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Research Article

AN EXPERIMENTAL EVALUATION OF ANTI-INFLAMMATORY ACTIVITY OF *MORINGA OLEIFERA* SEEDS

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ABSTRACT

Objective: To evaluate the anti-inflammatory activity of *Moringa oleifera* seeds in experimentally induced acute and sub acute models of inflammation in rats.

Material and methods: Albino wistar rats of either sex weighing 150-200g were used. Ethanolic and aqueous extracts of *Moringa oliefera* seeds (EEMO and AEMO) were prepared with the help of Soxhlet's apparatus. The anti-inflammatory activity was studied using carrageenan induced paw edema method and cotton pellet granuloma method. Histopathological examination of the paw biopsy was done by H&E stain. Statistical analysis was performed using One-way analysis of variance (ANOVA) followed by post hoc dunnett's test. $P < 0.05$ was considered statistically significant.

Results: In Carrageenan induced paw edema method, both EEMO and AEMO treated groups demonstrated dose dependent decrease in paw edema compared to control group. Histopathological examination of the paws showed marked decrease in edema and cell infiltration in extract treated groups compared to control. In Cotton pellet induced granuloma method all the EEMO and AEMO treated groups demonstrated significant dose dependent decrease in granuloma formation when compared to control.

Conclusion: Thus it can be concluded from our study that the both ethanolic and aqueous extracts of *Moringa oleifera* seeds possesses anti-inflammatory activity.

Keywords: Moringa oleifera, Carrageenan induced paw edema, Cotton pellet granuloma, Anti-inflammatory.

INTRODUCTION

Moringa oleifera Lam. commonly known as the drumstick tree or the horseradish tree is one of the most widely cultivated and best known of the thirteen species of the family Moringaceae [1- 2].It is grown throughout the subtropics and tropics of Africa and Asia [3]. *Moringa oleifera* is known as a 'Miracle tree' as almost every part of it is useful for humans. It is often attributed as "natural nutrition for the tropics" for its high nutritional value [4]. The leaves, immature pods and flowers are used as a nutritive vegetable in various Asian countries, particularly in India. The seeds and other parts of Moringa *oleifera* have long been used in traditional medicine for their medicinal values [5]. The whole plant is reported to have antimicrobial activity and various active principles like pterygospermin and 4-α-L -rhamnosyloxy benzyl isothiocyanate have been isolated which have powerful antibacterial

and antifungal activity[6-8]. The leaves are reported to have anti-inflammatory, diuretic, antispasmodic and hypotensive activity [5, 9]. The roots are reported to have antispasmodic, hepatoprotective, anthelmintic and anticonvulsant activity [10-11]. The pods are reported to have hypolipidemic and hypotensive effect [12-13]. The seeds are reported to have antiarthritic, antitumour, and antioxidant activities [14-16]. However there are very few studies reporting the anti-inflammatory activity of *Moringa oleifera* seeds. Hence this study was conducted for the scientific evaluation of anti-inflammatory activity of *Moringa oleifera s*eeds using in vivo acute and sub acute models of inflammation.

MATERIAL AND METHODS

Plant Material and Extraction

The pods of *Moringa oliefera* were collected from University campus and seeds were separated from them and shade dried. The seeds were identified and authenticated by Prof. S. H. Afaq, Department of Pharmacognosy, Ilmul Advia, A.K. Tibbya College, A.M.U. and voucher specimen was submitted (SC-0130/11). Seeds were powdered with the help of a mechanical grinder and 100 gm of seed powder was extracted separately with 300ml of distilled water and ethanol for aqueous and ethanolic extract respectively with the help of Soxhlet's apparatus. The extracts were collected in Petri dishes and evaporated till dryness at 40 ºC in an incubator. Then the extracts were sealed with aluminium foil and stored at 4 ºC for further experimental work.

Drugs and Chemicals used

Aspirin (Reckitt Benckiser, India), Propylene glycol (BDH, Mumbai) and Carageenan(Sigma Chemicals, USA) were used in the study. The other chemicals used were of analytical grades manufactured by Merck Laboratories (Mumbai, India).

