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EVALUATION OF ANTI-INFLAMMATORY ACTIVITY OF *MIMUSOPS ELENGI* EXTRACTS IN DIFFERENT IN-VITRO AND IN-VIVO MODELS

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ABSTRACT

In the present study the anti-inflammatory potential of Methanolic and Aqueous extracts of Leaves of *Mimusops elengi* L. was evaluated for their *in-vitro* and *in-vivo* anti-inflammatory activity. *In-vitro* anti-inflammatory activity was evaluated by using membrane stabilization assay, proteinase inhibitory activity and immunomodulatory activity using macrophages. *In-vivo* animal models involve carrageenan induced rat paw edema, in which the decrease in edema formation due to drug treatment (200 and 400 mg/kg) was measured using Plethysmometer and cotton pellet induced granuloma model in which the level of inhibition of granuloma was measured at different dose levels(200 & 400 mg/kg). Methanolic and Aqueous extracts of *Mimusops elengi* Leaves showed significant results in *in-vitro* and in-vivo anti-inflammatory models. The results of *in-vitro* and *in-vivo* models showed that *Mimusops elengi* has potential anti-inflammatory activity and acts through multiple mechanisms.

KEY-WORDS: **Antioxidant, anti-inflammatory, *Mimusops elengi***

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INTRODUCTION

Mimusops elengi Linn (family Sapotaceae) commonly known as Bakul and is found all over the different parts of India, Pakistan, Bangladesh[1]. The tree is of great significance to the Hindus and the dried twigs of the tree are used for various yajnas and religious rituals. Reports suggest that in the ancient Indian civilization, the fruits were a staple diet of the sages, hermits and people[2]. *Mimusops elengi* Linn. (M. elengi) is a large glabrous evergreen tree 12-15 m high, with a compact leafy head and short erect trunk, bark smooth, scaly and gray. Leaves 6.3-10 by 3.2-5 cm, elliptic shortly acuminate, glabrous, base acute or rounded, petioles 1.3-2.5 cm long[3]. It is cultivated in gardens as an ornamental tree for sweet-scented flowers. It has been used in the traditional Indian system of medicine for the treatment of various ailments. The different parts of the title plant (bark, flowers, seeds and fruits) have great therapeutic value. The bark, fruits and flowers of this plant are used in the treatment of diarrhea, dysentery[4], as a brain tonic and to relieve cephalalgia. The water distilled from the flowers is used as a stimulant medicine, calm anxiety, panic attacks and brain tonic as used by the traditional practitioners of Southern India[5,6,7]. Several other therapeutic uses such as cardiotonic, alexipharmic, stomachic, anthelmintic, astringent[7], antioxidant, antiurolithiatic[8], anti-amnesic[9], antiulcer[11], hypotensive[11], antidiabetic[13] have been reported. The plant is used in folk medicine for treatment of asthma, diarrhea, fever, headache, rheumatism, eye pain and strengthening of gums[13] . Phytochemical work on this plant has revealed the presence of various sugars, amino acids, free triterpenes, fatty acids, flavonoids and free sterols[14]. Several triterpenoids, steroids, steroidal glycosides, flavonoids, and alkaloids have been reported from this specie[4,15] . Other phytochemical constituents like taraxerol, taraxerone, ursolic acid, betulinic acid, V-spinosterol, W-sitosterol, lupeol, alkaloid isoretronecyl tiglate and mixture of triterpenoid saponins have also been reported in the bark of *Mimusops elengi*[16]. Pharmacological studies have shown that this plant possesses diuretic, spermicidal, spasmolytic and antimycotic activity[11].

