

Evaluation of anti-inflammatory, analgesic and antipyretic potential of perilla frutescens fixed oil

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Abstract

Background: The plant *Perilla frutescens* Linn .belongs to the family Labiatae or Lamiaceae also called as Shiso plant or beefsteak plant purple mint. Perilla seed oil contains high amount of omega-3 fatty acids, especially alpha-linolenic acid (ALA) and these have been well tested as anti-inflammatory compounds that are useful for a variety of inflammatory conditions. *Perilla frutescens* seeds oil are a good source of polyunsaturated fatty acids (PUFAs). ALA may also help to prevent and treat depression, reduce menstrual pain, reduce the risk for fatal heart attacks, prevent breast cancer and treat autoimmune diseases such as lupus and rheumatoid arthritis.

Objectives: To evaluate the analgesic, anti-inflammatory and antipyretic activity of *Perilla frutescens* fixed oil.

Materials and Methods: Albino Wistar rats of either sex weighing 150–200 g and Swiss mice weighing 25–30 g were used. For investigating the anti-inflammatory effect of natural products, carrageenan- induced paw edema is a suitable experimental animal model .The carrageenan-induced paw edema method and histamine induced paw edema methods were used for the evaluation of anti-inflammatory activity. Carrageenan is a mixture of polysaccharides composed of sulphated galactose units and is derived from Irish Sea moss, *Chondrus crispus*. Histamine is an organic nitrogen compound involved in local responses. It is a biogenic amine. The analgesic effect of *Perilla frutescens* seed oil was evaluated using acetic acid induced writhing test and tail flick test. Typhoid paratyphoid A/B Vaccine method is used for evaluation of antipyretic activity.

Results: Results were analyzed by one way ANOVA followed by Students Newman Keuls multiple comparisons tests. *Perilla frutescens* fixed oil showed significant anti-inflammatory activity from 60 min as compared to vehicle treated animals. This is as comparable to aspirin. The extract showed some significant effect on inflammation and also reduced the number of writhing after acetic acid administration. But the extract does not show any antipyretic activity on Typhoid paratyphoid A/B Vaccine induced pyrexia.

Conclusion: It is therefore concluded that *Perilla frutescens* fixed oil has an anti-inflammatory and peripheral analgesic potential.

Keywords: Anti-inflammatory, Analgesic, Antipyretic, *Perilla frutescens* Carrageenan, Histamine, Omega -3 fatty acids

Introduction-

Inflammation is characterized by increased blood supply and activation of defense mechanism. It produces redness, swelling, heat, pain and often accompanied by loss of function. Inflammation is protective and destructive response, local microcirculation to tissue injury arising from cell damages due to mechanical trauma, physical, thermal injury and infections (1). It may be acute or chronic. There are many players which play a role in inflammatory response

such as circulating cells, plasma proteins, vascular wall cells and extracellular matrix (ECM) of the surrounding connective tissue. Inflammation is of acute and chronic types. Acute inflammation is the immediate and early response to an injurious agent, while chronic inflammation is the inflammation of prolonged duration (weeks or months) in which there is active inflammation, tissue destruction, and attempts at repair, which proceed simultaneously. However, inflammation, if uncontrolled, can become a cause of suffering, leading to disabilities, contractures, disfiguring of body, and chronic pain. In such situations, the inflammation needs to be controlled or suppressed (2). Pain is defined as “an unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage.” Fever, on the other hand, is defined as the elevation of core body temperature above normal; in healthy adults, the average oral temperature is 37°C (98.6°F) (3). Drugs used for the management of pain and inflammation are either narcotics (e.g., opioid) or non-narcotics (e.g., salicylates and corticosteroids), both of which are well known for their side effects, such as intestinal tract ulcers and erosions of the stomach linings. As a result, there is greater interest in finding a safer and potent alternative, especially agents from natural sources. The plant *Perilla frutescens* Linn .belongs to the family Labiatae or Lamiaceae also called as Shiso plant or beefsteak plant purple mint (4). It is distributed throughout India extending to the China, Japan, Korea, Thailand, and other Asian countries.