Animals

Albino wistar rats of either sex weighing (150-200g) were procured from the Central Animal House, JNMC, Aligarh Muslim University. They were housed in polypropylene cages at ambient temperature (25± 2◦ C), relative humidity (55 ± 5%) and 12-hr light-dark cycle. Animals had free access to standard pellet diet and water ad *libitum*. The study protocol was approved by the Institutional Animal Ethics Committee (IAEC) and all animal experiments were carried out as per the rules and regulations of IAEC & CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals).

Experimental design

Animals were divided into eight groups of six animals each. Group-1 served as control and was given propylene glycol 2ml/kg, group-2 served as standard and was given asprin(100mg/kg), groups 3, 4 and 5 were given ethanolic extract of *Moringa oleifera*(EEMO) seeds at the dose of 50, 100 and 200mg/kg respectively and groups 6, 7 and 8 were given aqueous extract of *Moringa oleifera*(AEMO) seeds at the dose of 250, 500 and 750mg/kg respectively.

Carrageenan induced paw oedema method [17

This is one of the most commonly employed method for the screening of acute inflammation. All the groups were treated with single dose of respective drug and after 1 hour of the administration of the drugs, acute inflammation was produced by sub plantar injection of 0.1 ml of freshly prepared 1% suspension of carrageenan in normal saline in the right hind paw of the rats The paw was marked at the level of the lateral malleolus and was immersed every time up to this mark. The paw volumes were measured at 0h, 1h, 2h,

and 3h after the carrageenan injection using digital plethysmometer (Orchid scientific, India).

The percentage inhibition of paw edema at each time interval was calculated by using the following formula:-

$$\text{Percentage inhibition} = \frac{(Vt - Vo)control - (Vt - Vo)\text{ treated}}{(Vt - Vo)\,control} \times 100$$

Where

Vo = Paw volume of test/control group at 0 hr

Vt= Paw volume of test/control group at that particular time interval

For histopathological examination, biopsies of paws were taken, under deep ether anaesthesia 3 h after the induction of inflammation with carrageenan and then stored in 10% formalin prior to processing. The sections were stained with hematoxylin and eosin (H & E) for histopathological observations.

Cotton pellet induced granuloma method [18]

This method is commonly used for the screening of sub acute inflammation. The rats were anesthetized with light ether anaesthesia. Under aseptic conditions two sterilized cotton pellet (10mg) were implanted subcutaneously on either side of lumbar region in each rat. The incisions were sutured by silk 2.0 sutures and the wounds were sealed with betadine solution to prevent contamination. Bleeding was minimal and the animals recovered within 5 - 10 min from the effect of anaesthesia. All the groups were treated with drugs daily for 7 days including the day of implantation of pellets. On the eighth day the animals were anaesthetized with ether, the cotton pellets were removed and dried at 60 ^{0}C for 24 h. The dry weight of the granuloma was calculated by noting the difference in the dry weight of the cotton pellets recorded before and after implantation. The incisions were sutured by silk 2.0 sutures and sealed with betadine solution and animals were rehabilitated.

Percent inhibition was calculated by using the following formula.

$$\text{Percent inhibition} = \frac{WC - WT}{WC} \times 100$$

Where, WC = Weight of the cotton pellets in control animal.

WT = Weight of the cotton pellets in drug treated animals.

Statistical analysis

All the values are expressed as Mean ± SEM (n=6). Statistical significance was calculated by one way ANOVA followed by post hoc Dunnett's multiple comparison test. $p < 0.05$ was considered to be statistically significant.

