Anti-inflammatory Effect of Methanolic Extract of *Mangifera Indica* Leaves on Inflammed Rats in Comparison to Indomethacin

Nahar K[1], Chowdhury IH[2], Sultana S[3], Khan SJ[4], Fatema SK[5], Mamun MAA[6]

ABSTRACT

In the context of inflammation and immunity, there are fragmented and observational studies relating the pharmacological effects of Mangifera indica leaves and its main active component-mangiferin. Hence, this study aims to evaluate the anti-inflammatory effects of this leaf extract (MIE) in comparison to Indomethacin on inflammed rats. The methanolic extract of Mangifera indica leaves in different doses, Indomethacin and normal saline were orally administrated in experimental and control groups of rats. Then acute inflammation was induced by administration of 0.1 ml of 1% Carrageenan in sterile saline solution into the sub-plantar surface of the right hind paw of each rat. Anteroposterior diameter of rat paw oedema was measured at 0 hour and at the end of 3 hours of Carrageenan injection. In the group treated with higher dose of methanolic extract of Mangifera indica leaves, acute inflammation in rats was improved significantly. But in the group treated with lower dose acute inflammation was not improved significantly in comparison to Indomethacin administered group. The methanolic extract of Mangifera indica (MEMI) leaves has potential health benefits as it showed dose dependent anti-inflammatory activity. The mangiferin in the MEMI leaves inhibits COX 2 enzyme and exerts the anti-inflammatory effects.

Keywords: *Mangifera indica leaves, indomethacin, anti-inflammatory drug.*

Mugda Med Coll J. 2024; 7(1): 26-33

DOI: https://doi.org/10.3329/mumcj.v7i1.73966

INTRODUCTION

Inflammation is the immune response of body to injury or infection. At the site of injury cells release molecular signals that cause a number of changes in the affected area. These are vasodilation, increased blood flow, increased vascular permeability, exudation of fluids containing protein like antibodies and invasion by several different types of leukocytes including granulocytes, monocytes and lymphocyte. Neutrophils are the first leukocytes to appear at the injured site[1].

The occurrence of inflammatory disorder is seen worldwide with no racial predilection. However, the poor and developing countries are lacking proper management of inflammatory diseases. As a result, the prevalence of inflammatory conditions is considerably high in developing countries like Bangladesh. The anti-inflammatory drugs that are now available includes non-steroidal anti-inflammatory drugs, like indomethacin, corticosteroids, gold, disease modifying anti-rheumatic drugs (DMARD) such as methotrexate, cyclosporine etc. Indomethacin is mostly used worldwide as an anti- inflammatory drug[2].

Carrageenan-induced inflammation has long been used as an in vivo model of local inflammation.

1. Dr. Kamrun Nahar, Lecturer, Department of Pharmacology, Mugda Medical College, Dhaka-1214.
2. Dr. Iftekhar Hossain Chowdhury, Assistant Professor, Department of Pharmacology, Mugda Medical College, Dhaka-1214.
3. Dr. Sadia Sultana, Lecturer, Department of Pharmacology, Mugda Medical College, Dhaka-1214.
4. Dr. Sharmin Jahan Khan, Lecturer, Department of Pharmacology, Mugda Medical College, Dhaka-1214.
5. Dr. Syeda Kaniz Fatema, Lecturer, Department of Pharmacology, Mugda Medical College, Dhaka-1214.
6. Dr. Md. Abdullah Al Mamun, Junior Consultant, Department of Cardiology, Mugda Medical College Hospital, Dhaka-1214.

Address of Correspondence: Dr. Kamrun Nahar, Lecturer, Department of Pharmacology, Mugda Medical College, Dhaka-1214. Email-silvianahar@yahoo. com

Carrageenan can stimulate through TLR4 signaling pathways. It initiates an inflammatory response in these cells that differs from a typical endotoxin effect such as LPS stimulation in terms of the pathways and gene products altered, suggesting that activation of TLR2/6 and TLR4/6, the predominant pathways through which carrageenan induces inflammatory responses[3].

Numerous communities employ herbal medicines derived from medicinal plants to treat and avoid a wide range of illnesses[4]. Plant barks, leaves, flowers, and other parts have been utilized in medicine. The similar chemicals found in plants have just lately been used to generate synthetic pharmaceuticals[5]. Medicinal plants are a significant source of biologically active natural chemicals and are used as an alternate and/or supplementary treatment method due to their extensive pharmacological, therapeutic, and other biological properties[6].

Given the recent increase in microbial infections in humans, scientists have turned their attention to medicinal plants as low-cost and efficient forms of treatment[7]. Due to the development of microbial resistance to numerous antibiotics, the utilization of extracts and bioactive chemicals produced from medicinal plants has expanded[8]. Plant-based medicines are garnering popularity because of their minimal toxic effects and negligible health consequences[9].

