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Chemical composition, anti-inflammatory and antioxidant activities of the essential oil of *Piper cubeba* L.

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Abstract

Piper Cubeba (L.) is used as a remedy for various ailments. However, the scientific basis for its medicinal use, especially as anti-inflammation remains unknown. Therefore, the present study aims to investigate the anti-inflammatory and antioxidant activities of Piper cubeba essential oil (PCEO) in laboratory rodent models. The in vivo anti-inflammatory activity of PCEO at three doses (150, 300 and 600 mg /kg, p.o) was tested in carrageenan-induced rat paw edema, cotton pellet granuloma and carrageenan-induced pleurisy. The mechanism of inflammation using carrageenan-induced pleurisy in rat was further studied. The in vitro antioxidant activity was examined using DPPH. In parallel to that the oil was analyzed using GC/MS. PCEO at 600 mg/kg reduced the paw edema considerably (65%) and the weight of cotton pellet granuloma (46%). The pretreatment with PCEO also reduced exudate volume. The number of Polymorphonuclear (PMN) cells was reduced. Furthermore, dose dependent reduction in myeloperoxidase (MPO), nitric oxide and proinflammatory cytokine such tumor necrosis factor (TNFα) and interleukin-1 (IL-1β) were observed and supported by histological observation. Moreover, PCEO exhibited promising strong antioxidant activity. The GC/MS uncovered that monoterpenes e.g. sabinene, 4-terpineol, γ-terpinene and α-thujene were the fundamental part of the oil which could be in charge for the demonstrated activities.

Keywords: *Piper cubeba,* essential oil, anti-inflammatory, antioxidant, **p**roinflammatory cytokines

1. Introduction

Inflammation is a typical defensive reaction to tissue damage (wounds, cuts or burns), toxic chemicals, microbial organisms or even immune system illness. It is the most widely recognized reason for clinical pain. It is categorized as either acute or chronic, contingent upon whether it includes a short reaction or a delayed one, individually. Unfortunately, the medications as of now accessible to treat inflammation are connected with various symptoms and low effectiveness, particularly for chronic diseases (MOTHANA [1]). Along these lines, natural products including medicinal plants, with high efficacy and low side effects, are attractive as substitutes for chemically synthesized therapeutics. In spite of the enormous technological progression in pharmaceutical industry and medical implementation, the World Health Organization appraises that 80% of the world's occupants still depend for the most

part on traditional medication using medicinal plants for their human health services (GURIB-FAKI [2]). The investigation of natural products incorporating volatile oils utilized as a part in the folk medicine as anti-inflammatory agents ought to at present be seen as a productive research strategy, in the quest for anti-inflammatory drugs (CALIXTO & al. [3]).

Essential oils (EOs) or volatile oils (VOs) are concentrated natural complex aromatic compounds formed as secondary metabolites by aromatic plants. EOs are important ingredients in cosmetics, perfumes and are used for flavoring food, beverages and in pharmaceutical industries (MIGUEL [4]; ESPERNDIM & al. [5]). They are also known since ancient times for their antioxidant, antibacterial, antifungal, anti-inflammatory and many medicinal properties as well as in preservation of foods. They are used as pain killers, to reduce swelling and to relieve abdominal cramps (BAKKALI & al. [6]). In addition, they are also reported to be useful in viral diseases, cancers and as antioxidant agents (ADORJAN [7]). *Piper cubeba* L., a member of Piperaceae family, is valued as a spice and medicinal plant. In Arab traditional medicine, the essential oil of *P. cubeba* is used to cure various ailments including rheumatism, cough and intestinal disorders (BOS & al. [8]). Furthermore, the oil is reported to possess anti-leukemic, tumor inhibitory, antibiotic and antiparasitic potential (BOS & al. [8]). In addition to that, several extracts of the berries as well as of the leaves showed different pharmacological activities. An aqueous extract of *P. cubeba* showed a potent inhibitory activity on cytochrome P450 3A4 (GHOSH & al. [9]). Previous study demonstrated antigastric ulcer potential of an aqueous suspension of *P. cubeba* (ALSAID & al. [10]). Some constituents of *P. cubeba* fruits have also been shown to possess gastroprotective activity (ALSAID & al. [10]; MORIKAWA & al. [11]). Since there is no scientific data available so far on antioxidant and antinflamatory activities of *Piper cubeba* essential oil (PCEO), therefore the present study was undertaken to investigate the chemical composition, *in vivo* antinflamatory and *in vitro* antioxidant of *Piper cubeba* essential oil. To our knowledge, this is the first attempt addressing such ethno pharmacological properties of *Piper cubeba* essential oil in a comprehensive manner.