RESULTS

In Carrageenan induced paw edema method, aspirin (100 mg/kg) significantly decreased the paw edema at 1h ($p<0.05$) and 3h ($p<0.001$) after carrageenan injection compared to control and percentage inhibition of edema was 55.55% and 81.66% at 1h and 3h respectively. Both EEMO and AEMO treated groups demonstrated dose dependent decrease in paw edema compared to control group. EEMO at the 200mg/kg showed significant reduction of paw edema at 1h ($p<0.05$) and 3h ($p<0.001$) after carrageenan injection compared to control and percentage inhibition of edema was 52.77% and 70.65% at 1h and 3h respectively whereas EEMO at 50 mg/kg ($p<0.05$) and 100 mg/kg ($p<0.05$) doses showed significant reduction of paw edema only at 3h after carrageenan injection and percentage inhibition of edema was 30% and 51.62% for 50 and 100 mg/kg respectively. AEMO at 250 mg/kg ($p<0.05$), 500 mg/kg ($p<0.05$) and 750 mg/kg ($p<0.05$) doses also showed significant reduction of paw edema only at 3h after carrageenan injection and percentage inhibition of edema was 21.60%, 33.33% and 40% respectively **(Table 1 and 2).**

Histopathological examination

Histopathological examination of the paws in control group revealed extensive edema which appears as separation of layers of epidermis, dermis and collagen fibres with extensive infiltration of neutrophils in control group (Figure1. A). Group treated with Aspirin 100mg/kg showed normal epidermal morphology, mild dermal edema with few inflammatory cells (Figure 1. B). Whereas groups treated with EEMO and AEMO showed dose dependent decrease in edema and cellular infiltration compared to control group (Figure 1. C & D).

Table 1: Effect of Ethanolic extract of *Moringa oleifera* seeds on Carrageenan induced paw edema

Groups	Paw volume at different time interval (in ml)				% Inhibition of edema at 3h
	0h	1 h	2 h	3 h	
Control	0.86±0.03	1.22±0.02	1.31±0.03	1.46±0.03	---
Aspirin (100mg/kg)	0.87±0.04	1.03±0.04*	1.05±0.04*	0.98±0.04**	81.66
EEMO (50mg/kg)	0.78±0.05	1.08±0.05	1.14±0.05	1.20±0.05*	30
EEMO (100mg/kg)	0.88±0.03	1.13±0.03	1.14±0.04	1.17±0.04*	51.62
EEMO (200mg/kg)	0.83±0.05	1.00±0.06*	1.05±0.05*	1.00±0.05**	70.65

EEMO: Ethanolic extract of *Moringa oleifera* seeds, Values are given in mean ± SEM (n=6), *indicates $p<0.05$, ** indicates $p<0.001$ when compared to the control group.

Table 2: Effect of Aqueous extract of *Moringa oleifera* seeds on Carrageenan induced paw edema

Groups	Paw volume at different time interval (in ml)				% Inhibition of edema at 3h
	0h	1 h	2 h	3 h	
CONTROL	0.86±0.03	1.22±0.02	1.31±0.03	1.46±0.03	----
ASPIRIN (100mg/kg)	0.87±0.04	1.03±0.04*	1.05±0.04*	0.98±0.04**	81.66
AEMO (250mg/kg)	0.77±0.03	1.09±0.03	1.16±0.03	1.24±0.03	21.60
AEMO (500mg/kg)	0.82±0.06	1.11±0.06	1.18±0.06	1.22±0.05*	33.33
AEMO (750mg/kg)	0.82±0.06	1.09±0.06	1.11±0.06*	1.18±0.06*	40

AEMO: Aqueous extract of *Moringa oleifera* seeds, Values are given in mean ± SEM (n=6), *indicates $p<0.05$, ** indicates $p<0.001$ when compared to the control group.

