ScienSage

Journal of Advanced Scientific Research

Available online through http://www.sciensage.info

ISSN
0976-9595
Research Article

ANTI-INFLAMMATORY POTENTIAL OF ESSENTIAL OIL FROM *POGOSTEMON BENGHALENSIS* (BURM.F.) KUNTZE. USING ANIMAL MODELS

Pradeep Damodaran Premakumari*[1], Murugan Kumaraswamy[2], Manoj Gopal Sarayu[1]

[1]*Department of Botany, Mahatma Gandhi College, Trivandrum, Kerala, India*

[2]*Center for Innovation in Science and Social Action, Trivandrum, Kerala, India*

**Corresponding author: prempradeep358@gmail.com*

ABSTRACT

Aromatic herbals are economically important as spices, flavor, crude essential oil and also for other therapeutic uses. The essential oils (Eos) continue to rise as a commodity and indirectly a range of beauty care and aromatherapy products. *Pogostemon benghalensis* (Burm.F.) Kuntze., a close relative of patchouli is a bushy herb of Lamiaceae family. The leaves are substituted as the source for essential oils which imparts the therapeutic properties to cure many health related issues among the human beings. Native people use the decoctions of this wild species for curing many skin related issues but not validated scientifically. In this scenario, the present study was undertaken to extract the Eos from *Pogostemon benghalensis,* fractionated by GC-MS and to evaluate the anti-inflammatory potentials using animal models. Anti-nociceptive role of Eos were analyzed in rats using the acetic acid-induced writhing test. Anti-inflammatory effects of Eos in four different concentrations, namely 100, 200, 300 and 500 mg/kg, were accessed in animal models representing various changes connected with inflammation such as carrageenan-induced paw edema, xylene induced ear edema, cotton pellet-induced granuloma and ethanol induced ulcer. No sign of toxicity was noticed in the Eos treated rats from both the species up to a concentration of 3000 mg/kg. The extract showed statistically significant inhibition of induced nociception and inflammation in a concentration dependent manner. The higher concentration of Eos from the species remarkably inhibited pain and inflammation as compared to control ($P< 0.01$). GC-MS analysis identified 41 volatile compounds as phytoconstituents in the species. The present study scientifically validated the anti-nociceptive and anti-inflammatory potential of Eos from *Pogostemon benghalensis*. These potentialities may be attributed by the presence of various classes of terpene phytoconstituents in the Eos.

Keywords: *Pogostemon benghalensis,* GC-MS, Essential oil, Carrageenan, Paw edema, Xylene, anti-inflammatory.

1. INTRODUCTION

The mammalian body is exposed to external stimuli, variety of hostile agents or toxic chemical substance and responds physiologically as inflammation. While the excessive or persistent inflammation leads to bacterial sepsis, arthritis or skin inflammations [1]. The inflammation is often represented as the accumulation of plasma fluid and blood cells at the inflammation site [2]. The skin provides the first line of defense against the invasion of foreign bodies [3]. The inflammation is generated as a result of migration of various cellular components to the site and the release of pro-inflammatory mediators such as cytokines, prostaglandins and leukotriene [4].

The current treatment methods are focused on the use of moisturizers, antihistamines, antibiotics and corticosteroids to cure inflammation and secondary infection. The use of synthetic drugs include steroids, disrupt many cytokine networks involved in lymphocyte function, leads to immune suppression and decrease collagen synthesis and finally to skin atrophy [5]. The use of synthetic drugs such as rofecoxib and celecoxib has limited due to side effect including gastric injury and ulceration, renal damage and cardiac abnormalities [6]. To eliminate such risks new therapeutic approaches are intensively needed to develop novel anti-inflammatory agents probably from the natural origin with more potentiality with lesser side effects. From long, natural products of plant origin have been a source of novel compounds exhibiting medicinal properties, anti-oxidative, anti-inflammatory and antimicrobial properties.

The essential oil refers volatile product obtained by hydrodistillation, dry distillation, steam distillation or by a suitable mechanical process without heating the plant or parts of it [7]. They are oily liquid, aromatic, lightly

coloured (pale yellow to orange), commonly lower density than water and composed of few to several hundred com-ponents. It is synthesized in all parts of the plant and stored in secretory cells, cavities, canals, epidermic cells or glandular trichomes [8]. The oil is mainly composed of terpenes (monoterpene, sesquiterpene and their oxygen-nated forms or derivatives). Different phytochemicals such as α-pinene, α-terpinene, terpinen-4-ol, α-terpineol and linalool present in Eos may have direct or indirect anti-inflammatory activity [9].

