF-α and IL-6 in rats. Treatment with PPLME shows attenuation of the serum TNF-α and IL-6 levels against negative control rats. At the doses of 200mg/kg and 400mg/kg, PPLME significantly decreases ($p<0.01$) serum TNF-α to 2.25±0.14 pg/mL and 1.44±0.12 pg/mL respectively when compared to negative control rats (3.76±0.13 pg/mL). PPLME treatment with 200mg/kg and 400mg/kg dose also significantly decreases the serum IL-6 levels to 0.93±0.13 pg/mL ($p<0.05$) and 0.63±0.16 pg/mL ($p<0.01$) respectively as against negative control rats (1.67±0.23 pg/mL). Meanwhile, decrease in the serum TNF-α and IL-6 was 1.35±0.14 pg/mL and 0.54±0.17 pg/mL, respectively in Indomethacin treated rats (Figures 3 and 4).

PPLME elevates anti-inflammatory cytokine IL-10

The results revealed PPLME 200 mg/kg and 400 mg/kg dose and Indomethacin treatment strikingly increases the serum IL-10 levels to 23.71±0.23 pg/mL ($p<0.01$), 34.92±0.21 pg/mL ($p<0.01$) and 37.23±0.22 pg/mL ($p<0.01$) respectively as compared to negative control rats (16.64±0.25 pg/mL) in cotton pellet-induced granuloma model (Figure 5).

PPLME rectifies hematological parameters

The negative control group rats showed attenuated RBCs and Hb levels and elevated levels of WBCs and ESR level in blood in contrast to the normal control rats in cotton pellet induced granuloma model. However, PPLME and Indomethacin treatment provides significant protection in rats and positively changes the values from abnormal alterations in hematological indices (Table 3).

Table 1: Effect of PPLME on Carrageenan-induced paw edema in rats

SI. No.	Group	Paw volume after carrageenan injection (mL)				
		1 hr	2 hr	3 hr	5 hr	24 hr
01.	Negative control	0.54±0.03	0.61±0.02	0.75±0.03	0.59±0.04	0.45±0.02
02.	Indomethacin (10 mg/kg)	0.44±0.02 (18.52%)	0.41±0.01** (32.77%)	0.35±0.03** (53.33%)	0.32±0.02** (45.76%)	0.26.±0.03** (42.22%)
03.	PPLME (200 mg/kg)	0.48±0.01 (11.11%)	0.49±0.05 (19.67%)	0.43±0.03** (42.67%)	0.40±0.04** (32.2%)	0.34±0.05 (24.44%)
04.	PPLME (400 mg/kg)	0.45±0.03 (16.67%)	0.42±0.03** (31.15%)	0.38±0.04** (49.33%)	0.35±0.03** (40.68%)	0.28±0.02** (37.78%)

Values are expressed as mean ± SEM (n=6).**$p< 0.01$ indicates statistical significance when compared with negative control group.

Table 2: Effect of PPLME on exudate and granuloma formation in rats.

Sl. No.	Group	Weight of exudates (mg)	% Inhibition (exudate)	Weight of granuloma tissue (mg)	% Inhibition (granuloma)
01.	Normal control				
02.	Negative control	176.37±6.32		63.46±5.16	
03.	Indomethacin (10 mg/kg)	99.74±4.51**	43.45	34.15±3.65**	46.19
04.	PPLME (200 mg/kg)	125.97±7.74**	28.58	42.87±4.34*	32.45
05.	PPLME (400 mg/kg)	103.59±6.19**	41.27	35.72±5.16**	43.71

Values are expressed as mean ± SEM (n=6). *p<0.05, **p<0.01 indicate statistical significance when compared with negative control group.

Table 3: Effect of PPLME on alteration of hematological and serum enzyme parameters in granuloma-induced rats.