MATERIALS AND METHODS

i) Animals

Female Wistar rats (180-200 gm) were procured from Glenmark Pharmaceuticals, Navi Mumbai. Animals were housed in polypropylene cages (6animals/cage) under hygienic conditions in the CPCSEA registered animal house (CPCSEA/87/1999) of Institute of Chemical Technology, University of Mumbai, Matunga, Mumbai. Animals were fed with standard pelleted diet procured from Chakan Oil Mills, Sangli, Maharashtra and supplied with fresh water. Light cycle of 12 h light/dark was strictly followed in the animal house. The animals were observed for any infection or any metabolic diseases during the study period and respective physician was consulted in case of any critical conditions.

ii) Test substances

Leaves of *Mimusops elengi* L. were collected from Belapur village of Maharashtra, India and were authenticated by Dr. Ganesh Iyer, Department of Botany, Ruia College, Matunga, Mumbai. For further studies the leaves were dried using tray dryer. The following formulations were procured from local pharmacy shops - Indomethacin (INDOCAP 25 mg, Jagson Pal P'ceutical Ltd., New Delhi): at dose of 10 mg/kg was used as a positive control agent in carrageenan induced paw edema method. Aspirin (German Remedies Ltd. Mumbai) at the dose of 100mg/kg was used as a positive control in *In-vitro* Anti-inflammatory activity. Diclofenac (REACTIN-50, 50 mg, Cipla Ltd., Haridwar, Uttarakhand) at dose of 20 mg/kg was used as a positive control in cotton pellet granuloma model.

iii) Preparation of Mimusops elengi L leaves extracts

Aqueous Extract: Dried Leaves of *Mimusops elengi* L. were refluxed with distilled water. The aqueous extract was then evaporated until dry, which was used for further studies. Methanolic

extract: Dried *Mimusops elengi* L. leaves were refluxed in Soxhlet apparatus with methanol. The methanolic extract was concentrated, which was used for further studies.

iv) Acute oral toxicity studies

Acute oral toxicity studies for test extracts of Leaves of *Mimusops elengi* L. were carried out as per the OECD (Organisation for Economic Co-operation and Development) guideline no. 423. This guideline refers to those adverse effects occurring within a short time following oral administration of a single dose of a substance or multiple doses given within 24 hr. Various parameter includes loss of reflex, pinna reflex, righting reflex, corneal reflex, changes in body weight, clinical abnormality and mortality was observed[17].

IN VITRO ANTI-INFLAMMATORY METHODS

i) Membrane Stabilization Assay

Preparation of erythrocyte suspension:

Fresh whole human blood (10 ml) was collected and transferred to the heparinized centrifuged tubes. The blood was washed three times with 0.9% saline. The volume of saline was measured and reconstituted as a 40%(v/v) suspension with isotonic buffer solution (pH 7.4) which contained in 1 Liter of distilled water: NaH2PO4 • 2H2O, 0.26 g; Na2HPO4 , 1.15 g; NaCl, 9 g (10 mM) sodium phosphate Buffer).

Heat-induced hemolysis

Portions 5 ml. of the isotonic buffer containing 50, 150, 200 and 400 µg/ml of extracts solution of *M. elengi* were put into two duplicate sets of centrifuge tubes. The vehicle, in the same amount, was added to another tube as control. Erythrocyte suspension (30µl) was added to each tube and mixed gently by inversion. One pair of the tubes was incubated at 54˚C for 20 min in water bath. The other pair was maintained at 0-5˚C in an ice bath. The reaction mixture was centrifuged for 3 min at 1300 g and the absorbance (O.D) of the supernatant was measured at 540 nm using spectrophotometer. Aspirin 100 µg/ml was used as a reference standard[18]. The percentage inhibition or acceleration of haemolysis in tests was calculated according to the equation:

Inhibition of haemolysis= 100× [1-OD2-OD1/OD3-OD1]

Where, OD1-test sample unheated; OD2-test sample heated; OD3-control sample heated.

ii) Proteinase Inhibitory Activity

The test was performed according to the previously described method [19]. The reaction mixture (2 ml) contained 0.06 mg trypsin, 1 ml 25 mM tris-HCL buffer (pH 7.4) and 1 ml test sample of different concentrations. The mixtures were incubated at 37°C for 5 min and then 1 ml of 0.8% (w/v) casein was added. The mixture was incubated for an additional 20 min. 2 ml of 70% perchloric acid was added to terminate the reaction. Cloudy suspension was centrifuged and the absorbance of the supernatant was read at 280 nm against buffer as blank. The experiment was performed in triplicate.

