Dermatological Evaluation of Counter-Irritant and Anti-Inflammatory Effect of Ethanolic Seed Extract of *Mangifera Indica* in Rabbits

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Abstract
Purpose: This study was designed to assess the counter-irritant and anti-inflammatory activity of seed extract of *Mangifera indica* belonging to the cashews family of the plant kingdom.
Method: Fifteen rabbits of either gender with an average weight 1.5 kg ±1.5g for each inducer (phenol, acetic acid, formalin and sandpaper) were used to evaluate the effect of seed extract. Phenol, formalin, acetic acid and sandpaper were used to induce irritation and inflammation while distilled water and betamethasone were used as negative and positive control respectively. Different doses (25, 50, 75 µg/mL) of ethanolic extract were assessed to evaluate the dose-dependent response of *M. indica* seed. Maximum tolerated dose and minimum tolerated dose was calculated at an interval of 2 hours in (25, 50 and 75 µg/mL).
Results: These doses with the same pattern showed anti-inflammatory activity 65.13±2.3% to 78.21±2.4% in phenol, 79.31±2.5% to 94.92±2.4 % in acetic acid, 79.21±2.7 % to 98.02±2.2 % in formalin and 60.21±2.9 % to 97.31±2.2% in sandpaper respectively**.**
Conclusion: Ethanolic extract of *M. Indica* seed shows the ability to lighten the effect of irritation and inflammation.
Keywords: Rabbits' ear, Anti-inflammatory, Counter-irritant, Betamethasone, *M. Indica* seed.

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INTRODUCTION

In the case of topical inflammation, a high level of histamine has been noted in the skin [1]. Mast cells, which are generally found in the connective tissue close to blood arteries, have been shown to produce histamine as one of the key inflammatory mediators [1]. Basophils and platelets in the blood contain histamine. It is held in mast cell granules and released by degranulation in response to a range

of stimuli, including physical damage like trauma, cold or heat through unknown processes, or antibody attachment to mast cells, which causes acute hypersensitive responses. [1].

Histamine promotes arteriole dilation as well as increases venule permeability. Histamine is thought to be the primary mediator of enhanced vascular permeability in the short term, causing inter endothelial gaps in postcapillary venules. [1]. Its vasoactive actions are primarily mediated by binding to H1 receptors on microvascular endothelial cells. Antihistamines, such as brompheniramine, clemastine, and diphenylhydramine, are H1 receptor antagonists that bind to and inhibit the H1 receptor [2].

The presence of diverse elements such as flavonoids, steroids, saponins, tannins, alkaloids, terpenoids, glycosides, and carbohydrates were determined using conventional phytochemical identification. Mangiferin (1,3,6,7-tetrahydroxyxanthone C2-B-D-glucoside) is a polyphenolic compound extracted from various parts like roots, kernels, bark and fruit of *M. indica* belonging to cashews locally called chuansa in Pakistan.[3]. Mangiferin shows various pharmacological activities like anti-diabetic, anti-oxidant, anti-tumor, anti-inflammatory and anti-microbial. Mango seed is rich in Mangiferin component and showed excellent anti-inflammatory activity when compared with mango peel [4]. Mangiferin has the ability to reduce Ig E and suppress anaphylactic reactions. Expectedly, Mangiferin reduces the activity of the released histamine in different ways. First, it suppresses mast cells, basophils and plasma cells which usually release histamine, secondly, it prevents arachidonic acid from releasing prostaglandin E which in turn releases a higher concentration of histamine and finally, it blocks the activity of the H1 receptor due to its antagonistic effect [5].

Most probably, it is the mangiferin that is responsible to treat topical inflammation and is also beneficial as an anti-microbial agent. The current study was designed to evaluate the effect of ethanolic extract of *M. indica* seed on experimentally-induced irritation in rabbits.

