



Anti-inflammatory and Antioxidant Activities of *Mimusops elengi* L.

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Authors' contributions

This work was carried out in collaboration between all authors. Author HZ designed the study, wrote the protocol and wrote the first draft of the manuscript. Author GHR supervised the study and identified the plant. Authors MK and SK managed the literature searches. Author HZ performed all experimental process. All authors read and approved the final manuscript.

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ABSTRACT

Aim: *M. elengi* L. (Sapotaceae) has been used for rheumatism and pain. However the floral part of this plant is not scientifically explored yet for its anti-inflammatory and antioxidant activity. The present study is an endeavor to evaluate anti-inflammatory and antioxidant activities of methanolic extracts of flower and leaves (MFE and MLE) of *Mimusops elengi*.

Study Design: Assessment of anti-inflammatory and antioxidant activity.

Methodology: Anti-inflammatory activity was evaluated in albino species of rats by using carrageenan induced paw edema, where as *in vitro* antioxidant activity was also performed by DPPH radical and nitric oxide scavenging method.

Results: The anti-inflammatory activity of methanolic extract of *M. elengi* (MFE, MLE) against carrageenan induced paw edema in albino rats at a dose of (50, 100, 200 mg/kg) showed that the extracts have significant ($P < 0.01$; $P < 0.001$) effect on inflammation and markedly reduced the swelling. At a concentration of 20 $\mu\text{g/mL}$, 40 $\mu\text{g/mL}$, 60 $\mu\text{g/mL}$, 80 $\mu\text{g/mL}$ and 100 $\mu\text{g/mL}$ (MFE, MLE) extracts, scavenged of DPPH radical. While in nitric oxide scavenging assay, various concentrations of the extracts (10, 25, 50, 100 and 150 $\mu\text{g/mL}$) of *M. elengi* showed percentage inhibition in a dose dependent manner. These activities showed by the extracts of *M. elengi* due to the frequent occurrence of rich phenolic compounds such as, flavonoids,

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tannins, phenols, terpenoids and saponins.

Conclusion: In the light of research it seems close correlation between the powerful antioxidant and significant anti-inflammatory activities of the MFE and MLE.

Keywords: *Mimusops elengi* L.; anti-inflammatory; antioxidant; carrageenan induced paw edema.

1. INTRODUCTION

Inflammation is a pathophysiological response of living tissue to injuries that leads to the local accumulation of plasma fluid and blood cells. It is a defense mechanism of body, the mediator that involve in inflammation reaction can induced, maintain or aggravate many diseases [1]. Excess production of pro-inflammatory molecules such as nitric oxide (NO) is believed to be responsible for modulating inflammation besides their crucial role in immune-inflammatory response. These inflammatory molecules are also known to cause cell death and tissue damage because nitric oxide can react with the free radicals such as superoxides to produce peroxynitrite that can lead to irreversible damage to cell membranes [2-3]. Studies have uncovered that phenolic compound act as excellent anti-inflammatory agents and have been extensively studied and beneficial effects have been demonstrated in many animal models [4-5]. It is well known that reactive oxygen species (ROS) play a major role in the development of oxidative stress that can lead to many illnesses including cardiovascular diseases, diabetes, inflammation, degenerative diseases, cancer, anemia, and ischemia. Many synthetic antioxidant agents have been developed to remediate oxidative stress. However, the factors such as high cost, lack of availability and side effects remained as major setbacks in combating oxidative stress. Natural antioxidants received a prominence as they are often free from side effects, less expensive and abundant in many plant sources [6]. Plants based antioxidant compounds play a defensive role by preventing the generation of free radicals and hence are extremely beneficial to alleviate the diseases caused by oxidative stress [7-9]. Antioxidants, which scavenge these reactive oxygen metabolites, have been found to complement the anti-inflammatory process, promote tissue repair and wound healing [10-12]. A number of plant secondary metabolites such as apigenin, quercetin, luteolin and silymarin have been found to exhibit anti-inflammatory activities due to their antioxidant properties [13,14].