Perilla frutescens seeds are a good source of polyunsaturated fatty acids (PUFAs). These polyunsaturated fatty acids are most beneficial to human health and in prevention of different diseases like cardiovascular disorders, cancer, inflammatory, rheumatoid arthritis etc. Perilla oil is a very rich source of the omega-3 fatty acid. e.g. alpha-linolenic acid. It may be an excellent alternative to fish oil supplementation. Due to the high content of ALA present in perilla seed oil, the oil is attributed to the anti-inflammatory activity. ALA may also help to prevent and treat depression, reduce menstrual pain, reduce the risk for fatal heart attacks, prevent breast cancer and treat autoimmune diseases such as lupus and rheumatoid arthritis. In addition to the essential fatty acids in Perilla, there are also several pharmacologically active phenolic compounds, such as rosmarinic acid, apigenin, luteolin, chrysoeriol, quercetin, and catechin (5).

GLC report of *Perilla frutescens* fixed oil shows poly unsaturated fatty acids (PUFA) viz alpha linolenic acid (ALA) (64.73%), oleic acid (4.74%) and linoleic acid (18.13%) in *Perilla frutescens* fixed oil along with saturated fatty acids mainly palmitic acid (7.17%), stearic acid (2.70%). Considering the significant ALA content (64.73%) and previous reports for ALA exhibiting anti-inflammatory, analgesic and antipyretic activity, the present work was undertaken to evaluate the same using *Perilla frutescens* fixed oil (6).

For the extraction of *Perilla frutescens* seed oil, seeds were crushed and cold macerated in petroleum ether (40–60°C) for 7 days. Petroleum ether was evaporated from the extract and oil was filtered to clarity. The oil was stored at room temperature in amber-colored airtight bottle. The oil thus obtained was subjected for evaluation of anti-inflammatory, analgesic and antipyretic activity. However, there have been no other published reports on the anti-inflammatory, analgesic, and antipyretic activities of *Perilla frutescens* fixed oil. The present study was aimed at evaluating the possible anti-inflammatory, analgesic, and antipyretic effects of *Perilla frutescens* fixed oil (7).

Materials & Methods

Plant Material:

Perilla frutescens seeds were used for this study were procured from local market. The seeds were authenticated at National Botanical Research Institute (NBRI, CSIR), Lucknow, India and voucher sample (Ref. No. NBRI/CIF/300/2012) was deposited at National Botanical Research Institute (NBRI). Seeds were crushed and cold macerated with petroleum ether in amber colour bottle for seven days then filter and evaporate the petroleum ether then subjected to further studies. To avoid oxidation the oil was purged with nitrogen and was filled to the brim of the

bottle so that there was no head space.

Physicochemical characterization of extract: -

Gas Liquid Chromatography of *Perilla frutescens* was done from Arbro Pharmaceuticals Limited for the estimation of poly unsaturated fatty acid (PUFA's). Sequence file; G\GS\SEQUENCE\FID\2009\FAME.

Experimental Animals

72 Wistar albino rats (100-200 gm) and Swiss albino mice (20-30 gm), were used for anti inflammatory, analgesic and antipyretic activity, respectively.

Sources of Animals: Indian Veterinary Research Institute, Animal Resources, Izzatnagar, and Bareilly (UP). The study was approved by the Institutional Animal Ethics Committee (Ref:43/1279/ac/SU/IAEC/2012).

Animals were housed under standard conditions of temperature (25 ± 1°C) with 12hr light/dark cycle and had a free access to commercial pellet diet and water *ad libitum*. The animals were given week's time to get acclimatized with the laboratory condition, before experimentation. Baba Saheb Bhimrao Ambedkar University (A Central University) Lucknow, Uttar Pradesh, constituted in accordance with the guidelines of Committee for the Purpose of Control and Supervision of Experiment on Animals (CPCSEA), India.

Drugs & Chemicals

Perilla frutescens fixed oil, aspirin, chlorpheniramine maleate, nalbophine, typhoid paratyphoid vaccine.

Drugs solution-

1. Aspirin: aspirin was prepared in phosphate buffer saline
2. Normal saline: 90mg of sodium chloride was dissolved in 10ml of distill water.
3. Carrageenan: 1% solution of Carrageenan was freshly prepared in normal saline.
4. Histamine: 0.5µmol solution of Histamine was prepared using distilled water.