2. Materials and Methods

Plant materials

Piper cubeba fruits were purchased from a local herbal medicine material shop in Riyadh, Saudi Arabia. They were identified by an expert taxonomist (Dr. Mohammed Yusuf) of the Department of Pharmacognosy, College of Pharmacy, King Saud University, Riyadh. A voucher specimen (# 123) was deposited in the crude drug museum of the college.

Extraction of the essential oil

The dried and crushed fruits of *P. cubeba* were hydro-distilled for 3 h by utilizing Clevenger-type apparatus as indicated by the European Pharmacopoeia. The water was removed from the acquired oil by anhydrous sodium sulfate then the oil was filtered and preserved at +4°C until time of analyzing and testing.

Gas chromatography-mass spectrometry analysis (GC-MC)

The chromatographic examinations were directed on a Hewlett-Packard 5890 series II gas chromatograph connected to a VG Analytical 70-250S mass spectrometer. The gas chromatograph (GC) was furnished with a fused silica capillary Elite-5MS column (30 m × 0.25 mm i.d., film thickness 0.25 μm, from Perkin Elmer, USA). Helium was utilized as a transporter gas at a stream rate of 1 ml/min. Oven temperature was customized from 60°C (2 min hold) at 10°C/min to 300°C and lastly held isothermally for 20 min. Injector temperature was 200°C. An election impact ionization system, with ionization energy of 70 eV was utilized for GC-MS detection. A scan rate of 0.6 sec (process duration: 0.2 second) was connected, covering a mass extent from 35 to 600 m/z.

Animal experimentation

Male Wistar rats (180-200 g) were gotten from the Experimental Animal Care Center, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia. The rats were kept at 23ºC and a 12 hours light/dark cycle. All rats were freely fed standard certified laboratory diets and tap water. The study protocol (CBR 4537) was approved by the Research Ethics Committee of College of Pharmacy, King Saud University. The rats were taken care of as per the Guide for the Care and Use of Laboratory Animals by the Animal Care Center.

Acute toxicity assay

Male Wistar rats were separated into test bunch containing of six animals in every gathering. The test was performed utilizing increasing oral dose of PCEO from 50 mg/kg to 3000 mg/kg of rats. During the first hour of testing, the rats were watched persistently and afterward half hourly for 4 hours for any overall behavioral alteration and common motor changes such as convulsion, shaking, writhing, response to tail pinching, chewing, pupil size, fecal output, feeding behavior, nourishing conduct, and so on. Furthermore, any mortality was daily recorded for three days. The oil did not bring on any noteworthy behavioral changes and no mortality was watched. Along these lines three doses 150, 300 and 600 mg/kg p.o were selected for anti-inflammatory experiments.

Studies on anti-inflammatory activity

Acute inflammation study (Carrageenan-induced rat paw edema)

Pedal inflammation in male albino rats (8 to 10 weeks old), weighing (180-200 g) was incited according to the procedure applied by (WINTER & al. [12]). 0.05 ml/kg of carrageenan sodium salt solution (1%) was injected subcutaneously into the right hind paw of each rodent under the subplantar aponeurosis. The rats were partitioned into five gatherings of six animals each. Group I was the control group and received 0.5 ml of normal sterile saline solution. Group II to IV were treated p.o. with (150, 300 and 600 mg/kg p.o.) of the PCEO, 1 h before the carrageenan injection. The rats in group V received 100 mg/kg (b.w., p.o.) of phenylbutazone as positive control. After injection, a plethysmometer (Hugo basil, Italy) was used to measure the paw volume at 0 h and 3 h. The inhibitory activity was ascertained by taking after the following formula:

$$Percent \quad inhibition = \left(1 - \frac{a - x}{b - y}\right)$$

a and x = the mean paw volume of treated animals before and after carrageenan injection, respectively; *b and y* = the mean paw volume of control animals before and after injection, respectively.