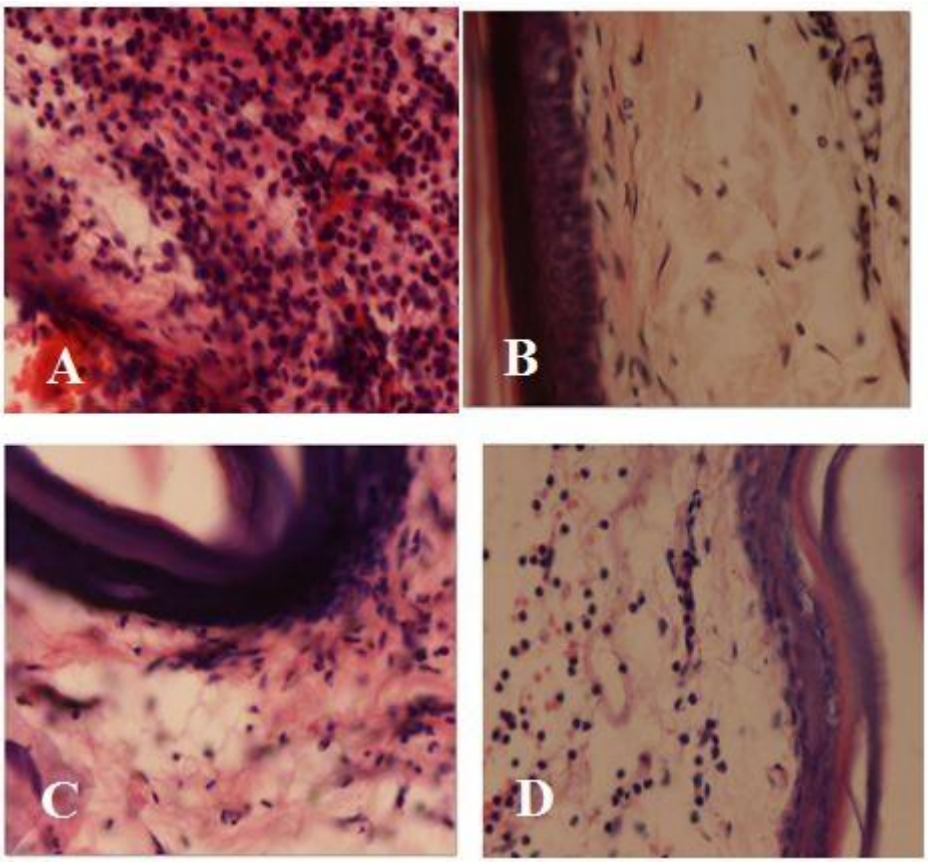

Fig. 1: Histological examination of paw sections after 3h of carrageenan injection. (A) Control group (B) Asprin Group, (C) Ethanolic extract 200 mg/kg and (D) Aqueous extract 750 mg/kg

In Cotton pellet induced granuloma method, Aspirin 100 mg/kg significantly inhibited the granuloma formation by 60.24% ($p<0.001$) when compared to control. Whereas EEMO significantly inhibited the granuloma formation by 18.22% ($p=0.05$), 35.86% ($p<0.001$), and 50.16% ($p<0.001$) compared to control group, at the doses of 50, 100 and 200mg, respectively. AEMO in doses 250, 500 and 750 mg/kg significantly ($p<0.001$) decreased granuloma formation by 21.37%, 32.03% and 62.33% respectively when compared to control (Figure 2 and 3).