The *P. benghalensis* leaf aqueous extract was applied for the various ailments by the ethnic community including food poisoning, vomiting, respiratory tract infections and uterine hemorrhage [10]. Eos has proven for its ability to scavenge free radicals using *in vivo* systems [11]. The antioxidant capacity of Eos may help the oils to act as anti-inflammatory agents through protecting the diverse cells (monocytes, neutrophils, eosinophils, and macrophages) from oxidative damage. Often the inflammation was accompanied by the increase in oxygen consumption resulting in to the formation of reactive oxygen species (ROS) or reactive nitrogen species (RNS). These ROS/RNS were scavenged by the Eos and successively suppress the inflammation [12].

However, there is no valid report available on anti-inflammatory activity of the leaf essential oil from *P. benghalensis*. Therefore, the aim of the present study was to evaluate the anti-inflammatory activity of essential oil from the leaf of *P. benghalensis*.

2. MATERIAL AND METHODS

2.1. Plant Materials

Fresh leaves of *Pogostemon benghalensis* used in the present study was collected from the natural habitats of Munnar Hills of Idukki district, Kerala, India on July 2019. The collected species were identified using flora and were authenticated at the Herbarium of Jawaharlal Nehru Tropical Botanic Garden and Research Institute (JNTBGRI), Palode, Trivandrum, Kerala, India. The voucher specimens were numbered (MGH 1456) and kept in the herbarium of the Department of Botany, M.G. College, Trivandrum, Kerala, India. The collected plants were washed thoroughly with running tap water and the fresh leaves were used for the extraction of Eos.

2.2. Extraction and analysis by GC-MS

Hydro-distillation of fresh leaves of the selected *P. benghalensis* was carried out using a Clevenger-type apparatus. GC-MS analysis was done using Hewlett-Packard 6890 gas chromatography (Agilent Technologies, USA). 1 µl of essential oil from the species was injected into the machine fitted with an HP-5 (5% phenyl methyl-polysiloxane, 30 m 0.32 mm *i.d.,* and 0.25µm film thicknesses) capillary column coupled with a model 5973 mass detector. GC-MS operation conditions were injector temperature: 220°C; transfer line temperature: 240°C; oven temperature programme: 60-246°C (3°C /min); carrier gas: helium (1.4 ml/min); detector temperature: 250°C; mass spectra, electron impact (EI+) mode, 70eV; ion source temperature: 240°C.

2.3. Animals

The healthy Wistar rats of 8 to 10 weeks aged with the body weight of 125-140 g were used for the present studies. They were given standard diet and water *ad libitum* [13, 14]. A total of 36 rats containing 6 rats per group were randomly categorized were used for anti-inflammatory studies. Ethical clearance for this experimental protocol was obtained from the Institutional Animal Ethics Committee (Reg.No.565/04 /c/CPCSEA).

2.4. Drug and Eos administration

The Eos extract was administered by suspending through 1% Carboxy methyl cellulose (CMC) solution. In carrageenan model, Eos extract of *Pogostemon benghalensis* at different doses of 100, 200, 300 and 500 mg/kg was administered orally using gastric canula.

2.5. Grouping of rats

Group I- Carrageenan control; Group II- indomethacin/ dexamethasone (10/ 5 mg/kg); Group III- Eos of *Pogostemon benghalensis* extract (100 mg/kg); Group IV- (200 mg/kg); Group V- (300 mg/kg); Group VI- (500 mg/kg).

2.6. Acute toxicity test

Acute toxicity test was done using the limit test dose of 3000 mg/kg as described by OECD guideline [13]. Six Wistar rats were fasted for 24 h but allowed free access to water. A limit dose of 3000 mg/kg of Eos from *Pogostemon benghalensis* was administered sequentially and animals were observed individually for behavioral profile (alertness, restlessness, irritability, and fearfulness), autonomic profiles (defecation and urination), neurologic profile (spontaneous activity, reactivity, touch response, pain response, and gait), physical states such as lacrimation, loss of appetite, tremors, hair erection, salivation, diarrhea, and for morbidity or mortality, after

dosing continuously for 2 h, periodically during the first 24 h (with special attention given during the first 4 h) and daily thereafter, for a total of 14 experimental days.

2.7. Analysis of *in vivo* anti-inflammatory potentiality

2.7.1. Acute inflammation models using carrageenan induced paw edema in rats

The experimental groups were treated with different concentrations of Eos (100, 200, 300 and 500 mg/kg) intraperitoneally before an hour of carrageenan induction for 2 consecutive days. After the second dose of Eos treatment, the sub plantar injection of 0.1ml carrageenan (1% w/v, in 0.1% carboxy methylcellulose) was done in the right hind paw of selected rats to induce the paw edema. The volume and thickness of right paw was measured at 0, 1, 3, 5 and 6 h using digital vernier caliper and compared with control group after carrageenan injection to determine the formation of edema. The percentage inhibition of edema was quantified using the formula [15].