1. Anti-inflammatory activity-

1.a. Carrageenan induced paw edema procedure was performed to measure the anti-inflammatory activity. Groups with 6 animals in each group were used:

Table 1: Drugs and dosage regimens:

S.No.	Group	Treatment	Dose & Route
1	I(Control)	Distilled water	3ml/kg, ip
2	II	<i>Perilla frutescens</i> seed oil	1ml/kg,ip
3	III	<i>Perilla frutescens</i> seed oil	2ml/kg, ip
4	IV	Aspirin	100mg/kg, ip

Control and test drugs were given orally before 30 min of carrageenan administration. A freshly prepared suspension of carrageenan (1% w/v, 0.1 ml) was injected subcutaneously in the plantar region of right hind paw of each rat. A mark was made on both hind paws just below the tibiotarsal junction, and paw volume was measured up to that mark in each animal. The paw volume was measured at the paw volume was measured plethysmographically, immediately and after 1, 2 and 3 hour after the carrageenan administration. One additional group of animals was given standard drug i.e. Aspirin (100mg/kg, ip).The difference between the initial and subsequent values gave the actual edema volume which was compared with control. The

inhibition of inflammation was calculated using the formula-

$$\% \text{ inhibition} = 100(V_c - V_t)/V_c$$

where, V_c represents edema volume in control and V_t edema volume in group treated with test.(8)

1.b. Histamine induced paw edema-

Overnight fasted rats were divided into 4 groups of 6 animals each. Group I (Control) was given distilled water (3ml/kg) whereas Group II-III were treated with *Perilla frutescens* seed oil (1, and 2ml/kg).The basal volume of the right hind paw was determined before the administration of any drug. After 30min. of drug administration, inflammation was induced by intraplantar injection of histamine (0.5 μ mol) into the rat hind paw. Chlorpheniramine maleate (25 mg/kg) was used as a positive control for the above experiment.

Table 2: Drugs and dosage regimens:

S. No.	Group	Treatment	Dose & Route
1	I	Control, Distilled water	3ml/kg, ip
2	II	<i>Perilla frutescens</i> seed oil	1ml/kg, ip
3	III	<i>Perilla frutescens</i> seed oil	2ml/kg, ip
4	IV	Chlorpheniramine maleate	25mg/kg, ip

The difference between the initial and subsequent values gave the actual edema volume which was compared with control. The inhibition of inflammation was calculated using the formula-

$$\% \text{ inhibition} = 100(V_c - V_t)/V_c$$

Where, V_c represents edema volume in control and V_t edema volume in group treated with test.

2. Analgesic activity

2a. Tail immersion test-

Swiss albino mice were divided into 4 groups of 6 animals each. Each mouse was inserted in a conoid paper receptacle with its tail protruding. The protruding tail was entirely immersed in a pot of water maintained at 58 °C. The time in seconds for withdrawal of the tail clearly out of water was taken as the reaction time and measured by a stopwatch. The cut off time for the experiment was 15sec. The reaction time was determined before and periodically (1.0, 2.0 and 3.0 h) after administration of *Perilla frutescens* seed oil (1 and 2 ml/kg, ip).Opioid analgesic, ip (Nalbuphine 2mg/kg) was used as positive control.

Table 3: Drugs and dosage regimens:

S.No.	Group	Treatment	Dose & Route
1	I	Control, Distilled water	3ml/kg, ip
2	II	<i>Perilla frutescens</i> seed oil	1mg/kg, ip
3	III	<i>Perilla frutescens</i> seed oil	2mg/kg, ip

4	IV	Nalbuphine	2mg/kg, ip
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2.b Acetic acid induced writhing test-

Swiss albino mice of either sex were divided into 4 groups of 6 animals each. Group I served as control and received distilled water (3 ml/kg, ip); group II to III received *Perilla frutescens* seed oil 1.0, and 2.0 ml/kg (ip), respectively. Group IV received aspirin, dissolved in phosphate buffer pH 7.4, at a dose of 100 mg/kg (ip). After 60 min, 0.6 % v/v acetic acid solution in normal saline (10 ml/kg) was administered ip to each animal. Immediately after the acetic acid administration, numbers of writhing or stretches (a syndrome characterized by the wave of contraction of the abdominal muscle followed by the extension of the hind limb) were counted for 15 min. Reduction in writhing number as compared to control was considered as the evidence for the presence of the analgesia. Percentage inhibition of writhing was calculated using formula-
%inhibition=100(Mean no. of writhings (control)-Mean no. of writhings (test)/Mean no.of writhing (control)