The practices of traditional medicine are based on hundreds of years. The world health

organization (WHO) estimates that two third of the world population still depends upon traditional medicines for the treatment of various types of diseases. One such important traditional medicinal plant is *Mimusops elengi* L. belongs to Sapotaceae family popularly known as Bakul or Spanish cherry or bullet wood. It is a small to large evergreen tree up to 15 m in height and found in different parts of the world i. e, Sothern India, Burma and Pakistan. It is also cultivated in gardens as an ornamental plant. All parts of the tree have medicinal properties [15]. The flowers are sweet, acrid, oleagenous; cooling, astringent to the bowels; good for the teeth, causes flatulence. They are used as an expectorant; cures biliousness, liver complaints, diseases of the nose, headache. The smoke of the flower is used in asthma. The leaves are traditionally used in treatment of different types of diseases such as fever, pustular eruptions of skin, ulcer, headache, dental diseases, bacterial diseases [16,17]. Leaves were found to have antioxidant, cytotoxic, analgesic, wound healing and antipyretic activities [18-20]. The presence of saponins, alkaloid, steroids and terpenoids has previously been reported form *M. elengi* [21]. Flower contains volatile oil [22], D-mannitol, beta-sitosterol and beta-sitosterol-D-glycoside [23]; leaves contain sterols, reducing sugar, tannins; stem bark contain tannins, spinosterol and taraxerol [24].

M. elengi has been used for rheumatism and pain *in vivo* and *in vitro* models previously [25-27]. However the floral part of this plant is not scientifically explored yet for its anti-inflammatory and antioxidant activity. Hence an effort has been made here for the first time to investigate the methanolic extracts of *M. elengi* (leave and flower) for its anti-inflammatory and antioxidant potentials.

2. MATERIALS AND METHODS

2.1 Plant Material and Preparation of Extracts

Fresh flowers and leaves of *Mimusops elengi* L. were collected from the premises of University of Karachi, Pakistan. The plant materials were

identified and authenticated by Prof. Dr. Ghazala H. Rizwani, Department of Pharmacognosy, Faculty of Pharmacy, University of Karachi, Pakistan. A voucher specimen no. 083 was deposited at the herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, University of Karachi, Pakistan.

After the collection of flower and leaves of *M. elengi*, plant parts were dried separately under shade. 500 g of flower and leaves were soaked in methanol (250 L each) at room temperature separately for seven to ten days. After that methanol was filtered through Whatmann filter paper No. 1 and the extract was concentrated under reduced pressure and controlled temperature ($40^{\circ}\text{C} \pm 0.5$) on a rotary evaporator (Buchi, Switzerland). The yield of flower extract (MFE) and leaves extract (MLE) obtained were 27.2 g (5.44%) and 60.0 g (12.0%) respectively.

2.2 Experimental Animals

Adult albino rats weighing 120-160 g of either sex were used for the experiments. They were housed in standard cages at a temperature of $27 \pm 2^{\circ}\text{C}$. The animals were exposed to the alternate cycle of 12 h of darkness and light and fed with standard laboratory diet (PCSIR Laboratories, Karachi, Pakistan) and water. The animals were fasted for at least 12 h before experiment. The set of rules followed for animal experiment were approved by the institutional animal ethical committee.

2.3 Chemicals

Absolute Methanol (Merck, Germany), Indomethacin (B.D.H. laboratory supplies, UK), Carrageenan (MP Biomedicals, LLC, USA), 2, 2-diphenyl- 1-picrylhydrazyl (B.D.H. laboratory supplies, UK), Ascorbic acid (Sigma Chemical, USA), sodium nitroprusside (B.D.H. laboratory supplies, UK), phosphate buffer (pH 7.4), sulfanilic acid reagent (B.D.H. laboratory supplies, UK), naphthyl ethylene diamine dihydrochloride (B.D.H. laboratory supplies, UK).