% inhibition = $[(P_C-P_T)/P_C] \times 100$

P_T =the thickness of paw of rats given test extract at corresponding time

P_C = the paw thickness of rats of control group at the same time.

2.7.2. Xylene-Induced Ear Edema

The Wistar rats were administrated with Eos orally on once daily dosage continued for 3 days (100, 200, 300 and 500 mg/kg) and the control groups were also maintained (both positive and negative control). Dexamethasone (5 mg/kg) was used as the standard drug in positive control group. The edema was induced by applying 50µl xylene to the Eos treated groups and positive control group at the inner surface of the right ear. 2h after xylene daubing, the rats were executed by cervical dislocation, and both ears were removed and weighed. The percentage of edema inhibition was determined for each group [16].

% inhibition = $[(E_C-E_T)/E_C] \times 100$

E_C= Difference of ear weight in control group

E_T= Difference of ear weight in test group

2.7.3. Cotton Pellet Induced Granuloma

Forty (40) mg weighed sterile cotton pellets were surgically implanted subcutaneously into the rat's groin under sterile condition. Subsequently standard drug, dexamethasone and Eos in different concentration (100, 200, 300 and 500 mg/kg) were administered orally for six consecutive days. The rats were sacrificed on the seventh day. The cotton pellets were removed surgically from extraneous tissues and were dried at 50°C for 24h. Then the weight of the cotton pellets was recorded. The weight difference between dry cotton and the cotton before implantation was considered as the weight of granuloma formed. Inhibition percentage was calculated using the following equation [17].

Inhibition % = $[(W_C-W_T)/W_C] \times 100$

W_C = Weight of pellet in control group

W_T= Weight of pellet in test group

2.7.4. Ethanol-induced gastric ulcer

The selected groups (Gr III to Gr VI) were administrated by gavage of single doses of 0.2 ml of Eos of different concentrations (100, 200, 300 and 500 mg/kg in 0.9% NaCl, containing 0.1% of Tween 20, as vehicle). Gr II was administrated with standard drug ranitidine (50 mg/kg, positive control) and Gr I was taken as negative control. All the groups except Gr I were received orally, 0.1 ml of 95% ethanol for gastric lesion induction. After 1h, all the animals were sacrificed. The stomachs were removed, opened along the greater curvature and fixed between two glass plates. Each stomach was scanned and was then examined for macroscopic gastric lesions. The number and severity of lesions/ulcer were noted and scored on an arbitrary 0–3 points. Mean scores for each group were calculated and expressed as ulcer index (UI).Then the glandular segments from stomach were removed and immediately homogenized in tubes with 4ml of distilled water and pH of the gastric juice was recorded [18].

2.7.5. Acetic Acid Induced Writhing

The rats in Gr III to Gr VI and Gr II were intragastrically administered with Eos of different concentration and standard drug indomethacin (10 mg/kg) respectively. Gr I was treated as negative control. After 1h an intraperitoneal injection of 0.6% acetic acid solution (10 ml/kg body weight) was given to test groups. The number of writhing responses was recorded for each animal after the administration of acetic acid, counted over a period of 10 min. The percentage inhibition of writhing was calculated using the following formula [19].

Inhibition (%) = $[(W_C-W_T)/W_C] \times 100$

W_C= Number of writhing in control

W_T= Number of writhing in test

2.8. Statistical analysis

The data is expressed as mean±Standard Deviation (SD). Results were analyzed using one-way ANOVA followed by Dunnet's test. Differences were considered as statistically significant at $P < 0.05$, when compared with control.

3. RESULTS AND DISCUSSION

3.1. GC-MS Analysis

The GC-MS analysis identified 41 volatile compounds from the essential oil (Eo) of *P. benghalensis*. α-Cadinol (35.78%) and patchouli alcohol (34.85%) were the major components in the oil. The other predominant components of *P. benghalensis* were 1,8 cineole (7.14%), aromadendrene (4.16%), þ-cymene (1.6%), born-ylacetae (2.15%), longicyclene (2.74%), β-elemene (1.56%), longifolene (1.18%), α-caryophyllene (1.08%), β-caryophyllene (1.14%), trans-β-farnesene (1.56%), α-patchoulene(2.39%), gurjunene (2.86%), valencene (2.21%), epi-cubedol (1.03%), bicyclogermacrene (2.78%), trans-β-guaiene(1.26%), α-bisabolene (1.16%) , ɗ-cadinene (2.45%), elemol (1.01%), spathulenol (1.16%), caryophyllene oxide (1.15%), guaiol (1.79%), isolongifol (1.20%), cubenol-1-epi (1.56%), α-murolol (1.45%), bulsenol(1.51%) and cadalene- 8,9-epoxide (2.95%) .