Table 4: Drugs and dosage regimens:

S.No.	Group	Treatment	Dose & Route
1	I	Control, Distilled water	3ml/kg, ip
2	II	<i>Perilla frutescens</i> seed oil	1mg/kg, ip
3	III	<i>Perilla frutescens</i> seed oil	2mg/kg, ip
4	IV	Nalbuphine	2mg/kg, ip

3. Antipyretic activity

3a. Typhoid paratyphoid A/B Vaccine-

Wistar albino rats were fasted overnight but the animals had free access to water. The animals were divided into 4 groups of 6 animals each. Group I served as a control and received only typhoid paratyphoid A/B vaccine, at dose of 1 ml/kg, sc. Group II to IV received *Perilla frutescens* seed oil in dose level of 1, 2, 3 ml/kg intraperitoneally along with the vaccine while group V received a standard drug, aspirin (dissolved in phosphate buffer of pH 7.4, 100 mg/kg) along with the vaccine. Rectal temperature was measured 1 min before the administration of vaccine and drugs, followed by hourly measurement for 3 h. The difference between the initial and subsequent values gave the actual temperature which was compared with control. The inhibition of temperature was calculated using the formula-

$$\% \text{ inhibition} = 100(T_c - T_t)/T_c$$

Where, T_c represents temperature of control and T_t temperature of group treated with test.

Table 5: Drugs and dosage regimens:

S.No.	Group	Treatment	Dose & Route
1	I	Control, Distilled water	3ml/kg, ip
2	II	<i>Perilla frutescens</i> seed oil	1mg/kg, ip
3	III	<i>Perilla frutescens</i> seed oil	2mg/kg, ip

4	IV	Nalbuphine	2mg/kg, ip
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Statistical Analysis

The data are presented as mean \pm SEM and analyzed by One way ANOVA followed by Students Newman Keuls multiple comparisons tests for the possible significance identification between the various groups .P<0.05 was considered statistically significant.

Result-

Anti-inflammatory activity

a. Carrageenan induced paw edema-

In carrageenan induced paw edema model 25.71% reduction in paw edema was observed after 3 hour at 1ml/kg dose of oil, while at a dose of 2ml/kg oil 34.28% reduction in paw edema was observed after 3 hour. Administration of standard drug aspirin produced 48.57% inhibition. The result suggests that the oil significantly inhibit the carrageenan induced paw edema i.e. oil has capacity to inhibit the synthesis of proinflammatory prostaglandins and leukotrienes (Table -6)

Table-6 Anti-inflammatory activity of Perilla seed oil against carrageenan induced paw edema in albino rats.

S.NO.	Treatment	Paw edema (mm)			
		0h	1h	2h	3h
1.	Control (Distilled water, 3ml/kg ,ip)	0.25 \pm 0.04	0.29 \pm 0.02	0.33 \pm 0.02	0.35 \pm 0.02
2.	Perilla seed oil (1ml/kg,ip)	0.24 \pm 0.02	0.28 \pm 0.04	0.27 \pm 0.01** (18.18%)	0.26 \pm 0.01*** (25.71%)
3.	Perilla seed oil (2ml/kg,ip)	0.22 \pm 0.02	0.25 \pm 0.02 ^{ns}	0.24 \pm 0.04** (36.36%)	0.23 \pm 0.02*** (48.57%)
4.	Aspirin (100mg/kg, ip)	0.20 \pm 0.01	0.25 \pm 0.01 ^{ns}	0.21 \pm 0.02** (27.27%)	0.18 \pm 0.02*** (34.28%)

Values are expressed as Mean \pm SEM (n=6). Statistical significance compared to control using Student Newman-Keul's test.

* P<0.05; **P<0.01;***P<0.001 were considered statistically significant

b. Histamine induced paw edema-

In histamine induced paw edema, oil inhibited the edema about 32.35% at dose of 1ml/kg after 3hour and 38.53% inhibition at dose of 2ml/kg after 3hour which was comparable to the inhibitory effect of standard drug i.e. chlorpheniramine maleate has 50.06% inhibition in paw edema. The result shows that oil has significantly inhibited the edema at different doses (1ml/kg and 2ml/kg) (Table 7).