Percentage Inhibition = (Abs sample –Abs control) x 100/ Abs control IC50 values were calculated as the average of triplicate analyses.

iii) Sulfarhodamine B (SRB) assay for Cell Viability on peritoneal macrophages

Isolation and culture of peritoneal macrophages

Peritoneal macrophages were isolated from mice which were injected intraperitoneally (i.p.) with 2 ml of 4% (w/v) fluid thioglycollate medium 3 days prior to peritoneal lavage with 10 ml of RPMI 1640 medium. The collected cells were washed with RPMI 1640 and cultured in RPMI 1640 supplemented with 10% FBS, 2mM L-glutamine, 100 U/ml penicillin, and 100mg/ml streptomycin (complete RPMI). The macrophage count was determined by using haemocytometer and cell viability was tested by trypan-blue dye exclusion technique. Then the cells were adjusted to required cell count ($1×10^5$ cells/ml) and plated on a 96-well flat-bottom culture plate (Tarsons products pvt. Ltd., india) and then incubated for 12 hrs at 37˚C in a 5% CO_2 humidified incubator. After removing the nonadherent cells, the mono-layered macrophages were treated with *M.elengi* extracts (10-100µg/ml) dissolved in complete RPMI medium containing 0.1% DMSO and maintained for 24 hrs at 37ºC in a 5% CO_2 humidified incubator.

Sulforhodamine B (SRB) assay

Peritoneal exudates cells (PEC) were isolated as above and were cultured (1×10^5cells/well) in complete RPMI and incubated for 2 hr at 37ºC in 5% CO_2 atmosphere. The SRB assay was performed as per the procedure described earlier by[20]. In Brief, the extract of *M.elengi* was dissolved in complete RPMI and added at various concentrations (100-6.25μg/ml) in triplicate. After 24 h incubation, cells were fixed by adding ice-cold 20% trichloroacetic acid (TCA) and incubating for 1 hr at 4ºC. The plates were washed two times with distilled water, air-dried and stained with 100 μl of SRB solution for 30 min at room temperature in dark. Unbound SRB was removed by washing thoroughly with 1% acetic acid and the plates were air-dried. The bound SRB stain was solubilised with 200μl of 10mM tris buffer (pH-10.4), and the optical density was read at 540 nm.

IN VIVO ANTI-INFLAMMATORY METHODS

i) Carrageenan Induced Rat Paw Edema

Anti-inflammatory activity was assessed by the method described by Winter et al[21]. The rats were divided into different groups of six animals each. First group (control) received equivalent volume of vehicle (0.5 % Na.CMC), second group (positive control) received 10 mg/kg p.o. Indomethacin. Group III & IV were treated with Methanolic extract of *M. elengi* Leaves 200 and 400 mg/kg (p.o) respectively.Group V & VI were treated with Aqueous extract of *M. elengi* Leaves 200 and 400mg/kg (p.o) respectively. After 1hr, the rats were challenged with subcutaneous injection of 0.1 ml of 1 % w/v solution of carrageenan (Sigma Chemical CO., USA) into the plantar side of the left hind paw. The paw was marked with ink at the level of lateral malleolus and immersed in mercury up to the mark. The paw volume was measured plethysmographically before and after injection (0 hr) and then every hour till 5 hrs after injection of carrageenan to each group.Inflammation was assessed as the difference between the zero time volume of the treated paw (Vo) and the volume at various times (Vt) after injection of the carrageenan, percent inhibition of edema was calculated using the relation:

Inhibition of edema (%) = 100× (1-a-x/b-y)

Where, a = mean paw volume of treated rats at various time after carrageenan injection

x = mean paw volume of treated rats before carrageenan injection

b = mean paw volume of control rats at various time after carrageenan injection;

y = mean paw volume of control rats before carrageenan injection.

ii) Cotton Pellet granuloma test in rats

The effect of extract on chronic inflammation was evaluated using cotton-pellet granuloma in rats[23]. Animals were divided in to six groups. Group I (Control) animals received equivalent volume of vehicle (0.5 % Na.CMC) and group II (positive control) received Diclofenc (20 mg/kg). Thirty minutes later, two autoclaved cotton pellets 30±1.0 mg were aseptically implanted under the previously depilated back of anaesthetized rats. The Methanolic extract of *M. elengi* Leaves Extract was administered in to group III & IV with 200 and 400 mg/kg (p.o) respectively. Group V & VI were treated with Aqueous extract of *M. elengi* Leaves 200 and 400 mg/kg(p.o) respectively once daily for the next 7 days. On day 8, animals were killed. The pellets were dissected out, freed of tissue attachments and dried in the oven overnight at 60˚C. The dry pellets were weighed and the mean weight of the granuloma tissue formed around each pellet determined. The level of inhibition of granuloma tissue development was calculated using the relation:

Inhibition= 100× (Tc-Tt/Tc)

Where, Tc = weight of granuloma tissue of control group;

Tt = weight of granuloma tissue of treated group.