3.2. Acute toxicity

In the acute toxicity test at the limit test dose of 3000 mg/kg of essential oil (Eo) of *P. benghalensis* neither mortality nor changes related to behavioral, autonomic, neurologic, nor physical profiles were observed within the first 24 h and further, during the 14-day follow-up of the experimental periods.

3.3. Anti-inflammatory analysis

From 1800s onwards, it was reported etiologically that inflammatory diseases leads to painful disorders including rheumatoid arthritis [20]. Severe side effects were recorded in connection with prolonged usage of synthetic drugs to relieve pain from inflammatory disorders. Therefore, search of safe and plant based drugs with minimum / no side effects are demanded globally. Traditional people employ diverse crude botanicals to relieve pain may be explored and to be validated scientifically. In this scenario, this part of the work is undertaken to analyze anti-inflammatory effect of essential oil (Eo) from *Pogostemon benghalensis* was carried using proven inflammatory animal models like paw edema, ear edema and peritonitis.

3.3.1. Carrageenan-induced paw edema

Carrageenan-induced paw edema is the most reliable experimental animal model in proving the efficacy of anti-inflammatory potential of natural phytochemicals [21]. In the present study, the Wistar rats were treated with different concentrations of Eo and evaluated from 1 to 6 h period of time. Eos from *Pogostemon benghalensis* reduced carrageenan triggered paw edema at varied levels. The effects of Eos and indomethacin on rat paw edema were displayed in Fig. 1. Administration of carrageenan into the sub-plantar tissue of the right hind paw of the experimental animals in the control group caused induction of edema which was peaked at 3 h (2.79 ml in paw volume) and increased steadily till the 6 h.

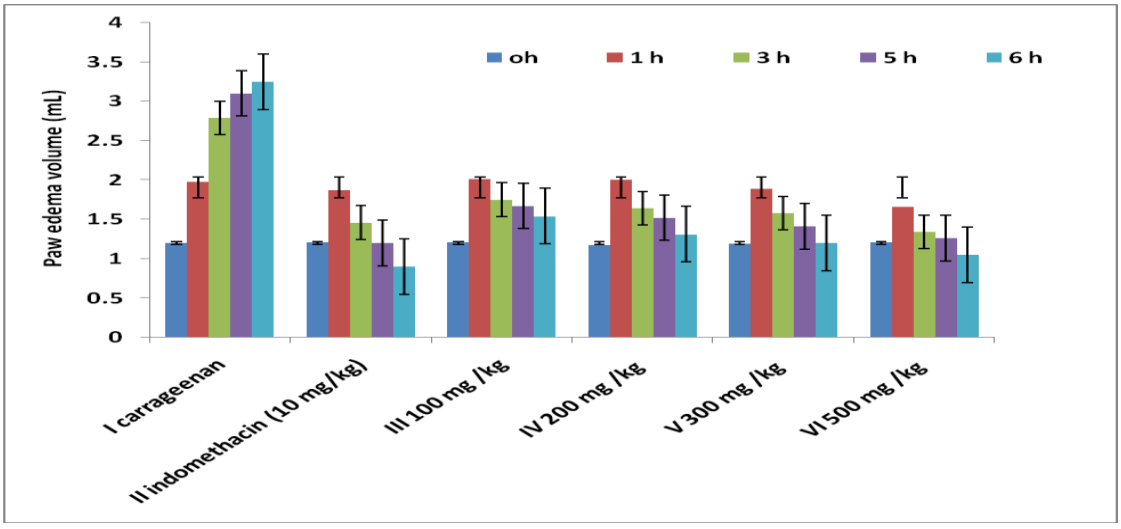

Fig.1: Paw edema in rats in carrageenan-induced paw edema regulated by Eo from *P. benghalensis*

The Eo from *Pogostemon benghalensis* remarkably reduced the paw edema in a concentration dependent manner from 1 to 6 h i.e., for example 300 mg/kg dosage showed 63.1% of inhibition. This effect was comparatively less than 10 mg/kg indomethacin treated groups (72.3 % inhibition). The impacts of Eo of the species at different concentrations and indomethacin were duration dependent.

The inflammatory reaction induced by carrageenan (sulphated polysaccharide) in the rat hind paw includes a sequence of multi-ordered events [22]. The initial phase (90 min) of reaction associated with carrageenan administration results in to the discharge of histamine and serotonin mediators. Subsequently, the second phase is activated by bradykinin from 90 to 150 min [23]. At the final phase (from 150 to 240 min), the mediator prostaglandins play its role. This phase seems to be more important as compared with the phase 1 and 2. The increased vascular response (maintained by kinins) accompanied with leukocyte leaching in to the inflamed region attains its maximal level in this phase. In the present study, the Eos from *P. benghalensis* was effective from 2 to 6 h suggests its plausible inhibitory roles between 2nd and third phases of inflammatory reactions.