Table-7 Anti-inflammatory activity of Perilla seed oil in histamine induced paw edema

S.No.	Treatment	Paw edema (mm)			
		0h	1h	2h	3h
1.	Control (Distilled water 3ml/kg ,ip)	0.24 \pm 0.01	0.28 \pm 0.02	0.31 \pm 0.02	0.34 \pm 0.01

2.	Perilla seed oil (1ml/kg,ip)	0.22± 0.02	0.24± 0.02 ^{ns}	0.23± 0.01** (22.58%)	0.23± 0.01** (32.35%)
3.	Perilla seed oil (2ml/kg,ip)	0.21± 0.04	0.24± 0.03 ^{ns}	0.23± 0.01** (25.80%)	0.21± 0.02*** (38.23%)
4.	Chlorpheniramine maleate (25mg/kg, ip)	0.21± 0.011	0.23± 0.004	0.20± 0.002** (35.48%)	0.17± 0.04*** (50.06%)

Values are expressed as Mean± SEM (n=6). Statistical significance(* P<0.05)(**P<0.01)(***P<0.001) compared to control using Newman-Keul's test.

Analgesic activity

a. Acetic acid induced writhing test-

The oil significantly inhibited the acetic acid –induced writhing response in a dose dependent manner. The percentage inhibition of writhing produced by oil was comparable to that of aspirin (100mg/kg) a known standard analgesic drug. The oil (2ml/kg) exhibited 49.85%, where as the standard drug aspirin demonstrated 80.28% inhibition of writhing count (Table 8).The results suggest the possible and significant analgesic activity for *Perilla frutescens* seed oil.

Table-8 Analgesic activity of Perilla seed oil in acetic acid induced writhing test

S.No.	Treatment	Writhing Count
	Control (Distilled water 3ml/kg ,ip)	53.250± 4.32
	Perilla seed oil (1ml/kg,ip)	26.57± 1.65*** (49.7%)
	Perilla seed oil (2ml/kg,ip)	19.75± 1.75*** (49.85%)
	Aspirin (100mg/kg ip)	10.50± 0.64*** (80.28%)

Values are expressed as Mean± SEM (n=6) . Statistical significance (***P<0.001) compared to control using student Newman-Keul's Test.

b. Tail Immersion Method-

The analgesic effect of perilla seed oil was further evaluated using tail immersion test. The oil exerted its analgesic action through mechanism of anti-nociception. The oil shows action by increasing the reaction time to heat. This indicates the involvement of narcotic or opioid receptors in analgesic effect of oil (10,11).The effect of perilla seed oil on tail flick test is shown in Table 9 and displayed marked analgesic effect. The dose of 2ml/kg showed significant analgesic effect (***p<0.001).

Table- 9 Analgesic activity of Perilla seed oil in tail flick test

S.No	Treatment	Basal reaction time (sec)		
		1h	2h	3h
1.	Control (Distilled water 3ml/kg ,ip)	8.0±0.577	7.50±0.250	8.262±0.006
2.	Perilla seed oil (1ml/kg,ip)	11.75± 0.478***	9.250± 0.629	8.0± 0.478** (12.22%)
3.	Perilla seed oil (2ml/kg,ip)	11.75± 0.478***	9.75± 0.75	7.25± 0.4087*** (3.14%)

4.	Nalbuphine (1mg/kg, ip)	12.75± 0.85***	10.0± 0.70	6.50± 0.288* (36.64%)
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Values are expressed as Mean± SEM (n=6) . Statistical significance (***)P<0.001,* P<0.0**P<0.01) compared to control using Newman-Keul’s test .

Antipyretic activity –

The antipyretic activity of the *Perilla frutescens* seed oil was evaluated by using typhoid paratyphoid A/B vaccine induced pyrexia in rats. 1ml perilla seed oil shows 0.51% inhibition after 3 hours, while 2ml dose of seed oil shows 0.074% inhibition after 3 hour (Table 10). The oil did not exhibit the antipyretic effect against typhoid paratyphoid induced pyrexia in rats.