2.4 Acute Toxicity (LD₅₀)

The acute toxicity (LD₅₀) study was carried on *M. elengi* extract (MFE, MLE) using a modified Lorke's method [28]. Animals (mice) of either sex were fasted overnight prior to the study. Oral doses of 10, 100, 1000 mg/kg were used in phase I, while 2000, 3000, 4000, and

5000 mg/kg were given in phase II to the treated groups; the control groups received 10 mL/kg of normal saline. The mice were closely observed, the toxic symptoms and behavioral changes for first 3 hrs after extract(s) administration. Behavior parameters of animals were observed including movement, sense of pain and touch, convulsion, phonation, aggression, increased or decreased respiration, lacrimation, social interaction, defecation and urination. All animals were observed for mortality for one week and their weights were registered.

2.5 Anti-inflammatory Activity

2.5.1 Carrageenan induced paw edema

Albino rats of either sex weighing 150-200 grams were divided into eight groups of six animals each. Group I served as control and administered with normal saline. Group II received indomethacin (10 mg/kg, body weight). While group III- VIII orally administered with extracts of *M. elengi* (50 mg/kg, 100 mg/kg and 200 mg/kg). After one hour of the administration of the extracts (MFE, MLE) and indomethacin, 0.1 mL of 1% w/v carrageenan solution in normal saline was injected into the sub plantar tissue of the hind paw of the rat. The paw volume of the rats were measured in the digital plethysmograph (Ugo basile, Italy), at 0 min, 30 min, 60 min, 90 min, 120 min, and 150 min. The percentage increase in paw edema of the treated groups was compared with that of the control and the inhibitory effect of the extracts was studied. The relative potency of the extracts under investigation was calculated based upon the percentage inhibition of the inflammation [29,30]. Percentage inhibition was calculated using the formula

$$\text{Anti - inflammatory activity (\%)} = \frac{V_c - V_t}{V_c} \times 100$$

Where, V_t the percentage represents the percentage difference in increased paw volume after the administration of test drugs to the rats and V_c represents difference of increased volume in the control groups.

2.6 Antioxidant Assay

2.6.1 DPPH radical scavenging activity

The free radical scavenging capacity of the extracts was determined using DPPH (2, 2-diphenyl- 1-picrylhydrazyl). DPPH solution was

prepared in methanol (0.004% w/v). MFE, MLE extract were mixed with 95% methanol to prepare the stock solution of 10 mg/100 mL or 100 µg/mL respectively. From stock solution 2 mL, 4 mL, 6 mL, 8 mL and 10 mL of this solution were taken in five test tubes and by serial dilution with same solvent was made the final volume of each test tube up to 10 mL whose concentration was then 20 µg/mL, 40 µg/mL, 60 µg/mL, 80 µg/mL and 100 µg/ml respectively. Freshly prepared DPPH solution was added in each of these test tubes and after 10 min, the absorbance was taken at 517 nm using spectrophotometer. Ascorbic acid was used as reference standard and dissolve in distilled water to make a stock solution of 100 µg/mL [31]. Scavenging of the DPPH free radical was measured using the following equation

$$\text{Radical scavenging (\%)} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

The relationship between percentage inhibition and sample concentration was plotted to determine the IC₅₀ value.

2.6.2 Nitric oxide scavenging activity

Sodium nitroprusside in aqueous solution at physiological pH spontaneously generated nitric oxide which interacts with oxygen to produce nitrite ions determined by the use of Griess reagents. Two mL of 10 mM sodium nitroprusside dissolved in 0.5 mL phosphate buffer saline (pH 7.4) was mixed with 0.5 mL of plant extracts at various concentrations (10, 25, 50, 100, 150 µg/mL). The mixture was incubated at 25°C after 150 min. From the incubated mixture 0.5 mL was taken out and added into 1.0 mL sulfanilic acid reagent (33% in 20% glacial acetic acid) and incubated at room temperature for 5 min. Finally 1.0 mL naphthyl ethylene diamine dihydrochloride (0.1% w/v) was mixed and incubated at room temperature for 30 min before measuring the absorbance at 540 nm was measured with a spectrophotometer [32]. The nitric oxide radical scavenging activity was calculated.

$$\text{Nitric oxide scavenging (\%)} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

2.7 Statistical Analysis

The data values were expressed as mean ± standard error of mean and analyzed using one

way ANOVA. *P* values lower than the appropriate levels of significance as designated were considered statistically significant.