3.3.2. Xylene induced ear edema in rats

The mean weight of the edema induced ear and the percentage inhibition of inflammatory reaction were displayed in the Table 1. Topical application of xylene on the right ear resulted in to an increase weight as compared to the left ear (control) in the vehicle group. Meanwhile, the synthetic drug the (positive control group) dexamethasone (5mg/kg) revealed a remarkable reduction in the mean ear weight as compared to the vehicle group.

Table 1: Effect of the essential oil (Eo) from *Pogostemon benghalensis* on xylene-induced ear edema in rats

Group	Edema (mg)	Inhibition (%)
Normal saline	18.70±0.24	---
Dexamethasone (5 mg/kg)	10.32±0.45**	68.2
Gr III 100 mg /kg	14.38±0.29**	40.7
Gr IV 200 mg /kg	12.40±0.06**	56.3
Gr V 300 mg /kg	10.79±0.52**	63.2
Gr VI 500 mg /kg	9.74±0.65**	69.8

***P < 0.01 compared with control, Groups treated with Eos were Gr III to VI*

Parallely, Eo from *Pogostemon benghalensis* at all the tested doses such as 100, 200, 300 and 500 mg/kg revealed remarkable reduction in the mean ear weight as compared to control group. Animals treated with Eo of 500 mg/kg from *P. benghalensis* showed the highest reduction as compared to 100 mg/kg application i.e., 69.8%.

Experimental proven documents have shown that exposure of skin to xylene/allied compounds induce a pleiotropic tissue response that culminates in to an inflammatory reaction similar to other skin prone disorders [24, 25]. Bagad *et al.*, [26] suggested that eicosanoids have a role in various skin inflammations. The mode of increase in eicosanoids is not completely elucidated but may include the induction of protein kinase C, mitogen-activated protein kinase and nuclear factor-κB, and the generation of mediators, like tumor necrosis factor-α, interleukin-1b, keratinocyte-derived chemokine, macrophage inflammatory protein-2 and prostaglandins [27, 28]. Zanini Jr *et al.*, [29] proved that xylene triggered ear edema is typical for establishing the anti-inflammatory potential of natural or synthetic drug's topical applications especially those arrests phospholi-pase A2 activities. Administration of xylene triggers acute neurogenic ear edema, which is linked with the phospholipase activity. Phospholipases are widely noticed in the central and peripheral nervous systems. Therefore, excessive discharge of these molecules from sensory neurons in the peripheral nervous system leads to vasodilatation and plasma extravasations and there by ear swelling. In addition, the ear edema connected with xylene comprises induction of inflammatory mediators like histamine, kinin and fibrinolysin. The sound inhibition of xylene triggered ear swelling in rats administrated with Eo from *Pogostemon benghalensis* tempt to suggest that the active fractions present in the Eo soundly inhibit the release of phospholipase or histamine, kinin, and fibrinolysin mediators.

3.3.3. Cotton pellet-induced granuloma test

Efficiency of Eo from *P. benghalensis* and indomethacin was carried by cotton pellet-induced granuloma test in terms of the proliferative stage of inflammation which includes tissue degeneration and fibrosis. The effects of Eo from *P. benghalensis* and indomethacin on the proliferative stage of inflammation were displayed in the Table 2. Anti-inflammatory effect of Eos was measured from the weights of moist and dry cotton pellets. Eo from *P. benghalensis* and dexamethasone (5 mg/kg b.w)

displayed remarkable inhibition of inflammation values *i.e.,* 61.0 and 67.6% respectively.

Table 2: Effect of Eo from *P. benghalensis* on cotton pellet-induced granuloma in rats

Group	Granuloma weight (mg)	Inhibition %
Distilled water	32.46±0.16	0.00
Dexamethasone (5 mg/kg)	2.10 ±0.31**	67.6
Gr III 100 mg /kg	2.57±0.16**	28.9
Gr IV 200 mg /kg	2.18±0.54**	45.0
Gr V 300 mg /kg	1.75±0.38**	53.2
Gr VI 500 mg /kg	1.44±0.12**	61.0

***P < 0.01 compared with control, Groups treated with Eo were Gr III to VI*

Generally, the cotton pellet-induced granuloma test is employed to evaluate chronic inflammatory reactions in terms of the transudative, exudative, and proliferative phases i.e., macrophage dysfunction and granuloma synthesis [30]. Inflammation consists of macrophage, neutrophil, fibroblast proliferations and also the multiplication of small blood vessels which forms the key source for the formation of a highly vascularized reddish mass of granulation tissue [31]. Thus, a reduction in granuloma weight may be due to the efficacy of Eos to decrease the fibroblast number and the synthesis of collagen and mucopolysaccharides which constitute the natural proliferative indicators of granulation tissue formation. Laaboudi, *et al.*, [32] substantiated the anti-inflammatory and analgesic activities of olive tree extract using ear edema model on animals. Yin *et al.*, [33] evaluated antinociceptive effects of dehydrocorydaline in mouse models of inflammatory pain *via* the opioid receptor and inflammatory cytokines. Anyasor and Ijituyi [34] proved hexane fraction balm from the leaves of *Costus afer* suppressed effectively the xylene induced topical inflammation in rat models. The present data strongly reflects the inhibitory potentiality of Eos against macrophage induction, infiltration and aggregation of inflammation related components in the site of injury.