Chronic inflammation study (Cotton pellet granuloma in rats)

The methods of Goldstein and his colleagues (GOLDSTEIN & al. [13]; GOLDSTEIN & al. [14]) were applied with a minor modifications. 30 mg of cotton-pellet were sterilized then embedded subcutaneously in the groin region of the rats. The rats were separated into five gatherings of six animals each. The rats in the control group (group I) received normal saline. Group II-IV was treated p.o. with (150, 300 and 600 mg/kg p.o.) body weight of PCEO once daily for four consecutive days. Group V was given 100 mg/kg of phenylbutazone (b.w., p.o.), as a positive control. On the fifth day, all rats were sacrificed then the sterile cotton-pellets were evacuated, liberated from unessential tissues, and weighted after drying at 60°C for 12 h. Ultimately the rate of the inhibition of chronic inflammation was figured and looked at. In general, the weight of the amount of granulomatous tissue formed is the variation between the initial and last weights.

Carrageenan-induced pleurisy in rats

The rats were grouped into five groups (N=5): Group I received 0.5% sodium carboxymethyl cellulose in saline solution, Group II received only carrageenan and served as toxic control, Group III-V received pretreatment of PCEO (150, 300 and 600 mg/kg) were managed orally

once day by day and went on for 4 days. Control and Carrageenan groups were treated with a proportional volume of sodium carboxymethyl cellulose solution (0.5%). On the fourth day, 0.5 ml of saline containing 1% carrageenan (Sigma, USA) or saline without carrageenan was injected into the pleural cavity under anesthesia. The animals were sacrificed at 4 h, after the injection with carrageenan. Two ml of sterile saline solution containing heparin (5 U/ml) was used to rinse the pleural cavity of chest. The exudate was expelled and the aggregate volume measured. The outcomes were figured by subtracting the volume injected from the aggregate volume recuperated. The cell pellet was separated from the samples by centrifuge and resuspended in phosphate buffer saline. The exudates were stained with Giemsa staining on cell counting chamber. The total count of neutrophils was enumerated by light microscope.

Determination of Myeloperoxidase MPO level of lung tissue

Myeloperoxidase (MPO) examination was used as a part of which neutrophil recruitment was indirectly estimated by means of MPO activity and measured by a according technique depicted formerly by (CAMPOS & al. [15]). Lung tissues were mixed in EDTA/NaCl buffer (pH 4.7) and centrifuged at 10,000 rpm for 15 min at 4°C. 0.5% hexadecyltrimethyl ammonium bromide buffer (pH 5.4) was used to resuspend the pellet. The suspensions were frozen and defrosted three times in liquid nitrogen. After defrosting, the suspensions were centrifuged at 10,000 rpm for 15 min. at 4°C. To perform the MPO test, 25 µl of the supernatant were treated with 1.6 mM tetramethylbenzidine, 80 mM $NaPO_4$, and 0.3 mM hydrogen peroxide. The absorbance of enzymatic response was measured at 690 nm and the outcomes were expressed as optical density per mg of tissue.

Determination of Proinflammatory Cytokines

A colorimetric ELISA kit (R&D systems, USA) was used to analyze TNF-α and IL-1β concentrations in lung tissues. The test was carried 4 h after prompting of pleurisy via carrageenan.

Determination of Nitric oxide

Nitric oxide Production was measured by evaluation of its related final products, nitrite/nitrate. In this test, nitrate was changed over to nitrite by nitrate reductase and total nitrite was measured utilizing the Griess reaction (GREEN & al. [16]). Briefly, lung tissue were reacted with FAD (5 mM), nitrate reductase (0.2 U/ml), and NADPH (50 mM) at 37°C for 20 min. To halt the reaction, lactate dehydrogenase (0.24%) and sodium pyruvate (10 mM) were added at 37°C for 5 min., after that the precipitation was performed with $ZnSO_4$ solution (1.4%). Griess reagent (sulphanilamide (1%), PO_4H_3 (2.5%), n-naphthyl-ethylene-diamine (0.1%)) reacted with total nitrite at 37°C for 10 min., and was perused utilizing the 540 nm filter in a titrated Biotek ELISA reader.

Histological examination of the lungs

The lung tissues were fixed in formalin solution (10%) at 25°C for a week. The tissues dried out and hence implanted in paraffin blocks. Sections were deparaffinized with xylene, and stained with hematoxylin-eosin. All thin sections were examined utilizing optical microscopy.