Sl. No.	Parameters	Normal control	Negative control	Indomethacin	PPLME (200 mg/kg)	PPLME (400 mg/kg)
01.	HB (gm/dL)	14.31±0.37**	9.24±0.41	13.67.±0.32**	10.71±0.34*	12.27±0.39**
02.	RBCs (x10³/μL)	9.23±0.43**	6.24±0.47	8.97±0.44**	8.14±0.45*	8.73±0.46**
03.	WBCs (x10³/μL)	7.5±0.36**	11.3±0.45	7.9±0.38**	8.9±0.33**	8.3±0.39**
04.	ESR (mm/1ˢᵗ hr)	4.1±0.43**	9.3±0.5	4.5±0.47**	5.3±0.43**	4.7±0.41**
05.	ALT (U/L)	23.16±0.63**	64.32±0.74	33.12±0.67**	45.36±0.7**	37.15±0.65**
06.	AST (U/L)	123.56±0.57**	163.46±0.64	131.14±0.54**	148.52±0.62**	137.43±0.58**
07.	ALP (U/L)	163.25±0.57**	308.34±0.69	169.32±0.63**	214.61±0.61**	174.41±0.54**

Values are expressed as mean ± SEM (n=6).*p<0.05, **p<0.01 indicates statistical significance when compared with negative control group.
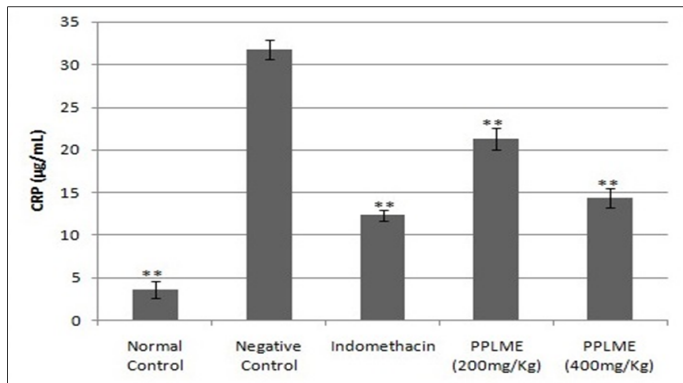

Figure 1: Effect of PPLME on Serum CRP levels. Values are expressed as mean ± SEM (n=6). **p<0.01 indicates statistical significance when compared with negative control group.
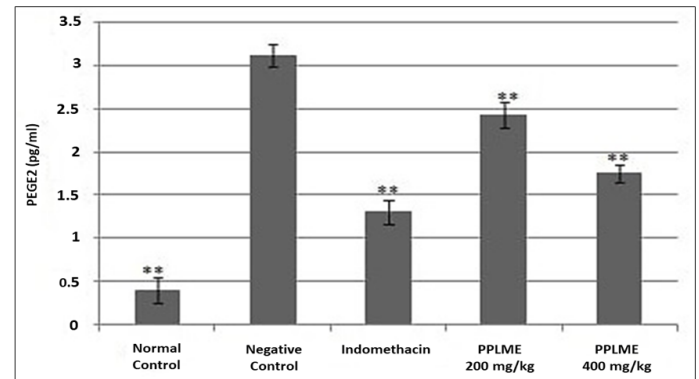

Figure 2: Effect of PPLME on Serum PGE2 levels. Values are expressed as mean ± SEM (n=6). **p<0.01 indicates statistical significance when compared with negative control group.
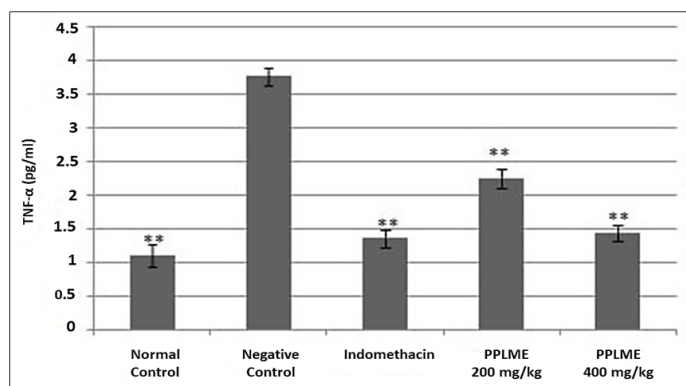
Figure 3: Effect of PPLME on Serum TNF-α levels. Values are expressed as mean ± SEM (*n*=6). ******p<0.01 indicates statistical significance when compared with negative control group.
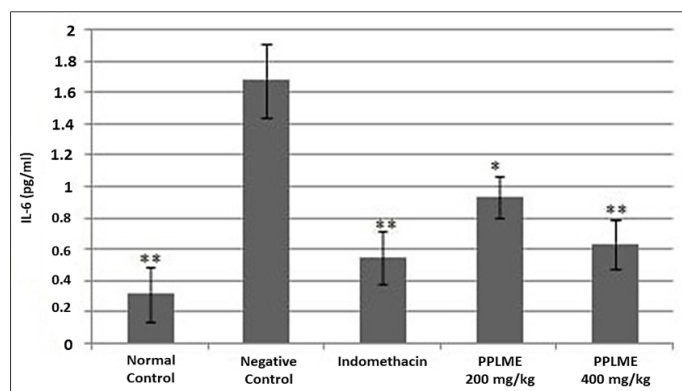