3.3.4. Ethanol-induced gastric ulcer

Oral application of ethanol in the control rats resulted in to lesions in the glandular region of the rat stomach and was visualized as long thick, reddish bands. Eo from *P. benghalensis* has displayed optimal ulcer protection indices of 62.9% with the dosage of 500 mg/kg in comparison to control. Ranitidine 50 mg/kg as standard drug showed significant reduction of ulcer (70.3%).

Generally, ethanol triggered gastric ulcer formation was used to analyze the cyto-protective efficacy of the plant based drugs. Ethanol can damage the mucous membrane of the stomach and also induces gastric blood flow which results in to haemorrhage and necrotic injuries. The drastic penetrative power of ethanol leads to enhanced intra cellular membrane permeability to sodium and water. The intense intracellular loading of calcium is another marked effect of ethanol which induces pathogenesis of gastric mucosal membranes. The final end point will be cell death and surface epithelium exfoliation [35].

Table 3: Effect of Eo from *P. benghalensis* on ethanol-induced gastric ulcer in rats

Treatment groups	Ulcer index	Protection (%)	pH of gastric juice
Control	12.80**	------	3.20
Ranitidine 50 mg/kg	3.25**	70.3	5.50
Gr III 100 mg /kg	4.80**	45.6	3.78
Gr IV 200 mg /kg	4.20**	50.2	3.97
Gr V 300 mg /kg	3.73**	57.4	4.22
Gr VI 500 mg /kg	3.41**	62.9	5.00

***P < 0.01 compared with control, Groups treated with Eos were Gr III to VI*

The Eo from *P. benghalensis* to an extent safe guarded the stomach from lesions produced by ethanol toxicity. The mode of action of Eo as antiulcer may be due to the reductions in gastric acid secretion and there by gastric cyto-protection. Further studies are warranted for analyzing the pathway of action on gastric acid secretion and gastric cyto-protection by Eo. Sahoo *et al.*, [36] analyzed the antiulcer activity of ethanolic leaf extract of *Salvadora indica* on rats. Abebaw, *et al.*, [37] validated the anti-ulcer activity of the leaf extract from *Osyris quadripartite* in rats. Raju *et al.*, [38] proved the anti-ulcer activity of methanolic fruit extract of *Terminalia chebula* in rats.

3.3.5. Acetic acid-induced abdominal writhing in rats

Eo administration at variable concentrations of *P. benghalensis* remarkably reduced the abdominal writhing number by 79.9******% (at 500 mg/kg Eos) as compared to the synthetic drug indomethacin (10 mg/kg) 75.9******%, despite of the initially noted induction in the number of spasms after Eos administration (20.2±0.38

treated with 100 mg /kg of *P. benghalensis*). This may be probably due to the initial irritating effect caused by the Eo (Table 4).

The main features of acute inflammation are vasodilatation, the exudation of plasma, increase of vascular permeability and cell migration (primarily neutrophil) into the site of inflammation [39]. Increased vascular permeability occurs as a result of contraction and separation of endothelial cells at their boundaries to expose the basement membrane, which is freely permeable to plasma proteins and fluid [40]. Phlogistic agents increase vascular permeability at various times after injury in inflammation condition. Chemically induced vascular permeability (such as seen with acetic acid) causes an immediate sustained reaction that is prolonged over 24 h and its inhibition suggests that the Eo of *P. benghalensis* may effectively suppress the exudative phase of acute inflammation [41]. The Eo of *P. benghalensis* reduces the dye leakage into the peritoneumin dose dependent manner. Reduction of dye leakage indicates its anti-inflammatory action due to reduced vascular permeability. In this method, Eo of *P. benghalensis* with 100, 200, 300 and 500 mg/kg and indomethacin (10 mg/kg) dose resulted in the significant ($P < 0.05$) inhibition.