Determination of antioxidant activity

For the determination of the antioxidative efficacy, DPPH free radical scavenging assay was utilized to quantify the antioxidant activity. The technique was done as depicted by (BRAND-WILLIAM & al. [17]). Briefly, numerous concentrations of PCEO (10, 50, 100, 500 and 1000 µg/ml) were prepared in methanol. Ascorbic acid was used as a positive control. 500 µl of the oil, 125 µl prepared DPPH (1 mM in methanol) and 375 µl solvent (methanol) were mixed and incubated at 25°C for 30 min. The diminishing in absorbance was measured at λ = 517 nm. The radical scavenging activity was figured from the equation:

$$\% \text{ of radical scavenging activity} = Abs_{control} - Abs_{sample} / Abs_{control} \times 100$$

Statistical analysis

The data were arranged as Means±SEM. Multiple comparison procedure (Tukey post hoc test) in one-way ANOVA was used to determine the significant difference between

groups. A *p*-value of less than 0.05 was considered significant. All statistical examinations were carried out utilizing GraphPad Prism 5 (Graphpad Software, Inc., San Diego, CA).

3. Results and discussion

The present study evaluated the *in vivo* anti-inflammatory and *in vitro* antioxidant activity of essential oil of *Piper cubeba* L. a valued spice and medicinal plant. Hydro distillation of the berries of *Piper cubeba* afforded yellow oil with a yield of 9.6% (w/w) on dry weight basis. The retention times, percentage composition and identification methods are shown in Table 1, where the identified components are listed in order of their elution on the Elite-5MS column. The GC-MS analysis led to the identification of 32 compounds representing 93.4% of the total oil of *P. cubeba*. The monoterpene hydrocarbones (61.5%) and oxygenated monoterpenes (22.9%) were determined as the main groups, among which sabinene (46.3%), 4-Terpineol (17.0%), γ-Terpinene (4.2%) and α-Thujene (2.6%) were found to be the main compounds (Table 1).

The results of the acute anti-inflammatory effect of the investigated PCEO on carrageenan-promoted edema in hind paws of the rats appear in Table 2. Carrageenan affected paw edema stayed even 3 h after its injection into the subplantar area of rat paw. PCEO demonstrated a significant ($P < 0.001$) decrease in the edema paw volume in a dose-dependent manner (Table 2). Remarkably, PCEO at the doses of 300 and 600 mg/kg showed the highest anti-inflammatory activity where the edema formation was inhibited to the extent of 58.1 and 65.2% respectively. Phenylbutazone as a reference drug (100 mg/kg) showed a significant inhibitory effect (75.85%). The results of the chronic anti-inflammatory activity of the investigated PCEO against cotton pellet-induced granuloma are displayed in Table 2. PCEO demonstrated a noteworthy ($P < 0.001$) decrease in weight of cotton pellet granuloma in a dose-dependent manner. The data given in Table 2 demonstrate that PCEO at the dose of 600 mg/kg was fit to demonstrate the greatest granuloma inhibition (46.5%), Phenylbutazone (100 mg/kg) showed the most astounding decrease in weight of cotton pellet granuloma (69.9%).

Table 1. Essential oil composition of PCEO

No.	Compounds	RT	Area (%)	Identification
1	α-Thujene	10.4	2.6	1,2
2	α-Pinene	10.7	0.8	1,2,3
3	Sabinene	12.7	46.3	1,2
4	β-Myrcene	13.3	0.9	1,2
5	Phellanderen	14.1	0.3	1,2
6	α-Terpinene	14.6	1.3	1,2,3
7	*p*-Cymene	15.0	0.8	1,2,3
8	β-Phellanderene	15.2	1.2	1,2
9	γ-Terpinene	16.7	4.2	1,2,3
10	*t*- Sabinene	17.2	1.1	1,2
11	hydrate	17.9	0.8	1,2
12	α-Terpinolene	18.8	2.8	1,2,3
13	Linalool	19.8	1.1	1,2
14	Terpinen-4-ol	22.8	17.0	1,2,3
15	4-Terpineol	22.9	0.4	1,2
16	*p*-Cymene-8-ol	23.3	0.8	1,2,3
17	α-Terpineol	24.1	0.3	1,2
18	γ-Terpineol	28.7	0.5	1,2
19	Safrole	31.4	0.2	1,2
20	δ-Elemene	32.2	1.9	1,2
21	α-Cubebene	33.9	1.6	1,2
22	α-Copaene	34.6	0.7	1,2,3
23	(*E*)- β-	36.3	0.3	1,2