Figure 4: Effect of PPLME on Serum IL-6 levels. Values are expressed as mean ± SEM (*n*=6). **p*<0.05, ***p*<0.01 indicates statistical significance when compared with negative control group.
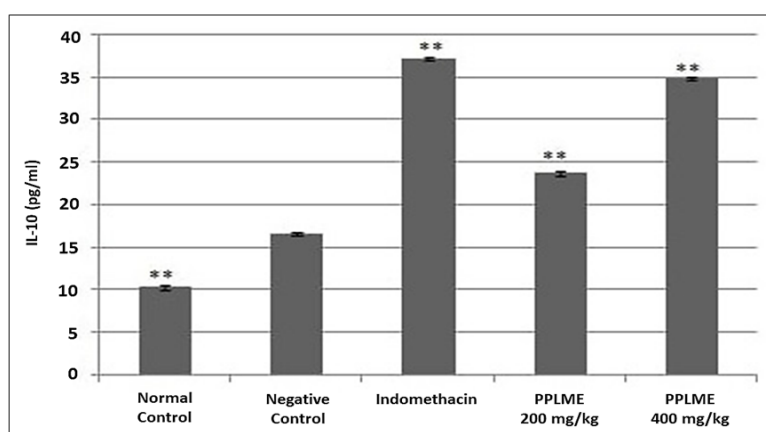

Figure 5: Effect of PPLME on Serum IL-10 levels. Values are expressed as mean ± SEM (*n*=6). ***p*<0.01 indicates statistical significance when compared with negative control group.

PPLME normalize serum hepatic enzymes levels

Increased serum levels of ALT, AST, and ALP enzyme levels were observed in negative control rats in contrast to normal control rats in cotton pellet induced granuloma model. Contrastingly, PPLME treatment dose dependently and Indomethacin significantly restores the serum enzyme values to near normal levels from abnormal rise (Table 3).

DISCUSSION

The current investigations establishes the *in vivo* anti-inflammatory activity of PPLME in the Carrageenan-induced paw edema model and Cotton pellet-induced granuloma model which simulate acute and chronic inflammatory situations in rats respectively.

Carrageenan-induced paw edema model is one of the most accepted phlogistic tool for the pharmacological evaluation of acute inflammation and anti-inflammatory potential of drugs. Carrageenan injection in rat paw causes inflammation by developing cardinal signs of inflammation i.e., edema, erythema and hyperalgesia. The Carrageenan-induced model develops distinct pathophysiological phases of inflammation. The early

phase initiates within one hour and is mainly due to release of cytoplasmic enzymes, histamine, serotonin, from the mast cells. Late phases are caused by prostaglandin like mediators, bradykinin, leukotrienes, polymorphonuclear cells, and also tissue macrophages.[9-11] In the present study, the anti-edematous effect of the PPLME was maintained during the different the phases of edema occurance which was also reflected by Indomethacin. The findings suggest the possible mediators inhibition activity like cyclooxygenase inhibition of the PPLME extract as like that of NSAIDS such as Indomethacin.