STATISTICAL ANALYSIS

The observations were expressed as mean ± S.D the difference in response to test drugs and control was determined by one-way analysis of variance (ANOVA) followed by Dunnett's t-test. $P<0.05$ was considered as significant. The software used is Graph Pad Prism version 5.03 for the statistical analysis.

RESULTS

INVITRO ANTI-INFLAMMATORY ACTIVITY

Membrane Stabilization Assay

The Methanolic and Aqueous extracts of *M. elengi* leaves showed dose dependent inhibition on the haemolysis of erythrocytes induced by heat. The IC_{50} value for methanolic extract was found to be 364.56±1.21 µg/ml which is lower than the aqueous extract which is 379.02±1.05 µg/ml.

Table 1
Effect of M. elengi extracts on Membrane Stabilization Assay

Conc. µg/ml	% Inhibition	
	Methanolic	Aqueous
50	12.32±1.27	10.43±1.24
150	22.45±1.06	27.51±2.06
200	37.51±1.34	33.67±1.34
400	52.17±0.07	50.19±1.05
IC50	364.56±1.21 µg/ml	379.02±1.05 µg/ml

Values represents in the results are mean ±SD of three replicates; linear regression analysis was used to calculate IC50 value).

Proteinase Inhibitory Activity

Results of proteinase inhibitory activity reveals that both the extracts posses significant anti-proteinase activity. The concentration of methanolic and aqueous extracts of*M. elengi* leaves needed for 50% inhibition was found to be 416.03 µg/ml and 492.92 µg/ml respectively. The results suggest that methanolic extract is a more potent proteinase inhibitor than aqueous extract.

Table 2
Effect of M. elengi extracts on Proteinase Inhibitory activity

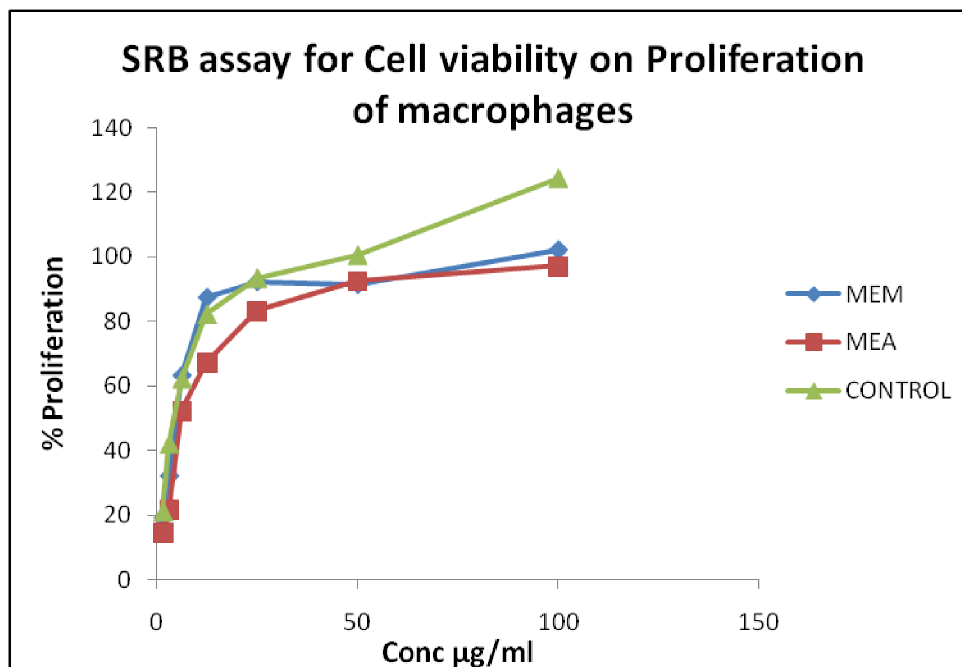
Conc.(µg/ml)	% inhibition	
	Methanolic	Aqueous
50	10±3.61	7±5.04
100	19±1.69	12±3.08
200	27±6.69	16±5.77
400	44±5.29	38±1.29
500	62±3.21	54±0.92
IC 50	416.03 µg/ml	492.92 µg/ml

Values represents in the results are mean ±SD of three replicates; linear regression analysis was used to calculate IC_{50} value).