Several Mangifera species have been discovered to offer therapeutic benefits as antidiabetic, antiviral, antibacterial, anti-Alzheimer, antioxidant, and anticancer agent[10]. Furthermore, this has a wide range of bioactive compounds, including vitamins A and C, protein, carotenoids, benzoic acid, gallic acid, carbohydrates, fiber, minerals, and phenolic compounds (such as iriflophenones, quercetin, catechin, and gallotannins). Several pharmacological actions are hypothesized to originate from these bioactive molecules[11].

Mangiferin (C19H18O11), a natural glucoxanthone, is one of the major bioactive compounds present in different parts of M.indica, including the leaves, barks, and peels, as well as many other plants[12]. Mangiferin has been shown in numerous studies to have a wide spectrum of biological actions, making it a viable agent for the food and pharmaceutical sectors. It offers several health-promoting properties, such as anti-inflammatory, antiviral, immunoregulatory, and anticancer capabilities[13].

The side effects of the currently available anti-inflammatory drugs pose a major problem during their clinical uses. Therefore, the development of newer and more potent anti-inflammatory drugs with lesser side effects is necessary[14].

METHODS

This experimental study was carried out at the Department of Pharmacology, Dhaka Medical College, Dhaka during the period from July 2015 to June 2016. Sample size was 28 Long Evan Norwegian rats. Rats were divided into 4 groups, 7 rats each for each group of treatments. The experimental rats were of either sex, weighing between 150 to 200gm. They were allowed to feed on standard laboratory diet and to drink water ad libitum. The animals were maintained at room temperature under condition of natural light and dark schedule. The leaves of Mangifera indica were cut into pieces, shade-dried and grounded to coarse powder. The leaves of Mangifera indica was soaked in methanol (900ml) with continuous shaking at 25.C for 3 days and filtered. The organic extract was evaporated under vacuum to obtain a semisolid residue (10g). The methanolic extract of Mangifera indica leaves was given orally by nasogastric tube at doses of 200 mg/kg body weight and at a dose of 500 mg/kg body weight. Indomethacin was given orally at a dose of 10 mg/kg body weight as standard anti-inflammatory drugs. The group of rats given only saline solution was served as control group. After one hour of drug administration, 0.1 ml of 1% Carrageenan in sterile saline solution was injected into the sub-plantar surface of the right hind paw for the production of acute inflammation. Antero-posterior diameters of paw was measured by slide calipers at zero hour and at the end of three hours of carrageenan injection. Progress of the inflammatory exudative lesion - oedema was assessed by measuring the maximum linear cross section of the joint at zero hour and at the end of three hours.

Experimental design: Carrageenan induced rat paw edema in control group (Group I) and experimental group (Group II, III, IV) (Fig.-1)