The Cotton pellet-induced granuloma model well demonstrates the conditions of sub-acute and chronic inflammation, mainly characterized by the monocyte infiltration, fibroblast proliferation, angiogenesis, exudates formation, etc.[12,13] It has mainly three phases of inflammation. The first (transudative) phase is permeation of low protein content fluid takes place into the inflammation site. The Second (exudative) phase, starts after two to three days, displayed by exudation of fluid containing protein. In the third (proliferative) phase, develops after three days, shows mucopolysaccharide and collagen appearance and fibroblasts increase.[14-16] In this study, the PPLME decreases the

exudates and granuloma weight which indicates its ability to the inhibit exudative and proliferative phases of inflammation.

Inflammatory process involves a complex network of large number of inflammatory markers, mediators and cytokines and interconnected positive and negative feedback loops that augment or inhibits the inflammatory responses and signs.[17] CRP is an acute plasma phase protein and considered as a clinical marker of inflammation, also plays major role in inflammatory process, which is upregulated during inflammation, tissue damage and infections. Inflammatory cytokines like IL-1 and IL-6 plays vital role in CRP gene induction and regulation.[18,19] PGE2 is one of the major pro-inflammatory lipid mediator. Degradation of membrane phospholipid produces arachidonic acid further forms PGE2 catalyzed by COXs and PGE synthetases. PGE2 by various intricate mechanisms, triggers vasodilation, fluid extravasation and leukocyte recruitment which develops symptoms like redness, swelling and pain in tissues. Anti-inflammatory drugs decreases PGE2 levels by mainly inhibiting COX activities.[20,21]

In the course of inflammation, the increased release of the pro-inflammatory cytokines, such as TNF-α, IL-1β, IL-6, etc. leads to the exacerbation of inflammatory conditions.[22,23] TNF-α and IL-6 in particular, induces the secretions of other cytokines, chemokines and pro-inflammatory mediators like PGE2 and nitrous oxide and plays central role in the development of inflammation.[24,25] TNF-α inhibition suppress the release of IL-1β, IL-6, and IL-8, thus inhibiting the release of other inflammatory mediators like COX-2 and PGE2, which in turn inhibits inflammation and edema.[26] Conversely, cytokines such as IL-10 implicit anti-inflammatory effect, by inhibiting expressions of pro-inflammatory cytokines.[27] Reports has explored that IL-10 possesses ability to reduce the inflammatory cytokines IL-1, TNF-α, IL- 6, IL-8, and IL-12, and also to suppress the synthesis of nitric oxide, gelatinase, and collagenase.[28]

Thus, inhibition of pro-inflammatory mediators and cytokines and increased IL-10 is considered as practical therapeutic strategy to control inflammatory disorders. In the present study, the anti-inflammatory effect of PPLME as like that of Indomethacin (NSAIDs) in the Carrageenan-induced paw edema and Cotton pellet-induced granuloma experiment appears to result from the modulation of key mediators and cytokines in inflammatory pathogenesis, such as decreasing the levels of CRP, PGE2, TNF-α, IL-6, IL-1β while increasing the level of IL-10 in serum.

Alterations in basic hematologic indices such as Hb content, RBC count, WBC count, and ESR have been associated with inflammation pathobiology. Anemia (decreased Hb content and RBC count) is one of the implications of sub-chronic and chronic inflammatory conditions. Anemia in inflammation is multifactorial, develops mainly due to decreased erythropoietin levels, increased erythrocyte destruction, decreased plasma iron, disturbances in reticuloendothelial system leading to

failure of bone marrow to counter anemia.[29] Inflammatory cytokines, particularly TNF- α also reported to modulate macrophage iron metabolism and causes bone marrow erythropoiesis inhibition.[30] Erythrocyte Sedimentation Rate (ESR) as a biomarker of inflammation, is altered as sequels of rise in the plasma concentrations of acute-phase reactant proteins such as fibrinogen, α- and β-globulin in response to inflammation. Elevated levels of ESR indicated presence of active inflammation.[31] Elevated levels of total leukocyte (WBC) count is considered as harbinger of systemic inflammation and diseases progression. During inflammatory responses, infiltration of WBCs from circulatory system is essential process, which is stimulated by various chemotactic agents, interleukins, complement system, histamines and other factors.[32,33]

Therefore, examining the influence of test agent in rectifying altered hematologic indices would be of great value for developing a new therapeutic approach for inflammatory conditions. The results of the study revealed that PPLME and Indomethacin treatment augmented Hb and RBC levels, thereby reversing the anemic condition possibly by stimulating erythropoiesis, and by arresting TNF-α release. Decrease of ESR and WBC count from abnormal raise by PPLME and Indomethacin treatment displayed recovery from inflammatory process along with reduction in leukocyte migration.