Sulfarhodamine B (SRB) assay for Cell Viability on peritoneal macrophages

Effect of methanolic and Aqueous extract of *M. elengi* Leaves on cell viability. Macrophages were incubated with *Mimusops elengi* extracts and cell viability was determined by SRB Assay. Both the extracts showed dose dependent proliferation of macrophages at 100µg/ml and Standard control Phytohaemaggulutinin which has proliferative action on macrophages.

Graph 1
Proliferation of M. elengi extracts and PHA on macrophages



*(Each point is the mean ±S.D in duplicate, *p<0.05, as compared to control using one-way ANOVA followed by Dunnett's test).*

ACUTE ORAL TOXICITY STUDY

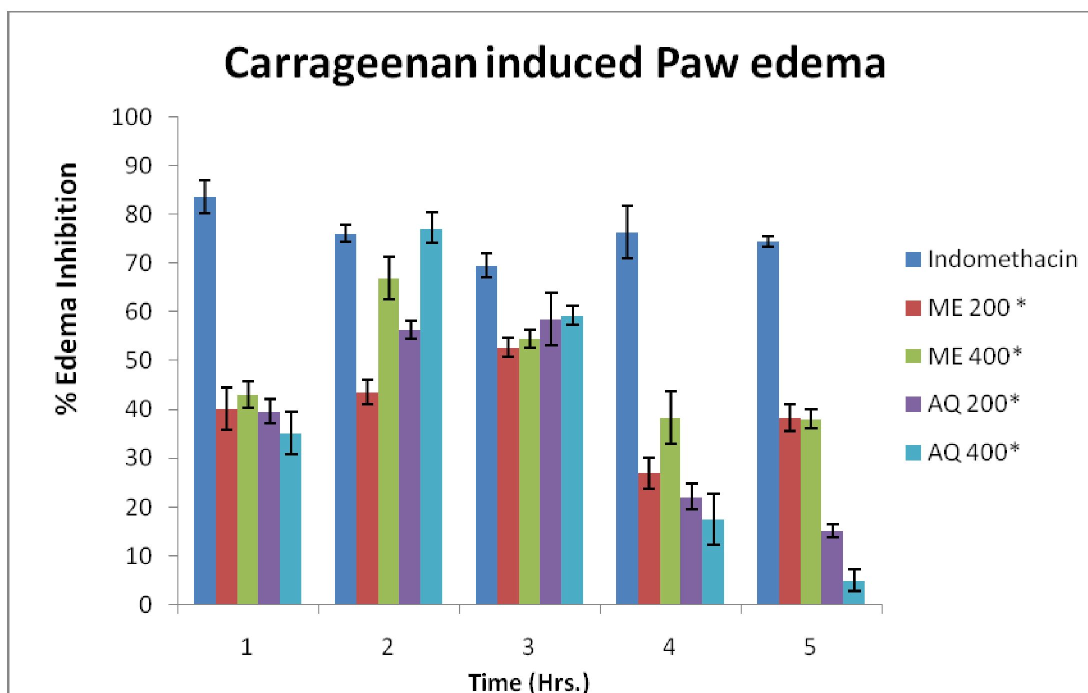
LD_{50} for methanolic and Aqueous extracts of *M. elengi* Leaves was calculated as greater than 2000mg/kg. Acute toxicity studies revealed that ME was safe at a maximum oral dose of 2000 mg/kg b.w. in mice. No lethality or toxic reactions were observed in 14 days period. However, the animals showed slight sedation after 1 h of MEEE administration. Based on this data, 1/10th and 1/5th dose i.e. 100 and 200 mg/kg was selected for the further study.
Dose I (Low Dose) = 2000 mg x 1/10 = 200 mg/kg
Dose I (High Dose) = 2000 mg x 1/5 = 400 mg/kg

Table 3
Acute Oral Toxicity study of extracts of M.elengi Leaves according to OECD Guideline 423.