Table-10 Antipyretic activity of Perilla seed oil in typhoid paratyphoid A/B vaccine induced pyrexia

S. No	Treatment	Rectal temperature in °c			
		0h	1h	2h	3h
1	Control (Distilled water (1ml/kg ,sc)	33.375±0.047	34.10±0.070	34.225±0.93	33.875±0.025
2	Perilla seed oil+vaccine (1ml/kg,ip)+(1ml/kg,sc)	33.575±0.062	34.12±0.11	33.825±0.047 (1.68%)	33.700±0.000 (0.50%)
3	Perilla seed oil+vaccine (2ml/kg,ip)+(1ml/kg,sc)	33.325±0.047	34.290±0.004	34.350±0.119	34.850±0.075 (0.074%)
4	Aspirin+vaccine (100mg/kg ip)+(1ml/kg)	33.475±0.047	33.325±0.0478 (2.27%)	33.150±0.028 (3.14%)	33.150±0.028 (3.14%)

Values are expressed as Mean ±SEM (n=6) .No statistical significance when compared to control using student Newman-Keul’s Test.

Discussion-

The GLC report of *Perilla frutescens* fixed oil shows the presence of poly unsaturated fatty acids (PUFA) via alpha linolenic acid (ALA) (64.73%), oleic acid (4.74%) and linoleic acid (18.13%) along with saturated fatty acids mainly palmitic acid (7.17%), stearic acid (2.70%). Considering the significant ALA content (64.73%) and previous reports of ALA exhibiting anti-inflammatory, analgesic and antipyretic activity, the present work was undertaken to evaluate the same using *Perilla frutescens* fixed oil.

The anti-inflammatory activity of *Perilla frutescens* seed oil was evaluated against carrageenan and histamine induced paw edema in rats. Inflammation is characterized by redness, swelling and pain which occur due to local vasodilatation, increased capillary permeability and infiltration of leucocytes .The key inflammatory mediators are the n-6 eicosanoids, PGE2 and leukotrienes B4 derived from polyunsaturated fatty acid arachidonic acid metabolism by the action of cyclooxygenase and lipoxygenase (12).

The most effective and widely used model for inflammation is carrageenan- induced paw edema. Carrageenan is a mixture of polysaccharides composed of sulfated galactose units and is derived

from Irish Sea moss, *Chondrus crispus*. It is used as an endemogen. For investigating the anti-inflammatory effect of natural products, carrageenan- induced rat paw edema is a suitable experimental animal model which is believed to be biphasic. The early phase (1-2hr) of the carrageenan model is mainly mediated by histamine, serotonin and increased synthesis of prostaglandins in the damaged tissue surroundings. The late phase is sustained by prostaglandin release and mediated by bradykinine, leukotrienes, polymorph nuclear cells and prostaglandins produced by tissue macrophages (13,14,15).

In carrageenan induced paw edema model 25.71% reduction in paw edema was observed after 3 hour at 1ml/kg dose of oil, while at a dose of 2ml/kg oil 34.28% reduction in paw edema was observed after 3 hour. Administration of standard drug aspirin produced 48.57% inhibition. In our body tissue inflammation starts from arachidonic acid which could be metabolized by cyclooxygenase and lipoxygenase pathways. Activation of cyclooxygenase pathway produces prostaglandins while lipoxygenase pathway yields proinflammatory leukotrienes (16). The result suggests that the oil significantly inhibit the carrageenan induced paw edema i.e. oil has capacity to inhibit the synthesis of proinflammatory prostaglandins and leukotrienes.

The oil was further evaluated for its anti-inflammatory potential against histamine induced paw edema. Histamine is an organic nitrogen compound involved in local [immune responses](#) as well as regulating physiological function in the gut and act as a [neurotransmitter](#) (17,18). It is a biogenic amine. Histamine triggers the [inflammatory response](#). Histamine exerts its actions by combining with specific cellular [histamine receptors](#). The four histamine receptors that have been discovered in humans are designated H₁, H₂ and all [G protein-coupled receptors](#). Swelling occurs primarily due to action of histamine which is released following mast cell degranulation by number of inflammatory mediators including substances P, IL-1, IL-10 and TNF- α . This is likely to evoke the release of neuropeptide as well as release of prostaglandins and monohydroxyeicosatetranoic-acid from endothelial cell leading to hyperalgesia and other pro-inflammatory effects. The acute inflammatory process, in which vascular permeability increases and leukocyte migration occurs, involves several mediators including neutrophil-derived active oxygen species and free radicals, such as hydrogen peroxide, superoxide and the hydroxyl radical, which finally damage the molecules within cells. Histamine causes increase in vascular permeability and polymorph nuclear leucocytes (PNL) infiltration. Histamine and histamine- activated PNL can trigger the production of oxygen derived free radicals (19,20). In histamine induced paw edema, oil inhibited the edema about 32.35% at dose of 1ml/kg after 3hour and 38.53% inhibition at dose of 2ml/kg after 3hour which was comparable to the inhibitory effect of standard drug i.e. chlorpheniramine maleate has 50.06% inhibition in paw edema. The result shows that oil has significantly inhibited the edema at different doses (1ml/kg and 2ml/kg).