Furthermore, serum hepatic enzyme (ALT, ALP and AST) estimations are used as conventional markers in diagnostic evaluation of liver function and anti-inflammatory drug screening process. Inflammatory processes and disease progression has positive correlation with elevated serum hepatic enzyme levels. Abnormal rise of serum hepatic enzyme activities are considered as indicators of hepatocellular tissue injury as a consequence from oxidative and inflammatory stressors, which can cause increased release of these cytoplasmic enzymes into the blood circulation.[34,35] In the current study, higher levels of ALT, AST and ALP from baseline level in negative control rats is a reflection of hepatic derangement. However, PPLME treated rats shows significant decrement in elevated serum hepatic enzymes in dose dependent manner to near normal levels, which reflects simultaneous anti-inflammatory and hepatoprotective effect of PPLME.

CONCLUSION

The current study suggested that the PPLME possess effective anti-inflammatory property in both paw edema and granuloma models used and also gives insights into its mechanism of anti-inflammatory action. The anti-inflammatory mechanisms of PPLME is due to its potential to inhibit and modulate various inflammatory mediators and cytokine pathways. Furthermore, the study provide a scientific background for the validity of the traditional use of the plant leaves in inflammatory conditions.

However more detailed studies are required for the further exploration of the therapeutic properties hidden in the plant.

ETHICS APPROVAL

The Institutional Animal Ethical Committee of Dibrugarh University, Dibrugarh, Assam, India approved the experimental protocol (Approval No. IAEC/DU/189).

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

AUTHORS CONTRIBUTIONS

DJK conceptualized, designed the study, experiments, data analysis, manuscript preparation. KD performed experiments and analyzed the data. NS performed experiments and analyzed the data. SD performed experiments, analyzed the data and manuscript preparation. BBK designed the study, manuscript preparation and supervision.

ABBREVIATIONS

ALT: Alanine transaminase; **ALP:** Alkaline phosphatase; **AST:** Aspartate aminotransferase; **COX:** Cyclooxygenase; **CRP:** C-reactive protein; **ELISA:** Enzyme-linked immunosorbent assay; **ESR:** Erythrocyte sedimentation rate; **NSAID:** Non-steroidal anti-inflammatory drugs; **PGE2:** Prostaglandin E2; **TNF:** Tumor necrosis factor; **PPLME:** *Piper pedicellatum* C. DC. Leaf methanol extract; **Hb:** Hemoglobin; **IL:** Interleukin; **RBC:** Red blood cells; **WBC:** White blood cells; **LD$_{50}$:** Lethal dose 50; **SEM:** Standard error of mean; **L:** Litre; **dL:** Decilitre; **μL:** Microlitre; **mm:** Millimetre; *p.o.*: per oral; **U:** Unit; **pg:** Picogram; **μg:** Microgram.

SUMMARY

The methanol extract of the plant leaf was prepared by successive solvent extraction and investigated for *in vivo* anti-inflammatory activity in rats by Carrageenan-induced paw edema and Cotton pellet-induced granuloma models. The methanol extract inhibited the paw edema in rats at different time intervals and attenuates exudates and granuloma formation in rats. The methanol extract de-escalated key inflammatory mediators and pro-inflammatory cytokines and interleukins levels, also increases the anti-inflammatory interleukin levels in serum, which provides insight into the mechanism of its anti-inflammatory action. Treatment with methanol extract also corrected disturbed hematological and serum hepatic enzyme parameters to near normal values. The research work validates the ethno medicinal use of the plant leaves in inflammatory conditions.

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