Parameters	Methanolic	Aqueous
Loss of reflex	-	-
Righting reflex	-	-
Pinna reflex	-	-
Corneal reflex	-	-
Changes in body weight	-	-
Clinical abnormality	-	-
Mortality	-	-

IN-VIVO ANTI-INFLAMMATORY ACTIVITY

Graph 2
Comparative Effect of M. elengi extracts and Indomethacin on Carrageenan induced Paw edema



(Value are expressed as Mean ±S.D; *p<0.05 as compared to control using one-way ANOVA followed by Dunnett's test; N=6 in each group).

Pretreatment with aqueous and methanolic extracts of *M. elengi* showed dose dependent decrease in paw edema on carrageenan induced rat paw edema. The percentage edema inhibition was found to be higher in methanolic extract where as aqueous extract showed less percentage edema inhibition as compare to methanolic.

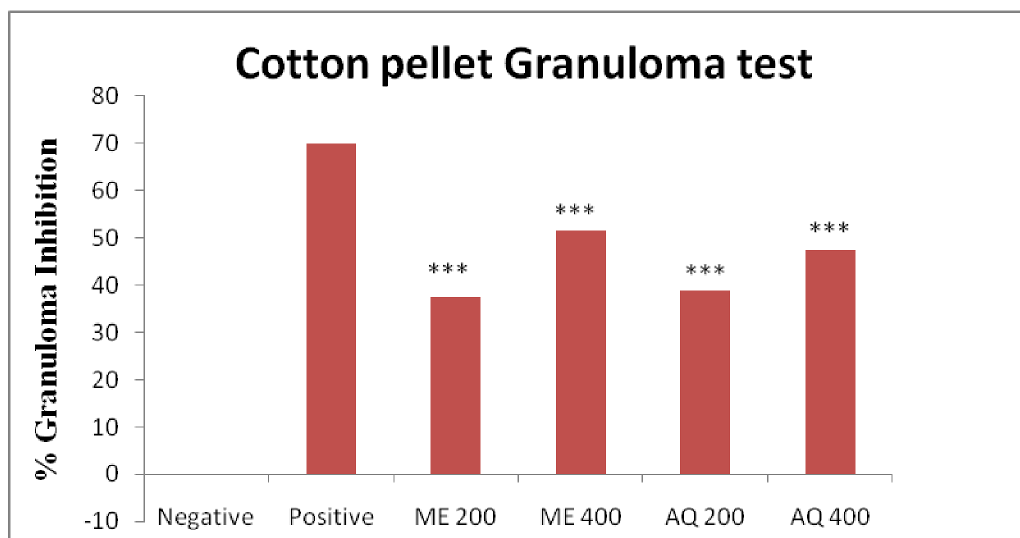
COTTON PELLET INDUCED GRANULOMA TEST IN RATS

The Methanolic and Aqueous extracts of *M. elengi* leaves decreased dry weight of the cotton pellets compared to control groups. The methanolic extract showed highest 51.44 % Granuloma Inhibition at the dose of 400 mg/kg whereas aqueous extract at dose of 400 mg/kg inhibited the granuloma formation by 47.42 %. Both the extracts caused a significant ($p<0.001$) dose related inhibition of granuloma formation.

Table 4
% inhibition of aqueous and MeOH extract of M. elengi Leaves on cotton pellet induced granuloma in Rats

Drug	Dose (mg/kg)	Granuloma tissue wt (gms)	% Granuloma Inhibition
Aqueous	200	0.121±0.02	38.75±0.04
Aqueous	400	0.104±0.005	47.42±0.01
Methanolic	200	0.124±0.01	37.5±0.02
Methanolic	400	0.096±0.09	51.44±0.05
Negative control	-	0.199±0.02	-
Positive control (Diclofenac)	20	0.059±0.005	70.06±0.12

Graph 4
Comparative effect of M. elengi extracts and Diclofenac on cotton pellet granuloma test in rats