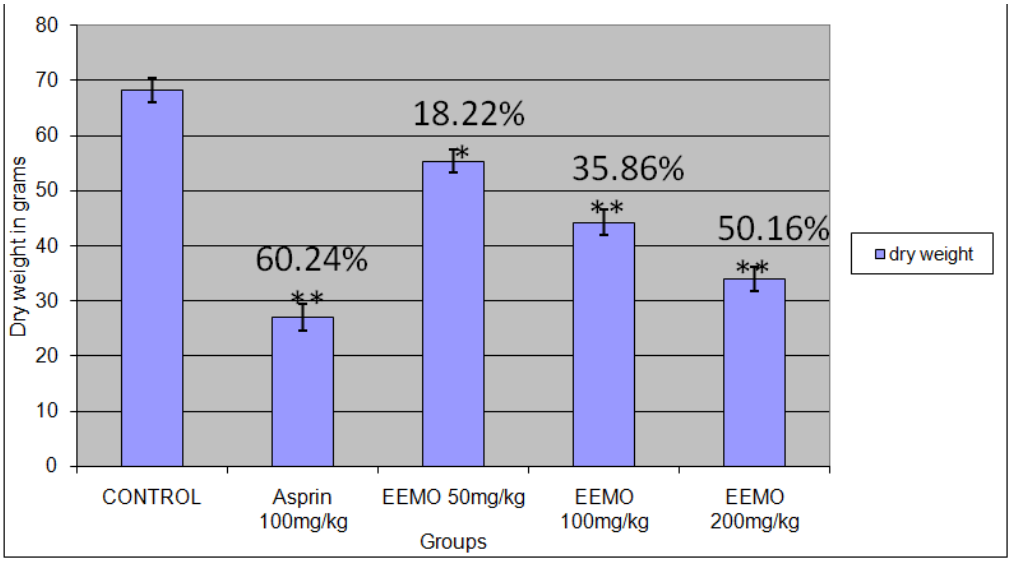

Fig. 2: Effect of Ethanolic extracts of *Moringa oleifera* seeds on granulation tissue formation in Cotton pellet granuloma method.

EEMO: Ethanolic extract of *Moringa oleifera* seeds, Values are given in mean ± SEM (n=6).

*indicates $p<0.05$, ** indicates $p<0.001$ when compared to the control group, using Values given on top of each bar represents percentage inhibition of granuloma formation compared to control group.

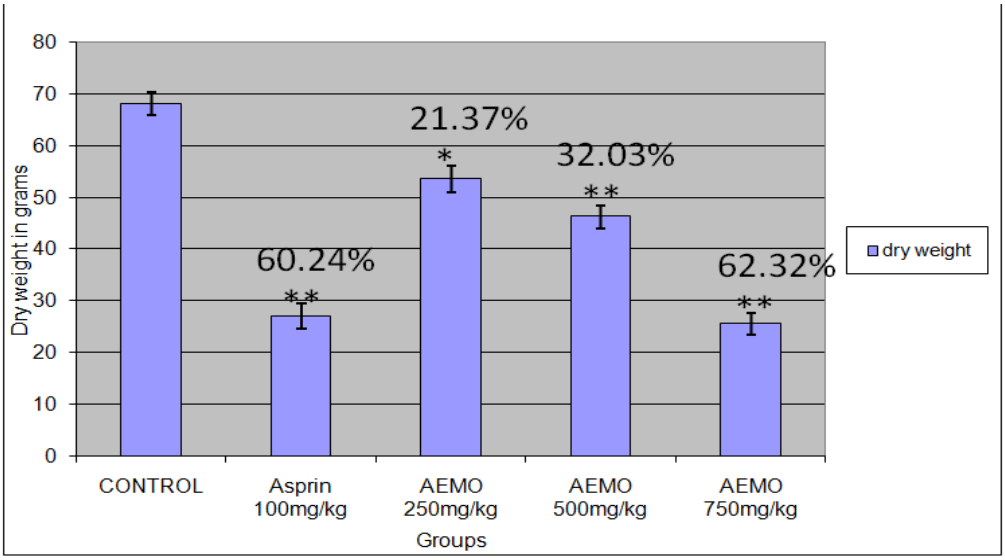

Fig. 3: Effect of Aqueous extracts of *Moringa oleifera* seeds on granulation tissue formation in Cotton pellet granuloma method.

AEMO: Aqueous extract of *Moringa oleifera* seeds, Values are given in mean ± SEM (n=6) *indicates p<0.05, ** indicates p <0.001 when compared to the control group.

Values given on top of each bar represents percentage inhibition of granuloma formation compared to control group.

DISCUSSION

The most widely used primary test for screening of anti-inflammatory agents is carrageenan induced edema in the rat hind paw. The development of edema in the paw of the rats after injection of carrageenan is a biphasic event [19]. The initial phase of inflammation which is observed during the first hour is attributed to a release of histamine and serotonin and the second phase is due to a release of prostaglandin like substances. In the present study, ethanolic extract *Moringa oleifera* seeds showed significant reduction of edema in both the phases of inflammation but maximum reduction was observed in the second phase of inflammation (70.65%) which was comparable with aspirin (81.66%). Aqueous extract showed significant (p<0.01) reduction of edema mainly in the second phase of inflammation. The effect of *Moringa oleifera* seeds lasted for 3 hours parallel to that of aspirin. Histopathological examination of the paws of EEMO and AEMO treated groups further confirmed above results as evidenced by decrease in edema and cellular infiltration compared to control group (Fig. C& D).