24	Caryophyllene	37.1	0.2	1,2
25	γ -Elemene	38.4	0.2	1,2
26	α-Humulene	38.7	0.6	1,2
27	Aromadenderene	39.5	0.3	1,2
28	δ-Cadinene	40.0	0.5	1,2
29	γ -Cadinene	42.1	1.3	1,2
30	Germacrene D	44.9	0.2	1,2
31	Globulol	45.4	0.1	1,2
32	Spathulenol	51.2	0.9	1,2
	Caryophyllene oxide		61.5	
			22.9	
	Asarone		6.5	
	Monoterpene hydrocarbons		2.5	
			93.4	
	Oxygenated monoterpenes			
	Sesquiterpene hydrocarbons			
	Oxygenated sesquiterpenes			
	Total			

RT: retention time, 1: comparison with bibliography, 2: mass spectrum, 3: co-injection with authentic compound.

As shown in Figure 1A and 1B, carrageenan induced significant ($P < 0.01$) increase in the exudate volume from 0.38 to 1.23 ml/rat in pleural cavity of rats. Moreover, the number of polymorpho-nuclear neutrophils (PMN cells) has been significantly ($P < 0.01$) increased to 57.58×10^6 cells/rat. Pretreatment with PCEO at the doses 150, 300 and 600 mg/kg reduced significantly the exudate volume to 1.15, 0.89 and 0.73 ml respectively. The number of PMN cells was also reduced to 52.9×10^6, 43.49×10^6 and 37.37×10^6 respectively. As presented in Figure 1C and 1D, the levels of tumor necrosis factor (TNF-α) and interleukin-1β (IL-1β) were significantly ($P < 0.01$) increased in the carrageenan-induced rat lungs. Pretreatment with PCEO at doses of 150, 300 and 600 mg/kg showed significant decrease the release of TNF-α and IL-1β in a dose-dependent manner ($P < 0.05$, $P < 0.01$). As shown in Figure 1E, carrageenan induced a significant ($P < 0.01$) increase in the level of myeloperoxidase (MPO) from 39 to 283 U/l in lung tissue.

Table 2. Effect of PCEO on carrageenan-induced paw edema and cotton pellet granuloma in albino rats

Group (n=6)	Dose (p.o)	Carrageenan-induced edema in right hind paw		Cotton pellet-induced granuloma	
		Mean increase in paw volume ml ± S.E (3h)	Inhibition in %	Mean increase in weight of pellet mg ± S.E	Inhibition in %
Control (1% carrageenan)	0.05 ml	0.82 ± 0.016		48.00 ± 1.82	
PCEO + carrageenan	150 mg/kg	0.54 ± 0.036***	34.00	41.65 ± 1.97*	13.22
PCEO + carrageenan	300 mg/kg	0.34 ± 0.030***	58.14	34.46 ± 1.91***	28.19
PCEO + carrageenan	600 mg/kg	0.28 ± 0.017***	65.19	26.56 ± 2.02***	46.56
Phenylbutazone+ carrageenan	100 mg/kg	0.20 ± 0.01***	75.85	14.43 ± 1.20***	69.93

*All values represent mean ± SEM. *p < 0.01, ***p < 0.001; ANOVA, followed by Dunnett's multiple comparison test. Percent Inhibition*

The results showed that pretreatment with the PCEO at doses of 150, 300 and 600 mg/kg could decrease significantly ($P < 0.01$, $P < 0.001$, $P < 0.01$) MPO level to 269, 189, and 165 U/l respectively. As shown in Figure 1F, the level of nitric oxide was significantly ($P < 0.01$) increased in the lungs by carrageenan. Pretreatment with the PCEO at doses of 150, 300 and 600 mg/kg p.o. could significantly decrease the release of nitric oxide in a dose-dependent manner ($P < 0.01$).