3. RESULTS

3.1 Acute Toxicity (LD₅₀)

No death was recorded in the acute toxicity testing for all animals and there was also no obvious signs of toxicity in all treatment groups in both species (rat and mice) and phases (1 and 2) when observed within 24 hours of post administration of the extracts of *Mimusops elengi* (MFE and MLE). The LD₅₀ of the extracts was greater than 5000 mg extract/kg body weight (Tables 1-2).

3.2 Carrageenan Induced Paw Edema

The anti-inflammatory activity of methanolic extracts (MFE, MLE) of *Mimusops elengi* against carrageenan induced paw edema in albino rats showed that the extracts have significant effect on inflammation and markedly reduced the swelling. The maximum percentage reduction in the paw volume in the group of animals treated with a dose of 100 mg/kg of extracts (MFE, MLE) was 65.57%, 72.13% and for the 200 mg/kg was 78.68%, 75.40% at 3 hours respectively. It shows that the plant extracts have significant (*P* < 0.01; *P* < 0.001) anti-inflammatory effect and the results were compared with indomethacin (10 mg/kg, body weight) used as standard (Fig. 1 and Fig. 2).

3.3 DPPH radical Scavenging Activity

The capacity to scavenge by the methanolic flower and leaves extracts of *M. elengi* Linn (MFE and MLE) was presented in Fig. 3. At a concentration of 20 µg/mL, 40 µg/mL, 60 µg/mL, 80 µg/mL and 100 µg/mL extracts, scavenged of DPPH radical were 25.90%, 53.34%, 56.99%, 70.58%, 72.58% (MFE); 32.33%, 62.08%, 77.64%, 89.45%, 90.46% (MLE) respectively. Ascorbic acid used as a standard for DPPH scavenging activity, showed 43.41%, 55.24%, 76.43%, 86.36 and 91.88% percentage scavenging at same concentrations. IC₅₀ calculated by the regression curve for extracts were 50±0.11 µg/mL and 32±0.06 µg/mL respectively. While IC₅₀ calculated for ascorbic acid was 28±0.04 µg/ml (Fig. 5).

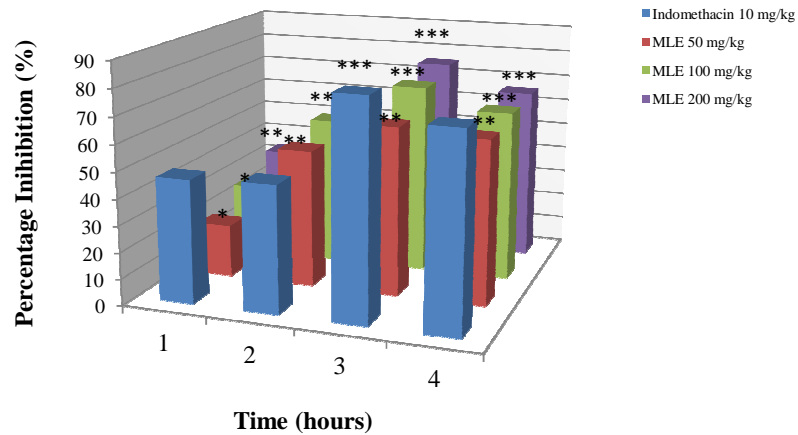


Fig. 1. Effect of *M. elengi* flower extract (MFE) on carrageenan- induced rat paw edema induced. Each value represents as mean ± SEM (n=6); *P< 0.05 **P< 0.01 ***P< 0.001 as compared with control group

Table 1. Acute oral toxicity with behavioural parameters of *M. elengi* (MFE, MLE) extracts