The inhibitory effect of *Moringa oleifera* seeds on first phase inflammation could be due to inhibition of the serotonin and histamine mediated effect and on second phase could be due to the inhibition of the prostaglandin synthesis as suggested by the mechanism of edema formation by carrageenan [20]. This anti-inflammatory activity can be attributed to various phytochemicals like alkaloids, flavonoids, sterols, glycosides, tannins and terpenoids reported in *Moringa oleifera* seeds.[14,21] Sterols like β-sitosterol and flavonoids present in *Moringa oleifera* seeds are known to target prostaglandin synthesis which are involved in acute inflammation **[21-22].** *Moringa oleifera* seeds are also rich in glycosides like benzyl isothiocyanate which have been reported to possess anti-inflammatory activity by decreasing various mediators of inflammation like prostaglandins, NO, IL-6, IL-1β and TNF-α [23]. These inflammatory mediators are responsible for edema formation by increasing vascular permeability and recruitment of various inflammatory cells. The cotton pellet granuloma method has been widely employed to assess the transudative, exudative and proliferative components of sub acute inflammation. The increase in dry weight of the granuloma measures the proliferative phase due to monocyte infilteration and fibroblast proliferation that take place in chronic inflammation [24]. In this study, the ethanolic and aqueous extracts of *Moringa oleifera* seeds significantly decreased the dry weight of the granuloma when compared to the control group. The percentage inhibition for the ethanolic and aqueous extracts was highest at the doses of 200mg/kg and 750mg/kg i.e. 50.16% and 62.32% respectively (p<0.001) **(Figure 2 and 3)** which was comparable to that of aspirin (60.24%) and the effect of AEMO 750mg/kg was better than standard drug asprin.

This anti-inflammatory action may be due to the ability of *Moringa oleifera* seeds in reducing the number of fibroblasts and synthesis of collagen and mucopolysaccharide, which are natural proliferative agents of granulation tissue formation. The inhibition of production of proinflammatory cytokines, such as IL-1, IL-6 and TNF-α which are powerful chemotactic agents to macrophages and fibroblasts by *Moringa oleifera* seeds may be responsible for antiinflammatory effect [14]. The anti-inflammatory action may also be due to antioxidants like flavonoids, tocopherols and vitamin c present in *Moringa oleifera* seeds which decrease oxidative stress generated during inflammation [22].

CONCLUSION

Thus it can be concluded from our study that the both ethanolic and aqueous extracts of Moringa *oleifera* seeds possesses anti-inflammatory activity. Further more extensive studies are required to elucidate the exact mechanisms and active principle responsible for anti-inflammatory activity of *Moringa oleifera* seeds so that new potent and safe anti-inflammatory agents can be developed from it.

REFERENCES

1. Ramachandran C, Peter KV, Gopalakrishnan PK: Drumstick (*Moringa oleifera*): a multipurpose Indian vegetable. Econ Bot 1980; 34: 276–283.
2. Nadkarni, K. M, and Nadkarni, A. K. *Indian Materia Medica. Vol.1.* Mumbai: Popular Prakashan; 1976. p. 811–816.
3. Morton JF: The horseradish tree, *Moringa pterigosperma* (Moringaceae). A boon to arid lands. Econ Bot 1991; 45: 318–333.
4. Fahey J. W. *Moringa oleifera*: A Review of the Medical Evidence for Its Nutritional, Therapeutic, and Prophylactic Properties. Part 1. Trees for Life Journal 2005; 1:5.
5. Faizi S, Siddiqui BS, Saleem R, Siddiqui S, Aftab K, Gilani AH: Fully acetylated carbamate and hypotensive thiocarbamate glycosides from *Moringa oleifera*. Phytochemistry 1995; 38: 957–963.
6. Caceres A, Cebreva O, Morales O, Miollined P, and Mendia P: Pharmacological properties of M. oleifera 1. Preliminary

screening for antimicrobial activity. J. Ethnopharmacol 1991; 33:213–216.