METHODS

Animals

The study was conducted at Muhammad Institute of Medical and Allied Sciences, Multan, in October 2021 and consisted of 15 rabbits of either gender of six months old. The rabbits were taken from Multan pet market, Punjab. The standard betamethasone was purchased from Ethical Laboratories (Pvt.) Ltd. Pakistan, while all other chemicals were purchased from Solex Chemical (Pvt) Ltd-Multan. Before measuring the counter-irritant activity, the 15 rabbits weighing 1.5 kg ±1.5g were put under two-hour observation for an initial assessment of dermatological and allergic behaviour. Animals were maintained at the Muhammad Institute of Medical and Allied Sciences' Animal House in Multan. They were housed in stainless steel cages and given ad libitum access to commercially available food and tap water. The temperature was kept at 25 °C. The tests were carried out in accordance with National Research Council norms [6] and were authorized by the Muhammad Institute of Medical

and Allied Sciences' Animal Ethical Committee in Multan, Pakistan. MIMAS/03/Biochem/315/20 (Approval No. MIMAS/03/Biochem/315/20).

The fresh seeds of the plant *M. indica* were collected from Khand Mangoes Farm, Multan, Pakistan. Seeds were authenticated with the cooperation of an expert taxonomist at the Department of Botany, Bahauddin Zakariya University, Multan and seed specimens (org/tpl 101/record/ kew-2362844) were subjected for the record.

Preparation of Extract

For the preparation of extract, first of all, the fresh seeds of *M. indica* were shade dried for one month. All the adulterants and vegetative wastes were removed from vegetative material by manual picking before grinding into a coarse powder (250 g) with the help of a special herbal grinder. A solution of the coarse powder was prepared with 70% ethanol and stored in airtight jars for three weeks. The filtrate was evaporated at a temperature of 37 ºC under reduced pressure in a rotary evaporator following the literature [7]. The extract yield (10 %) obtained was stored at -2 ºC in amber colour airtight jars in laboratory refrigerators.

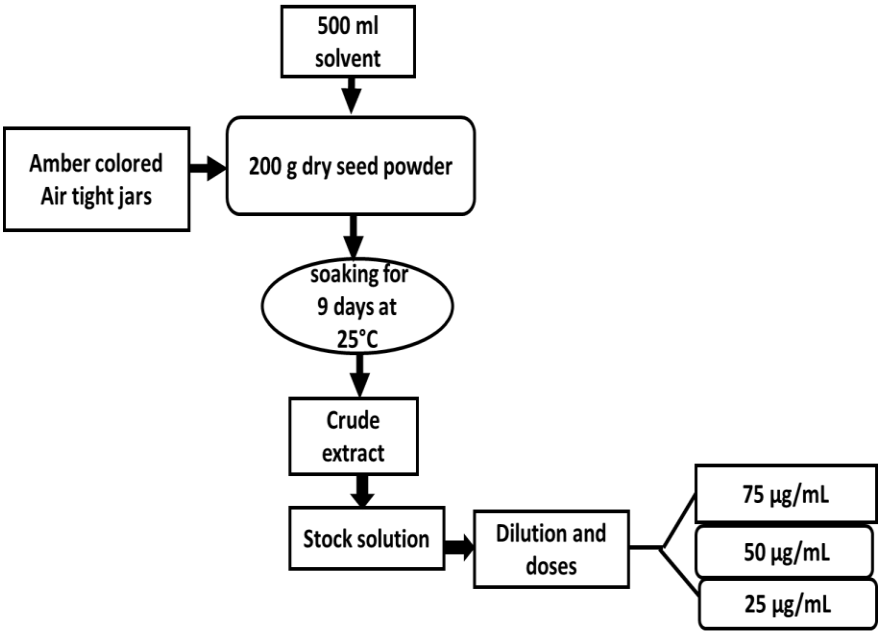

Figure 1: Preparation and Formulation of Extract

Anti-inflammatory Screening

The anti-inflammatory activity of the crude extract was determined and compared with negative control (distilled water) and standard (betamethasone). The potency of its anti-inflammatory activity

was measured by inducing inflammation with four different inducers i.e., phenol, formalin, acetic acid and sandpaper in the experimental animals according to the stated protocol [8].