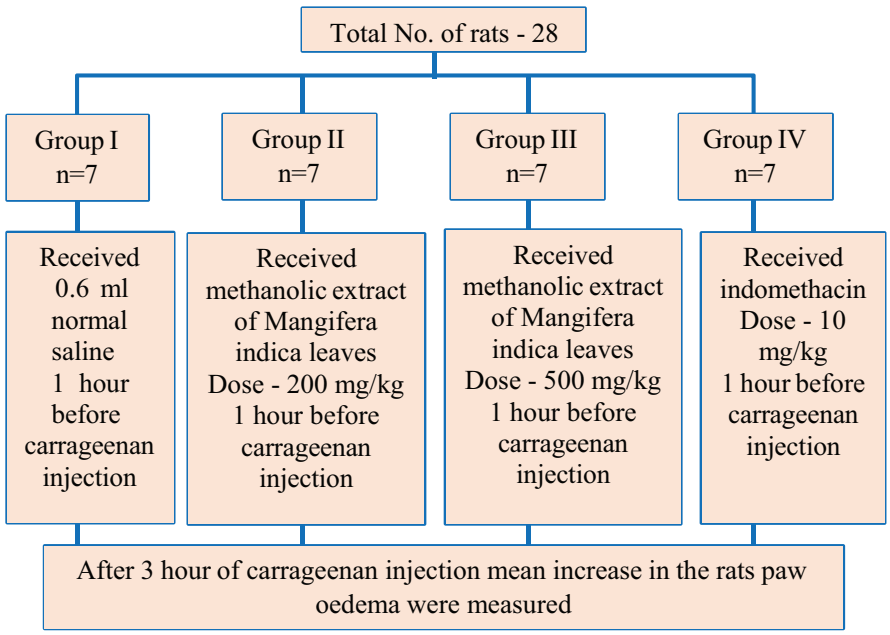

Fig.-1: *Design of experiment for acute inflammation*

RESULTS

The mean initial antero-posterior diameter of rat paw of control group (group I) and experimental group (group II, III, IV) were 3.86+0.03 mm, 3.82±0.04 mm, 3.81±0.02 mm and 3.84±0.02 mm respectively. The mean antero-posterior diameter of rat paw of control group (group I) and experimental group (group II, III, IV) after 3 hours of carrageenan injection were 7.49±0.03 mm, 5.86±0.03 mm, 5.49±0.04 mm and 5.39±0.03 mm respectively. The percentage of inhibition of edema was in case of Indomethacin 57.3%, in case of test extract (200 mg/kg) 43.8%, in case of test extract (500 mg/kg) 53.7%. The extract showed dose dependent anti-inflammatory activity, which was found to be statistically significant at higher concentration.

Table I: *Comparison between Control group and Experimental group (500 mg/kg body wt. of methanolic extract of Mangifera indica leaves) (n=7)*

Group	Group I Control group	Group III Experimental group	P value
Initial antero-posterior diameter (mm)(mean + SEM)	3.86±0.03	3.81±0 .02	0.12
Antero-posterior diameter after 3 hrs of carrageenan (mm) (mean±SEM)	7.49±0.03	5.49±0 .04	0.04

Table II: *Comparison between Control group and Experimental group (Indomethacin 10mg/per kg body weight) (n=7)*

Group	Group I Control group	Group IV Experimental group	P value
Initial antero-posterior diameter (mm)(mean + SEM)	3.86 + 0.03	3.84 + 0.02	0.09
Antero-posterior diameter after 3 hrs of carrageenan (mm)(mean ±SEM)	7.49 + 0.03	5.39 + 0.03	0.02

Table III: *Comparison between 2 Experimental groups (200 mg/kg body wt and 500 mg/kg body wt methanolic extract of Mangifera indica leaves) (n=7)*

Group	Group II Experimental group	Group III Experimental group	P value
Initial antero-posterior diameter (mm)(mean±SEM)	3.82±0.04	3.81±0.02	0.08
Antero-posterior diameter after 3 hrs of carrageenan (mm) (mean±SEM)	5.86±0.03	5.49±0.04	0.05
P value	0.04	0.05	

Table IV: *Comparison between 2 Experimental groups (500mg/kg body wt. of methanolic extract of Mangifera indica leaves and Indomethacin 10mg/per kg body weight) (n=7)*

Group	Group III Experimental group	Group IV Experimental group	P value
Initial antero-posterior diameter (mm)(mean ± SEM)	3.81±0.02	3.84±0.02	0.06
Antero-posterior diameter after 3 hrs of carrageenan (mm) (mean±SEM)	5.49±0.04	5.39±0.03	0.04
P value	0.05	0.04	

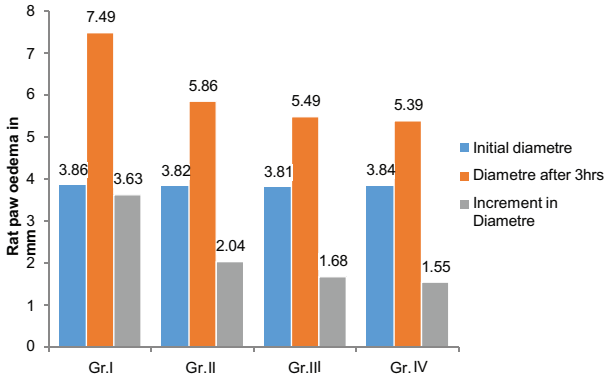

Fig.-1: *Bar diagram showing initial, after 3 hour of carrageenan injection & increment of antero-posterior diameter of rat paw*

DISCUSSION

The present study was carried out to evaluate the anti-inflammatory effect of methanolic extract of Mangifera indica leaves. Its anti-inflammatory effects were tested on Long Evan Norwegian rats. The crude methanolic extract showed presence of chemical constituent with presence of mangiferin. The methanolic extract of mangifera indica leaves is devoid of toxicity up to 5 g/kg in Long Evan Norwegian rats. Carrageenan induced rat paw edema was taken as a prototype of exudative phase of acute inflammation. Inflammatory stimuli like microbes, chemicals and necrosed cells activate the different mediator systems through a common trigger mechanism. The development of carrageenan induced edema is believed to be biphasic. The early phase is attributed to the release of histamine and serotonin and the delayed phase is sustained by the leukotrienes and prostaglandins[15]. In case of carrageenan induced acute rat paw edema technique: the percentage inhibition of edema- in case of standard (Indomethacin; 10 mg/kg) is 57.3%, in case of test extract (200 mg/kg) is 43.8%, in case of test extract (500 mg/kg) is 53.7%. The extract showed dose dependent anti-inflammatory activity, which was found to be statistically significant at higher concentration, 500 mg/kg, in acute carrageenan induced rat paw oedema model. The extract as well as indomethacin showed anti-inflammatory activity. The present study shows that the activity profile of extract at 500 mg/kg closely resembled to that of Indomethacin (standard drug). However, this activity was less potent as compared to indomethacin in low dose extract. This activity appears not to be significant in early phases of acute inflammation. Thus, extract shows anti- inflammatory activity at various acute phases of inflammation[16].