Effect of Methanolic and Aqueous extracts of M. elengi Leaves on Cotton pellet induced granuloma test in rats. Pretreatment with methanolic and aqueous extracts showed dose dependent decrease in Granuloma formation.
(Value are expressed as Mean ±S.D;p<0.001 as compared to control using one-way ANOVA followed by Dunnett's test; N=6 in each group)***

DISCUSSION

The erythrocyte membrane is analogous to the lysosomal membrane and its stabilization implies that the extract may as well stabilize lysosomal membranes. Stabilization of lysosomal membrane is important in limiting the inflammatory response by preventing the release of lysosomal constituents of activated neutrophils such as bactericidal enzymes and proteases, which cause further tissue inflammation and damage upon extracellular release. Some of the NSAIDs are known to possess membrane stabilization[18]. *M. elengi* was found to inhibit the haemolysis of erythrocytes induced by heat at a dose dependent manner, as concentration increased above 400 µg/ml extracts causes reduction in inhibition. From the results of the present study both the extracts were found to possess inhibition at concentration up to 400 µg/ml. Proteinases have been implicated in arthritic reactions. Neutrophils are known to be a rich source of proteinases which carry in their lysosomal granules many neutral serine proteinases. It was previously reported that leucocyte proteinases play an important role in the development of tissue damage during inflammatory reactions and significant level of protection was provided by proteinase inhibitors[19]. Both the extracts exhibited significant dose dependent anti-proteinase activity up to a concentration of 500 µg/ml. Methanolic and Aqueous *M. elengi* extracts showed proliferation of macrophages at concentration of 100µg/ml when compared to Phytohaemoagglutinin which induces proliferation of Macrophages. Extracts of *M. elengi* were found to be safe up to the dose of 2000 mg/kg since no mortality and abnormal behavioural pattern was observed.

Carrageenan-induced inflammation is useful in detecting orally active anti-inflammatory agents. Edema formation due to carrageenan in the rat paw is a biphasic event[23]. The initial phase is attributed to the release of histamine and serotonin. The edema produced at the peak (3 h) is thought to be due to the release of kinin-like substances, especially bradykinin. The second phase of edema is due to the release of prostaglandins, protease and lysosome. The second phase is sensitive to most clinically effective anti-inflammatory drugs. The anti edematous effect was significant during the first phase of inflammation, indicating the inhibition of

histamine release and during second phase due to the inhibition of cyclooxygenase enzymes that are involved in the formation of prostaglandins. Pretreatment with both Methanolic and Aqueous extracts of *M. elengi* showed significant ($P<0.05$) dose dependent decrease in rat paw edema from 2nd to 3rd hour. The cotton pellet granuloma method has been widely employed to assess the transudative, exudative and proliferative components of chronic inflammation. The fluid absorbed by the pellet greatly influences the wet weight of the granuloma and the dry weight correlates well with the amount of granulomatous tissue formed[24]. Monocyte infilteration and fibroblast proliferation rather than neutrophil infilteration and exudation take place in chronic inflammation. In this study, the Methanolic and Aqueous extracts of *M. elengi* decreased dry weight of the cotton pellets compared to control groups. The aqueous extract at dose of 400mg/kg inhibited the granuloma formation by 51.44 % which is higher than at dose of 200mg/kg. Thus extract caused a significant ($p<0.001$) dose related inhibition of granuloma formation. This may be due to the ability of *M. elengi* in reducing the number of fibroblasts and synthesis of collagen and mucopolysaccharide, which are natural proliferative agents of granulation tissue formation.

CONCLUSION

The *M. elengi* L. methanolic and aqueous extracts showed significant results in all the in-vitro and in-vitro methods at different dose level. The results of the present study have empirically indicated that *M. elengi* L. Leaves is effective in the treatment of inflammatory diseases and supported the common uses in tradition medicines worldwide.