Source	Test groups	Time (min)	Behavioural signs													
			A	B	C	D	E	F	G	H	I	J	K	L	M	N
<i>Minusops elengi</i>	MFE 1 g/kg	30 min	↑	+	++	+	+	-	-	-	↓	n	n	n	-	-
		60 min	↑	+	++	+	+	-	-	-	n	n	n	n	-	-
		120 min	↑	+	+	+	++	-	-	-	n	n	n	n	-	-
		180 min	n	+	+	+	++	-	-	-	n	n	n	n	-	-
	MFE 3 g/kg	30 min	↑	+	+	+	+	-	-	-	↓	n	n	n	-	-
		60 min	n	+	++	+	++	-	-	-	↓	n	n	n	-	-
		120 min	n	+	++	++	++	-	-	-	n	n	n	↓	-	-
		180 min	n	+	+	++	++	-	-	-	n	n	n	n	-	-
	MFE 5 g/kg	30 min	n	+	+	+	+	-	-	-	↓	n	n	n	-	-
		60 min	n	+	+	+	++	-	-	-	↓	n	n	n	-	-
		120 min	↑	+	++	+	++	-	-	-	↓	n	n	n	-	-
		180 min	↑	+	++	+	++	-	-	-	↓	n	n	n	-	-
	MLE 1 g/kg	30 min	↓	+	+	+	n	-	-	-	↓	n	n	n	-	-
		60 min	↓	++	++	+	n	-	-	-	↓	n	n	n	-	-
		120 min	↓	++	++	+	n	-	-	-	n	n	n	n	-	-
		180 min	↓	++	++	+	n	-	-	-	n	n	n	n	-	-
	MLE 3 g/kg	30 min	↓	+	+	++	++	-	-	-	↓	n	n	n	-	-
		60 min	↓	++	++	+	+	-	-	-	↓	n	n	n	-	-
		120 min	↓	++	++	+	+	-	-	-	n	n	n	n	-	-
		180 min	↓	+	++	+	+	-	-	-	n	n	n	n	-	-
MLE 5 g/kg	30 min	↓	+	+	+	+	-	-	-	↓	n	n	n	-	-	
	60 min	↓	+	+	+	+	-	-	-	↓	n	n	n	-	-	
	120 min	↓	+	+	+	+	-	-	-	n	n	n	n	-	-	
	180 min	↓	++	+	+	++	-	-	-	n	n	n	n	-	-	

↑= Increase in activity, ↓= Decrease in activity, -=No change in behavioural activity, += Slightly increase or decrease, ++ = Moderately increase, n = Normal activity, A= Movement, B= Phonation, C= Sense of pain, D= Sense of Touch, E= Social interaction, F= Aggression, G= Convulsion, H= Eye irritation, I= Laccrimation, J= Pupil size, K= Defecation, L= Urination, M= Respiration, N= Abnormal tail, O= Death

Table 2. Acute oral toxicity of *M. elengi* extracts

Source	Treatment group	First Phase		Second phase	
		Dose (mg/kg)	Mortality	Dose (mg/kg)	Mortality
<i>Mimusops elengi</i>	MFE	10	0/6	1500	0/6
		100	0/6	3000	0/6
		1000	0/6	5000	0/6
	MLE	10	0/6	1500	0/6
		100	0/6	3000	0/6
		1000	0/6	5000	0/6