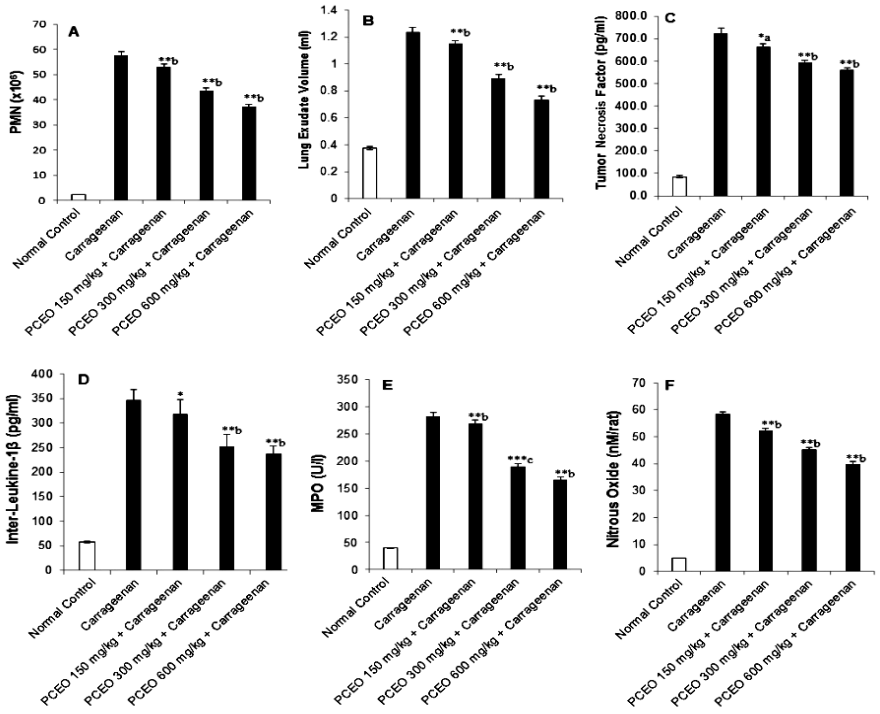

Figure 1. Effect of PCEO (150, 300 and 600 mg/kg) on carrageenan-induced pleurisy. Exudate volume (A), Polymorphonuclear Neutrophils (B), TNF-α (C), IL-1β (D), MPO level (E), and NO level (F) in pleural cavity after carrageenan injection. Values are mean ± SEM. of 6 rats in each group, P values: * < 0.05, ** <0.01, *** <0.001 compared with respective normal control, P values: a <0.05, b <0.01, c <0.001 compared with toxic control (Carrageenan).

In comparison with the saline group (Fig. 2A), the lungs of the rats treated with carrageenan showed pathological changes with inflammatory cells infiltration and local edema (Fig. 2B). The results showed that PCEO pretreatment at doses of 150, 300 and 600 mg /kg p.o. could decrease lung inflammation in a dose dependent manner (Fig. 2C, D, E). The potential antioxidant activity of the essential oil was determined on the basis of scavenging activity of the stable free radical DPPH. PCEO and ascorbic acid were able to reduce DPPH and to demonstrate antioxidant activity with IC_{50} values of 78.9 and 19.2 µg/mL respectively. In fact, PCEO at 500 µg/ml showed a strong radical scavenging activity (84%), compared with the highest antioxidant activity of ascorbic acid (92%) (Table 3).

Table 3. Free radical scavenging activity of the PCEO

Plant species	**Radical scavenging activity in %**				
	10 (µg/mL)	50 (µg/mL)	100 (µg/mL)	500 (µg/mL)	1000 (µg/mL)
P. cubeba	18.5	35.8	62.3	84.9	86.8
Ascorbic acid	45.2	69.9	88.5	92.8	94.2