CONCLUSION

Mangifera indica is a unique source of various types of compounds having diverse function. This study has revealed the anti-inflammatory effect of methanolic extract of Mangifera indica leaves. Anti-inflammatory drugs have many adverse effects but methanolic extract of Mangifera indica leaves have less significant side effects. Therefore, it can be used as alternative medicine for its easy availability, cost effectiveness and minimum side effects. However, extensive basic and clinical studies are required in order to identify the exact active ingredient responsible and to determine the precise mechanism of action.

REFERENCES

1. Scott A, Khan KM, Roberts CR, Cook JL, Duronio V. What do we mean by the term "inflammation"? A contemporary basic science update for sports medicine. Br J Sports Med. 2004; 38(3):372-80.
2. Sosa SI, Balick MJ, Arvigo R, Esposito RG, Pizza C, Altinier G, Tubaro A. Screening of the topical anti-inflammatory activity of some Central American plants. J Ethnopharmacol. 2002;81(2):211-5.
3. Myers MJ, Deaver CM, Lewandowski AJ. Molecular mechanism of action responsible for carrageenan-induced inflammatory response. Mol Immunol. 2019;109: 38-42.
4. Sharif MD, Banik GR. Status and utilization of medicinal plants in Rangamati of Bangladesh. Res J Agric Biol Sci. 2006;2(6):268-73.
5. Tower SJ, Hetcher WJ, Myers TE, Kuehl NJ, Taylor MT. Selective modification of tryptophan residues in peptides and proteins using a biomimetic electron transfer process. J Am Chem Soc. 2020; 142(20):9112-8.
6. Zhang A, Sun H, Wang X. Recent advances in natural products from plants for treatment of liver diseases. Eur J Med Chem. 2013;63: 570-7.
7. Liktor-Busa E, Keresztes A, LaVigne J, Streicher JM, Largent-Milnes TM. Analgesic potential of terpenes derived from Cannabis sativa. Pharmacol Rev. 2021;73(4):1269-97.
8. Dzotam JK, Kuete V. Antibacterial and Antibiotic-Modifying Activity of Methanol Extracts from Six Cameroonian Food Plants against Multidrug-Resistant Enteric Bacteria. Biomed Res Int. 2017;2017:1583510.
9. Kumar M, Saurabh V, Tomar M, Hasan M, Changan S, Sasi M, et al. Mango (Mangifera indica L.) leaves: Nutritional composition, phytochemical profile, and health-promoting bioactivities. Antioxidants. 2021;10(2):299.
10. Tegen D, Dessie K, Damtie D. Candidate anti-COVID-19 medicinal plants from Ethiopia: a review of plants traditionally used to treat viral diseases. Evidence-Based Compl Altern Med. 2021;2021:1-20.
11. Coelho EM, de Souza ME, Corrêa LC, Viana AC, de Azevêdo LC, dos Santos Lima M. Bioactive compounds and antioxidant activity of mango peel liqueurs (Mangifera indica L.) produced by different methods of maceration. Antioxidants. 2019;8(4):102.
12. Iseda S. On mangiferin, the coloring matter of mango (Mangifera indica Linn.). V. Identification of sugar component and the structure of mangiferin. Bull Chem Soc Japan. 1957;30(6):629-33.
13. Telang M, Dhulap S, Mandhare A, Hirwani R. Therapeutic and cosmetic applications of mangiferin: A patent review. Exp Opin Ther Patents. 2013;23(12):1561-80.
14. Sosa SI, Balick MJ, Arvigo R, Esposito RG, Pizza C, Altinier G, Tubaro A. Screening of the topical anti-inflammatory activity of some Central American plants. J Ethnopharmacol. 2002;81(2):211-5.
15. Vinegar R, Schreiber W, Hugo R. Biphasic development of carrageenin edema in rats. J Pharmacol Exp Ther. 1969;166(1):96-103.
16. Mujumdar AM, Naik DG, Dandge CN, Puntambekar HM. Anti-inflammatory activity of Curcuma amada Roxb. in albino rats. Indian J Pharmacol. 2000;32(6):375-7.