*LD*₅₀ > 5000 mg/kg

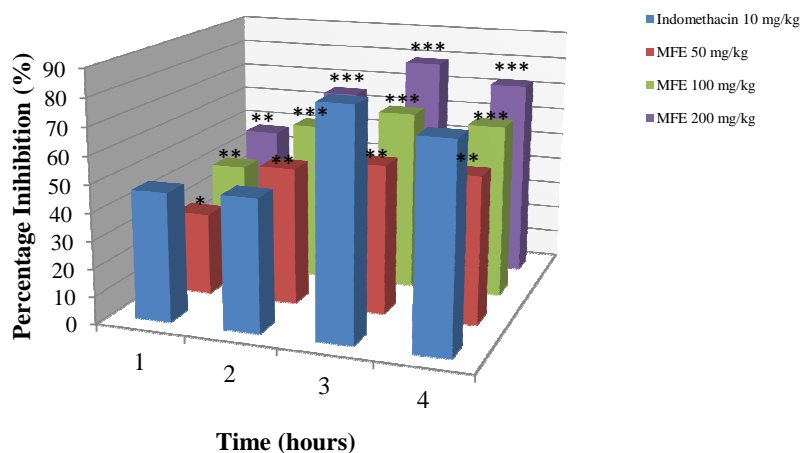


Fig. 2. Effect of *M. elengi* leaves extract (MLE) on carrageenan- induced rat paw edema induced. Each value represents as mean ± SEM (n=6); *P< 0.05 **P< 0.01 *P< 0.001 as compared with control group**

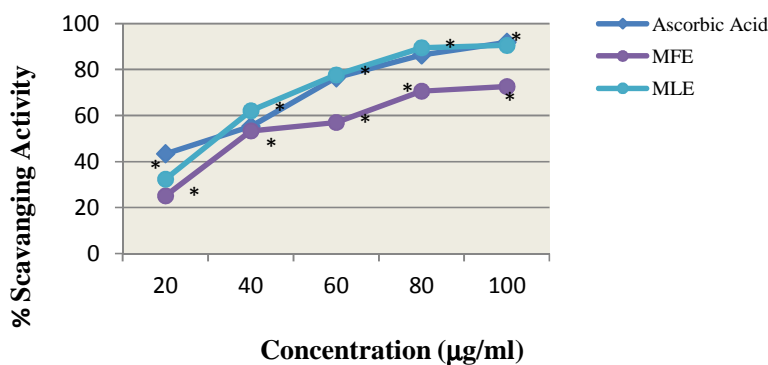


Fig. 3. DPPH scavenging activity of *M. elengi* (MFE, MLE) extracts

3.4 Nitric Oxide Scavenging Activity

While in nitric oxide scavenging assay, various concentrations (10, 25, 50, 100 and 150 µg/mL) of the extracts (MFE, MLE) showed percentage inhibition in a dose dependent manner depicted in Fig. 4. Ascorbic acid showed 81.02% nitric

oxide scavenging whereas extracts produced 56.01% and 57.13% activity. The extracts (MFE, MLE) and ascorbic acid needed for 50% inhibition (IC₅₀) was found to be, 81±0.01 µg/mL, 16±2.02 µg/mL and 12±2.13 µg/mL respectively (Fig. 5).