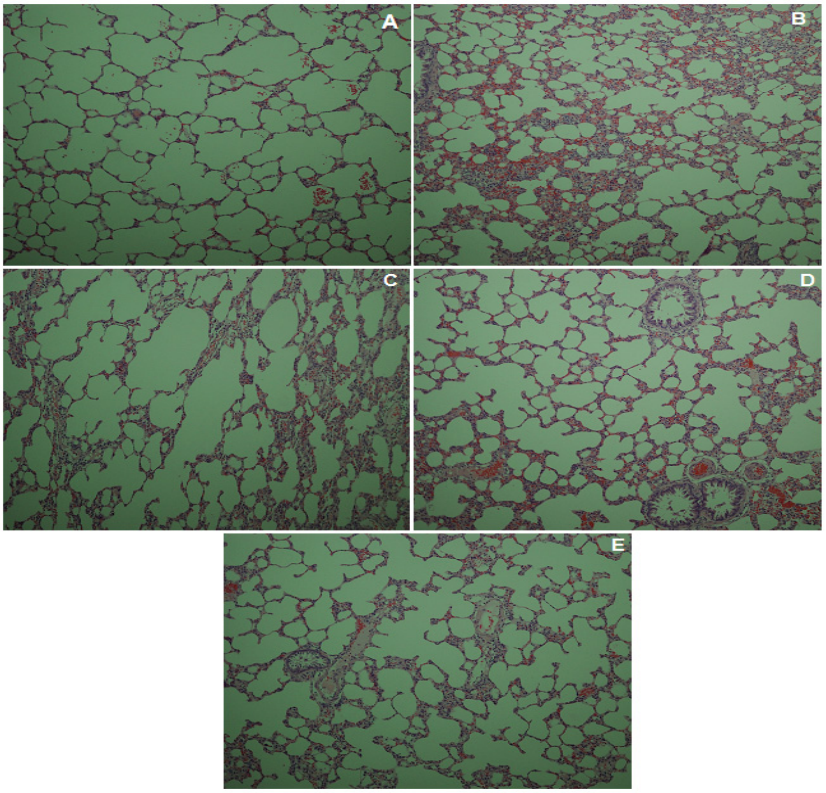

Figure 2. Effect of PCEO on lung inflammation (H & E stain ×100). Lung section of the rats treated with saline (A). Lung section of the rats treated with carrageenan (B). Lung section of the rats treated with carrageenan and PCEO (150 mg/kg, 300 mg/kg and 600 mg/kg p.o. C, D, E).

Reviewing the available current literature, it is crucial to bring up that this work speaks to the first report on *in vitro* antioxidant and *in vivo* anti-inflammatory activity of the essential oil of *Piper cubeba*. A previous study (YAM & al. [18]) reported on the anti-estrogenic and anti-inflammatory of the ethanolic extract of *Piper cubeba*. Our GC-MS investigation showed that sabinene (46.3%), 4-Terpineol (17.0%), γ-Terpinene (4.2%) and α-Thujene (2.6%) were found to be the main constituents. In previous studies (SINGH & al. [19]; MAGALHAES & al. [20]), the chemical composition of the essential oil of *P. cubeba* from different origins was investigated. In general, these studies revealed the predominance of monoterpene and sesquiterpene hydrocarbons e.g. sabinene, Eucalyptol, 4-terpineol, camphor, α-and β-cubebene as major compounds. A previous report on *P. cubeba* from Indonesia (BOS & al. [8]) showed little different chemical composition, where sabinene (9.1%), β-elemene (9.4%), β-caryophyllene (3.1%), *epi*-cubebol (4.3%), and cubebol (5.6%) were the major components of the berry oil.

The report by (ESPERNDIM & al. [5]; MAGALHAES & al. [20]) on the essential oil of *P. cubeba* from India showed similar chemical composition where monoterpene hydrocarbons and oxygenated monoterpenes predominated. It was shown that the main components were sabinene, eucalyptol, 4-Terpineol, γ-Terpinene and linalool. Thus, no large qualitative differences were found in the chemical composition between our results and the available current literature. The principal difference was of a quantitative nature. DPPH free radical scavenging activity has been used widely for the determination of anti-oxidant activity

(BRAND-WILLIAMS & al. [17]). DPPH free radical scavenging method is an easy, rapid and sensitive way to survey the antioxidant activity of pure compounds, plant extracts or essential oils (KOLEVA & al. [21]). It is believed that the DPPH free radical scavenging by antioxidants is due to their hydrogen donating ability. PCEO in the current study showed DPPH free radicals scavenging ability at different concentrations.

The probable mechanism of anti-inflammatory properties of PCEO used in this study needs more clarification. There are researches demonstrating that some monoterpenes e.g. euclyptol from EOs are great inhibitors of various inflammatory mediators for example prostaglandins and other arachidonic acid metabolites (JUERGENS & al. [22]).