Table 4: Effect of the administration of essential oil (Eo) / Indomethacin on acetic acid-induced abdominal writhing in rats

Group	Number of writhings (Mean±SD)	Inhibition (%)
Saline water	42.3±1.87	0
Indomethacin (10 mg/kg)	10.1±0.56**	75.9**
Gr III 100 mg /kg	20.2±0.38	42.7**
Gr IV 200 mg /kg	13.8±0.26	66.4**
Gr V 300 mg /kg	11.8±0.34	72.3**
Gr VI 500 mg /kg	9.4±0.02	79.9**

***P < 0.05 compared with control, Groups treated with Eos were Gr III to VI*

Lee *et al.*, [42] showed similar efficacy in terms of analgesic effects of *Eucalyptus* essential oil in mice. Many researchers like Vendruscolo *et al.*, [21] proved anti-inflammatory and antinociceptive activities of *Zingiber officinale* roscoe essential oil; Acha *et al.*, [22] using leaf essential oil from *Melaleuca quinquenervia;* monoterpenoid myrtenal by Dragomanova *et al.*, [23] in experimental animal models. Parandin and Daroogari [43] demonstrated that the ethanolic extract of *Propolis* as anti-inflammatory and antinociceptive in male mice and rats. Another study by Abbasloo *et al.*, [44] in the essential oil of *Satureja khuzistanica* Jamzad proved the anti-inflammatory potential and also attenuation of traumatic brain injuries in rats. Ilhan *et al.*, [45] proved the anti-inflammatory and antinociceptive features of *Bryonia alba* as an alternative to rheumatic pain. Synergistic effects of deuterium depleted water and essential oils from *Mentha longifolia* on sepsis induced liver injuries was narrated through cyclooxygenase-2 activities by Rasooli *et al.*, [46].

4. CONCLUSION

Based on the obtained results from the present study it can be summarized that Eo of *P.benghalensis* possesses remarkable anti-inflammatory potential and is also effective in inflammation associated pain. The present results indicate that Eo of *P. benghalensis* displayed anti-nociceptive and anti-inflammatory potentialities providing a scientific validation for its ethnobotanical usages for treating diverse ailments. This might be contributed by the presence of terpene constituents in the oils. Analysis of Eo of *P. benghalensis* by GC-MS identified 41 terpene fractions as major contributors for the *in vivo* anti-inflammatory activities. The obtained information could be applied to inhibit or alleviate chronic inflammatory conditions. The fractions in the Eo of *P. benghalensis* preclude inflammatory signaling. Hence an effort should be made to isolate and characterize the active agents responsible for the observed pharmacological activities.

Conflict of Interests

The authors report no conflict of interests.

5. REFERENCES

1. Palladino MA, Bahjat FR, Theodorakis EA, Moldawe LL. *Na.t Rev. Drug. Discov.*, 2003; **2**:736-746.
2. Sobota R, Szwed M, Kasza A, Bugno M, Kordula T. *Biochem. Bioph. Res. Co.*, 2000; **267**:329-333.
3. Kupper TS, Fuhlbrigge RC. *Biochem. Bioph. Res. Co.*, 2004; **4**: 211-222.
4. Briganti S, Picardo M. *J. Eur. Acad. Dermatol.*, 2003; **17**: 663-669.
5. Oikarinen A, Haapasaari KM, Sutinen M, Tasanen K. *Br. J. Dermato.l*, 1998; **139**:1106-1110.
6. Dogne JM, Hanson J, Supuran C, Pratico D. *Curr. Pharm. Des.*, 2006; **12**:971-975.