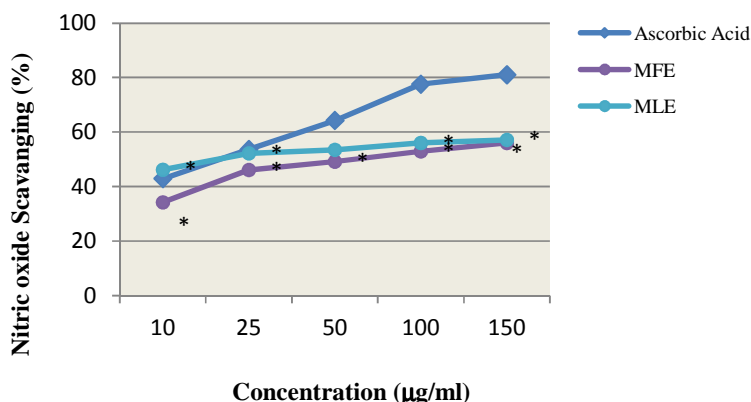


Fig. 4. Nitric oxide scavenging activity of *M. elengi* (MFE, MLE) extracts

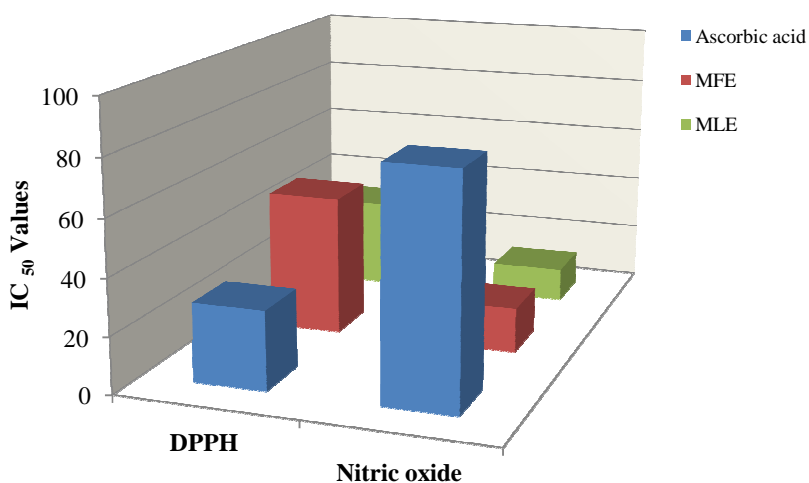


Fig. 5. The IC₅₀ values of *M. elengi* extract and Ascorbic acid (standard) in DPPH and nitric oxide scavenging activities

4. DISCUSSION

The use of traditional medicine is widespread and plants still represent a large source of natural antioxidants that might serve as leads for the development of novel drugs. Several anti-inflammatory drugs have recently been shown to have an antioxidant and radical scavenging mechanism as part of their activity [33-35]. Reactive oxygen species (ROS) propagate inflammation by stimulating release of cytokines such as interleukin-1, tumor necrosis factor- α , and interferon- γ , which stimulate recruitment of additional neutrophils and macrophages. Thus free radicals are important mediators that provoke or sustain inflammatory processes and, consequently, their neutralization by antioxidants

and radical scavengers can attenuate inflammation [36,37]. Inflammation is a complex process and ROS play an important role in the pathogenesis of inflammatory disease. Thus antioxidants which can scavenge ROS are expected to improve these disorders.

Carrageenan induced hind paw edema is the standard experimental model of acute inflammation. Carrageenan is the phlogistic agent of choice for testing anti-inflammatory drugs as it is not known to be antigenic and is devoid of apparent systemic effects. Moreover, the experimental model exhibits a high degree of reproducibility [29]. Carrageenan induced edema is a biphasic response. The first phase is mediated through the release of histamine,

serotonin and kinins whereas the second phase is related to the release of prostaglandin and slow reacting substances [38]. It has been reported that the second phase of edema is sensitive to drugs like hydrocortisone, phenylbutazone and indomethacin. Indomethacin is a cyclooxygenase inhibitor, the extract has activity which is comparable to indomethacin and can be said to inhibit the cyclooxygenase enzyme but lipoxygenase inhibitors also possess significant anti-inflammatory activity against carrageenan induced paw edema, so inhibition of carrageenan induced paw edema by the crude extract could also be due to its inhibitory activity on the lipoxygenase enzyme.

In humans the over-production of ROS can result in tissue injury and has been implicated in disease progression and oxidative damage of nucleic acids and proteins [36]. When there is a lack of antioxidants to quench the excess reactive free radicals, cardiovascular, cancer, neurodegenerative, Alzheimer's and inflammatory diseases may develop in the body [39,40]. The data presented in this study demonstrate that *M. elengi* flower and leaves extracts possess significant anti-inflammatory and antioxidant activities. These activities showed by the extracts of *M. elengi* due to the strong occurrence of rich phenolic compounds such as, flavonoids, tannins, phenols, along with terpenoids and saponins. The antioxidant effects produced by the MFE and MLE may be responsible for anti-inflammatory activity too.

5. CONCLUSION

In conclusion, the methanolic extracts of flower and leaves of this ornamental plant *Mimusops elengi* L. was able to significantly reduce inflammation and also have an ability to scavenge free radicals which plays a major role in many body metabolisms. This interesting source could be use as powerful of anti-inflammatory and antioxidant agents, along with its significance in food, cosmetics and pharmaceutical industries.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All experiments and procedures have been examined and approved by Ethical Committee of PCSIR Laboratories, Pakistan.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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