7. Das BR, PA Kurup, PL Narasimha Rao. Antibiotic principle from *Moringa pterygosperma.* VII. Antibacterial activity and chemical structure of compounds related to pterygospermin. Indian J of Med Res 1957; 45: 191-196.
8. Eilert U, Wolters B, Nadrtedt A: The antibiotic principle of seeds of *Moringa oleifera* and *Moringa stenopetala*. Planta Med 1981; 42: 55–61.
9. Caceres A, Saravia A, Rizzo S, Zabala L, De-Leon E, and Nave F: Pharmacological properties of M. oleifera 2. Screening for antispasmodic, anti-inflammatory and diuretic activity. J Ethnopharmacol 1992; 36:233–237.
10. Ruckmani K, Kavimani S, Anandan R, Jaykar B: Effect of Moringa oleifera Lam on paracetamol-induced hepatoxicity. Indian J Pharm Sci 1998; 60: 33– 35.
11. Ray R, Hazra and Guha D: Central inhibitory effect of *Moringa oleifera* root extract: possible role of neurotransmitters. Indian J of Exp Biol 2003; 41: 1279–1284.
12. Mehta LK, Balaraman R, Amin AH, Bafna PA, Gulati OD: Effect of fruits of *Moringa oleifera* on the lipid profile of normal and hypercholesterolaemic rabbits. J Ethnopharmacol 2003; 86: 191–195.
13. Faizi S, Siddiqui BS, Saleem R, Aftab K, Shaheen F, Gilani AH: Hypotensive constituents from the pods of *Moringa oleifera*. Planta Med 1998; 64: 225–228.
14. Mahajan SG, Mali RG, Mehta AA: Protective Effect of Ethanolic Extract of Seeds of *Moringa oleifera* Lam. against Inflammation Associated with Development of Arthritis in Rats. J Immunotoxicol 2007; 4(1): 39-47.
15. Guenera PA, Vergas C, Milagros UY: Anti-inflammatory and Anti-tumor activities of Seed extracts of Malunggay, *Moringa oleifera* L (Moringaceae). Philli J Sc 1996; 125: 175-84.
16. Hamza Alaaeldin A: Ameliorative effects of *Moringa oleifera* Lam seed extract on liver fibrosis in rats. Food and Chemical Toxicology 2010; 48: 345-355.
17. Winter CA, Risley EA, Nuss GW: Carrageenan-induced edema in hind paw of rat as an assay for antiinflammatory drugs. Pro Soc Biol Med 1962; 11: 544-7.
18. Winter CA, Porter CC: Effect of alterations in side chain upon anti-inflammatory and liver glycogen activities of hydrocortisone esters. J Am Pharm Assoc 1957; 46: 515-20.
19. Vinegar R, Schreiber W, Hugo R. Biphasic development of carrageenin edema in rats. J. Pharmacol. Exp 1969; 166: 96–103.
20. Sanchez MD, Lopez CJ Rios V: High-performance liquid chromatography method to measure ∝- and ϒ-tocopherol in leaves and fresh beans from Moringa oleifera. J. Chromatogr A 2006; 1105: 111–114.
21. Li D, Sinclair AJ. Macronutrient innovations: the role of fats and sterols in human health. Asia Pac J Clin Nutr 2002; 11: 155–62.
22. Raj Narayana K, Sripal Reddy K, Chaluvadi MR, Krishna DR: Bioflavonoids Classification, Pharmacological, Biochemical Effects and Therapeutic Potential. Indian J of Pharmacol 2001; 33: 2-1.
23. Lee YM, Seon MR, Cho HJ, Kin J, Park HJY. Benzyl isothiocyanate exhibits anti-inflammatory effects in murine macrophages and in mouse skin. J Mol Med 2009; 87*:* 1251–126.
24. Swingle K F, Shideman F E: Phases of the inflammatory response to subcutaneous implantation of a cotton pellet and their modification by certain anti-inflammatory agents. The J of Pharmacl and Exp Therap 1972; 13, 226–234.