In the present study, the anti-inflammatory action of the *P. cubeba* essential oil was evident in the carrageenan-induced pedal edema in rats. It has been well established that carrageenan-induced pedal edema in rats is an appropriate, well accepted and reproducible *in vivo* model to assess the naturally occurring medicinal materials; as the carrageenan-induced inflammation model acts by blocking and/or inhibiting phlogistic mediators of acute inflammation (HAJHASHEMI & al. [23]). Carrageenan is believed to cause inflammation which is biphasic in nature. The initial or first phase is due to the release of histamine and serotonin; while in second phase, the protease, bradykinin, lysosome, leukotrienes and prostaglandins are released (HAJHASHEMI & al. [23]; Al-YAHYA & al. [24]; Al-HOWIRINY & al. [25]). Some reports indicated that most clinically effective anti-inflammatory drugs are sensitive to the second phase of edema process (SMUCKLER & al. [26]). Furthermore, PCEO also showed the ability to reduce the granuloma formation in rats. Proliferation of fibroblasts and small blood vessels multiplication are the important features at the repairing phase of inflammation; and forming a highly vascularized mass known as granulation tissue (KT [27]). In the current study, PCEO effectively reduced the cotton pellet granuloma suggesting its influence in the proliferative stage of inflammation process. The results obtained in the present investigation are in accordance with earlier reports as different extracts of *P. cubeba* have been shown to exert anti-inflammatory activity (CHOI & HWANG [28]).

Further investigations have been done to estimate the role of proinflammatory cytokines such as tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β) and myeloperoxidase (MPO) using carrageenan-induced pleurisy which is a well-established inflammation model commonly used to investigate the pathophysiology of acute inflammation (SEVERINO & al. [29]). The results showed that PCEO could decrease lung inflammation in a dose dependent fashion in rats treated with carrageenan. Polymorphonuclear neutrophils (PMN) are the first line of defense host immunity (KOBAYASHI & DELEO [30]). The myeloperoxidase is one of the essential enzymes released from PMN activation and it is used as a marker enzyme for estimating PMN accumulation. MPO could also cause tissue damage during inflammation. The results showed that PCEO could inhibit the level of MPO in pleural tissue of the rats treated with carrageenan. Acute inflammation of lungs in rats results in significant infiltration of polymorphonuclear neutrophils. The obtained results demonstrated that pretreatment with PCEO reduced infiltration of these inflammatory cells.

The activation of proinflammatory cytokines is main step of inflammatory reaction (UTSUNOMYIA & al. [31]). Proinflammatory cytokines can promote chemotaxis to attract migrating granulocytes. On the other hand, leukocytes could produce further cytokines and proinflammatory mediators (ZHAO & al. [32]). TNF-α and IL-1β have been implicated in lung inflammation (UTSUNOMYIA & al. [31]; GANTER & al. [33]). There were reports that carrageenan can induce proinflammatory cytokines such as TNF-α and IL-1β in the lungs, leading to inflammation and lung injuries (UTSUNOMIYA & al. [34]; YEH & al. [35]). Our study exhibited that PCEO altogether lessened the levels of TNF-α and IL-1β in the lungs of

carrageenan-injected rats promoting to anti-inflammatory activity. In this way, anti-inflammatory efficacy of PCEO might be because of the attenuation of chemotaxis and inhibition of TNF-α and IL-1β in a dose dependent manner. Nitric oxide (NO) has a significant role as much as in the regulation of vascular permeability as in cell migration induced by proinflammatory agents, including carrageenan (VINEGAR & al. [36]; COSTA & al. [37]). PCEO also showed significant dose dependent reduction in NO levels in lung tissue of carrageenan induced pleurisy rats. Histological analysis of lung sections detected significant tissue injury (Fig. 2). Thus, when compared with lung sections taken from saline-treated animals (Fig. 2A). Histological test of lung sections of rats treated with carrageenan manifested tissue damage, edema, and infiltration of the tissue with neutrophils (PMN) (Fig. 2B). PCEO significantly reduced the degree of injury as well as the infiltration of PMNs (Fig. 2C, D, E) in a dose dependent manner indicating the anti-inflammatory action of PCEO. The GC-MS examination uncovered that monoterpenes e.g. sabinene, 4-terpineol, γ-terpinene and α-thujene were the fundamental compounds in PCEO which could be in charge of the monitored activities.

4. Conclusion

In conclusion, the data reported in this work indicate that the essential oil of *P. cubeba* possesses significant antioxidant and anti-inflammatory activities. These findings confirm the traditional anti-inflammatory indications of *P. cubeba* and provide persuasive evidence and support its use in Arab traditional medicine for the adjunct treatment of inflammatory disorders. Nevertheless, further studies are needed to confirm its clear mechanism of action.

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