Pain is an unpleasant sensation that is a consequence of complex neurochemical process in the central and peripheral nervous systems. Non-steroidal anti-inflammatory drugs and opioid are used in management of mild to moderate and severe pain respectively. These drugs have serious limitations due to their side effects. Most of drugs used presently for the management of pain and inflammation possess some side effect and toxic effects. It is therefore, inevitable to search for new less toxic and more effective anti-inflammatory and analgesic agents (21,22).

These are the agent which relieves pain without causing loss of consciousness. Algesia produces due to some mediators like COX-1 and COX-2. Analgesic activity results due to inhibition of COX mediators. The classification of analgesic drugs is usually based on their mechanism of action either on the central nervous system or on the peripheral nervous system. The analgesic effect of *Perilla frutescense* seed oil was evaluated using acetic acid induced writhing test and tail flick test. Acetic acid causes inflammatory pain by inducing capillary permeability, while tail immersion pain indicates involvement of narcotics or opioids receptors (23,24). The oil significantly inhibited the acetic acid –induced writhing response in a dose dependent manner. The percentage inhibition of writhing produced by oil was comparable to that of aspirin

(100mg/kg) a known standard analgesic drug. The oil (2ml/kg) exhibited 49.85%, where as the standard drug aspirin demonstrated 80.28% inhibition of writhing count. The results suggest the possible and significant analgesic activity for *Perilla frutescens* seed oil.

The analgesic effect of perilla seed oil was further evaluated using tail flick test. The oil exerted its analgesic action through mechanism of anti-nociception. The oil shows action by increasing the reaction time to heat. This indicates the involvement of narcotic or opioid receptors in analgesic effect of oil⁽¹⁶⁾. The effect of perilla seed oil on tail flick test is shown in Table 9 and displayed marked analgesic effect. The dose of 2ml/kg showed significant analgesic effect (**p<0.001).

The oil was further evaluated for antipyretic activity, considering the fact that anti-inflammatory agents also exhibit some antipyretic action. An antipyretic is a type of medication that will prevent or reduces fever by lowering body temperature from a raised state. Mostly NSAIDS work by inhibiting prostaglandin synthesis within the hypothalamus. The antipyretic activity of the *Perilla frutescens* seed oil was evaluated by using typhoid paratyphoid A/B vaccine induced pyrexia in rats. 1ml perilla seed oil shows 0.51% inhibition after 3 hours, while 2ml dose of seed oil shows 0.074% inhibition after 3 hour. The oil does not exhibit the antipyretic effect against typhoid paratyphoid induced pyrexia in rats.

Perilla oil is rich in the omega-3 fatty acids, which when metabolized gives eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which can displace arachidonic acid (AA) from cell membranes. These omega-3 fatty acids are also released with AA by phospholipases and act as substrate inhibitors of conversion of AA by cyclooxygenase and the terminal synthesis to the pro-inflammatory oxygenated inflammatory mediators known as eicosanoids. Considering the above results, it is evident that *Perilla frutescens* seed oil exhibit marked anti-inflammatory and analgesic activity. Nevertheless, we did not observe any antipyretic activity of the same. The anti-inflammatory and analgesic effects of the oil could be attributed to high amount of ALA present in the oil.

Conclusion-

Considering the significant ALA content in *Perilla Frutescens* seed oil, it was concluded that ALA is responsible for anti-inflammatory and analgesic effect. However, further studies need to be done to corroborate these findings as future scope of this work.

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