7. Rubiolo P, Sgorbini B, Liberto E, Cordero C, Bicchi C. *Flavour. Frag. J.*, 2010; **25**:282-290.
8. Bakkali F, Averbeck S, Averbeck D, Idaomar MM. *Food. Chem. Toxico.l*, 2008; **46**:446-475.
9. Hart PH, Brand C, Carson CF, Riley TV, Prager RH, Finlay-Jones JJ. *J. Inflamm. Res.*, 2000; **49**:619-626.
10. Naise MG, Bhadange DG. *Int. J. Appl. Eng. Res.*, 2017; **3(3S)**:228-229.
11. Maestri DM, Nepote V, Lamarque AL, Zygadlo JA. *Research. Signopost.*, 2006; **37(2)**:105-135.
12. Miguel MG. *Molecules.*, 2010; **15**:9252-9287.
13. Saleem U, Amin S, Ahmad B, Azeem H, Anwar F, Mary S. *Toxicol. Rep.*, 2017; **4**:580-585.
14. National Research Council (NRC). Guide for the care and use of laboratory animals. 8th edition. Wash, DC, USA: National Academy Press; 2011.
15. Hajhashemia V, Ghannadib A, Hajilooa M. *Iran. J. Pharm. Res.*, 2010; **9(2)**:163-168.
16. Hosseinzadeh H, Haddadkhodaparast MH, Aras AR. *Phytother. Res.*, 2003; **17(4)**:422-425.
17. Gupta M, Mazumder UK, Gomathi P, Selvan VT. *BMC. Complement. Altern. Med.*, 2006; **6(36)**:1-6.
18. Monteiro MVB, de Meloleite AKR, Bertini LM, de Morais SM, Nunes-Pinheiro DCS. *J. Ethnopharmacol.*, 2007; **111**:378-382.
19. Tahiri O, Atmani-Kilani D, Sanchez-Fidalgo S. *J. Ethnopharmacol.*, 2017; **209**:210-218.
20. Silman AJ. *Arthritis. Rheum.*, 2002; **46(3)**:579-581.
21. Vendruscolo A, Takaki I, Bersani-Amado LE, Dantas JA, Bersani-Amado CA, Cuman RK, *Indian. J. Pharmacol.*, 2006; **38**:58-59.
22. Acha E, Anounou JF, Adoverlande J, Assogba MF, Agossou G, Sezan A, Dansou HP, Gbenou JD. *J. Chem. Pharm.*, 2019; **11(1)**:36-50.
23. Dragomanova S, Tancheva L, Georgieva M, Klisurov R. *J. of. IMAB.*, 2019; **25(1):**2408-2415.
24. Studzinska-Sroka E, Dudek-Makuch M, Chanaj-Kaczmarek J, Czepulis N, Korybalska K, Rutkowski R, et al., *Molecules.*, 2018; **23(9)**:2133-2138.
25. Altinyay C, Suntar I, Altun L, Keles H, Kupeli, Akkol E. *J. of. Ethnopharmacol.*, 2016; **4(192)**:148-160.
26. Bagad AS, Joseph JA, Bhaskaran N, Agarwal A. *Adv. Pharmacol. Sci.*, 2013: **ID 805756**.1-6.
27. Zhao J, Maitituersun A, Li C, Li Q, Xu F, Liu T. *Evid. Based. Complement. Alternat. Med.*, 2018; **5(7965306)**:1-7.
28. Guo D, Xu L, Cao X, Guo Y, Ye Y, Chan CO, et al., *J. of. Ethnopharmacol.*, 2011; **8(138)**:717-722.
29. Zanini Jr JC, Medeiros YS, Cruz AB, Yunes RRA, Calixto JB. *Phytother. Res.*, 1992; **6(1)**:1-5.
30. Kumar R, Gupta Y, Singh S. *Indian. J. Pharmacol.*, 2016; **48(2)**:155-161.
31. Nair V, Singh S, Gupta Y. *J. Ayurveda. Integr. Med.*, 2013; **4(1)**:13-18.
32. Laaboudi W, Ghanam J, Aissam H, Merzouki M, Benlemlih M. *Int. J. Pharm. Pharm. Sci.*, 2016; **8(7)**:414-419.
33. Yin ZY, Li L, Chu SS, Sun Q, Ma ZL, Gu XP. *Sci. Rep.*, 2016; **6(27129):**1-11.
34. Anyasor GN, Ijituyi OH. *A. J. Physiol. Biochem. Pharmacol.*, 2018; **7(2)**:54-60.
35. Soll AH. *N. Engl. J. Med.*, 1990; **322**:909-916.
36. Sahoo S, Sahoo HB, Priyadarshini D, Soundarya G, Kumar K, Rani KU. *J. Clin. Diagn. Res.*, 2016; **10**(**9**):7-10.
37. Abebaw M, Mishra B, Gelayee DA. *J. Exp. Pharmacol.*, 2017; **9**:1-11.
38. Raju D, Ilango K, Chitra V, Ashish KJ. *Int. J. Pharm. Sci. Res.*, 2009; **1(3)**:101-107.
39. Brown JN, Roberts J. Histamine, bradykinin, and their antagonists, in Goodman and Gilman's the Pharmacological Basis of Therapeutics, 10th ed. NewYork, NY,USA: McGrawHill; 2001; 645-667.
40. Sherwood ER, Toliver-Kinsky T. *Best. Pract. Res Clin. Anaesthesiol.*, 2004; **18(3)**:385-405.
41. Okoli CO, Akah PA, Nwafor SV, Anisiobi AI, Ibegbunam IN, Erojikwe O. *J. of. Ethnopharmacol.*, 2007; **109(2)**:219-225.
42. Lee G, Park J, Kim MS, Seol GH, Min SS. *Korean. J. Pain.*, 2019; **32(2)**:79-86.
43. Parandin R, Daroogari S. *Int. J. Res. Med. Sci.*, 2019; **21(2)**:1-7.
44. Abbasloo E, Dehghan F, Khaksari M, Najafipour H, Vahidi R, Dabiri S, et al. *Sci. Rep.*, 2015; **6(1866)**:1-15.
45. Ilhan M, Guragac FT, Dereli, Tumen I, Akkol EK. *Open. Chem.*, 2019; **17**:23-30.
46. Rasooli A, Fatemi F, Hajihosseini R, Vaziri A, Akbarzadeh K, Malayeri MRM, et al. *Pharm. Biol.*, 2019; **57(1):**125-132.