REFERENCES

1. Ghani A. Medicinal Plants of Bangladesh. The Asiatic Society of Bangladesh, Dhaka 2003; 303.
2. Mitra R. Bakula-A reputed drug of Ayurveda, its uses in Indian medicine, Indian Journal of History of Science, 16(2): 169-180, (1981).
3. Kirtikar KR, Basu BD. Indian medicinal plants with illustrations. Uttaranchal, India: Oriental Enterprises: 2001.
4. Jahan N, Ahmed W and Malik A New steroidal glycosides from *Mimusops elengi.* Journal of Natural Product's 8 (8):1244-1247, (1995).
5. Warier PK, Nambiar VPK, Ramankutty, C. (Eds.),. Indian Medicinal Plants, vol. 4, Orient Longman Pvt. Limited, Chennai: (2007).
6. Salgnero CP, A Thai Herbal, Traditional Recipes for Health and Harmony. Findhorn Press, Finland: (2003).
7. Kirtikar KR, Basu BD. Indian Medicinal Plants. M/s. Bishensingh Mahendra Palsingh, Dehradun: 1494, (1935).
8. Ashok P, Koti BC, Vishwanathswamy, AHM, Antiurolithiatic and antioxidant activity of *Mimusops elengi* on ethylene glycol-induced urolithiasis in rats. Indian Journal of Pharmacology 42: 380–383, (2010).
9. Hadaginhal RV, Tikare VP, Patil KS, Bhanushali MD, Desai NS, Karigar A, Evaluation of cognitive enhancing activity of *Mimusops elengi* Linn on albino rats. International Journal of Research in Ayurveda and Pharmacy, 1: 484–492, (2010).
10. Shah PJ, Gandhi MS, Shah MB, Goswami SS, Santani D, Study of *Mimusops elengi* bark in experimental gastric ulcers. Journal of Ethnopharmacology, 89: 305–311, (2003).
11. Dar A, Behbahanian S, Malik A, Jahan N, Hypotensive effect of the methanolic extract of *Mimusops elengi* in normotensive rats. Phytomedicine, 6: 373–378, (1999).
12. Jerline M, Jothi G, Brindha P, Effect of *Mimusops elengi* Linn. Bark extract on alloxan induced hyperglycemia in albino rats. Journal of Cell and Tissue Research, 9: 1985–1988, (2009).

13. Perry L M, Medicinal Plants of East and South East Asia. Cambridge University Press London, 1980, pp. 376-377.
14. Misra G, Nigam S K, Mitra CR, Studies on *Mimusops* species. PlantaMed, 26: 155, (1974).
15. Sahu NP, Koike K, Jia Z, Nikaido T. Triterpenoid saponins from *Mimusops elengi,* Phytochemistry, 44(6): 1145-9, (1997).
16. Rao KS, Munjuluri PR, Kumar BVVR, Keshar NK, Evaluation of *in vitro* Antioxidant Activity and Total Phenolic Content of Methanol Bark Extract of *Mimusops elengi.* 1(2): 62-71, (2011).
17. 18.Litchfield JT & Wilcoxon FA. A simplified method of evaluating dose effect experiments, Journal of Pharmacological and Experimental Therapeutics, 96: 99–113, (1949).
18. 19.Shinde UA, Phadke AS, Nari AM. Membrane stabilization activity-a possible mechanism of action for the anti-inflammatory activity of *Cedrus deodara* wood oil. Fitoterapia, 70: 251-257, (1999).
19. Chatterjee GK, Pal SP. Search for anti-inflammatory agents from Indian Medicinal Plants-A Review. Indian Drug, 21: 413-419, (1984).
20. Vichai V, Kirtikara K. Sulforhodamine B Colorimetric assay for cytotoxicity screening. Nature Protocols, 1: 1112-116, (2006).
21. Winter CA, Risley EA. Carrageenan-induced edema in hind paw of the rat as an assay for anti-inflammatory drugs. Proceedings of the Society of Experimental Biology and Medicine, 111: 544-546, (1962).
22. Dhan P, Kumar P, Kumar N. Antiioxidant and Hypoglycemic Activity Of Some Indian Medicinal Plants. Pharmacologyonline, 3: 513-521, (2009).
23. Vinegar R, Schreiber W, Hugo R. Biphasic development of carrageenan edema in rats. Journal of Pharmacology and Experimental Therapy, 166: 96-103, (1969).
24. Swingle KF, Shideman FE. Phases of the inflammatory response to subcutaneous implantation of a cotton pellet and their modification by certain anti-inflammatory agents. Journal of Pharmacology and Experimental Therapeutics, 13:226–234, (1972).

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