Maximum and minimum tolerated doses were calculated at an interval of 2 hours in (75, 50 and 25) µg/ mL pattern.

All rabbits were divided into four (G1, G2, G3 and G4) groups, each group contained 15 rabbits. Inflammation was induced in G4 by anti-clockwise application of sandpaper on both ears of all rabbits in G4. Induction of redness and erythema in ears were observed and recorded as stated in a previous research study. Standard betamethasone 0.08 mg was applied on both ears of three rabbits while the other three rabbits were treated with distilled water (1 mL) as a negative control. The remaining rabbits were arranged in subgroups each containing three rabbits [10]. These subgroups were treated with *M. indica* seed extract concentrations of 25 µg/mL, 50 µg/mL, and 75 µg/mL. The time, dosage and degree of counter-irritancy were noted for all rabbits in G4.

Formalin, acetic acid and phenol were topically applied as inducers in G3, G2 and G1 respectively on both ears of three rabbits belonging to all three groups. Standard (0.08 mg), negative control (1 mL) and plant extract concentrations (25 µg/mL, 50 µg/mL, 75 µg/mL) were applied by subgrouping of each group as stated above. Reduction in redness and erythema on rabbit's ears were observed and recorded by visually analogue. Recovery of inflamed areas was measured in cm with the help of scale. Percentage inhibition was calculated:

$$\text{Percentage inhibition (\%)} = \frac{A - B}{A} \times 100$$

A= Initial value of inflamed area

B= Final value of inflamed area

Figure 2: Evaluation of anti-inflammatory activity

Statistical Analysis

The data presented as mean ± standard deviation (SD). The data was subjected to one-way ANOVA followed by a Dunnett's multiple comparison test using SPSS version-20 software, $p< 0.05$ was considered statistically significant and appropriate while greater than this was considered insignificant.

RESULTS

Different ethanolic extract concentrations of *M. indica* seed 25 µg/mL, 50 µg/mL and 75 µg/mL topically applied to rabbit's ear showed gradually increasing anti-inflammatory effect while remarkable extract concentration used for irritation inhibition found against four inducers by *M. indica* was 75µg/mL and the percentage inhibitions for all extract concentrations are given in Table 1.

Table 1: Counter-irritant activity of *M. indica* seed extract in rabbits

Groups	G1 n=15	G2 n=15	G3 n=15	G4 n=15
Applied Conc.	Phenol Irritation Inhibition (%)	Acetic acid irritation inhibition (%)	Formalin irritation inhibition (%)	Sand paper irritation inhibition (%)
Seed extract 25 µg/mL	65.13±2.3 (p=0.06)	79.31±2.5 (p=0.05)	79.21±2.7 (p=0.003)	60.21±2.9 (p=0.08)
Seed extract 50 µg/mL	71.09±2.3 (p=0.05)	83.09±2.4 (p=0.05)	87.03±2.3 (p=0.002)	63.44±2.1 (p=0.06)
Seed extract 75 µg/mL	78.21±2.4 (p=0.02)	85.20±2.3 (p=0.03)	94.92±2.4 (p=0.001)	66.45±2.6 (p=0.04)
Betamethasone (0.08 mg)	98.03±2.5 (p=0.000)	97.31±2.1 (p=0.000)	98.02±2.2 (p=0.001)	97.31±2.2 (p=0.001)
Distilled water (1 mL)	3.03±2.1 (p = 0.06)	3.12±2.3 (p =0 .07)	2.50±2.3 (p = 0.07)	2.02±2.3 (p =0.06)

Data are shown as Mean ± S.D, $p < 0.05$ statistically significant $p > 0.05$ was considered insignificant and $p < 0.000$ was recorded as highly significant.

According to the results, irritation inhibition percentage by *M. indica* seed extract concentration 75 µg/mL in G4 group of rabbits was 66.45±2.6 %, in G2 rabbits was 85.20±2.3 % and in G1 rabbits was 78.21±2.4 % with significant (p=0.04), (p=0.03) and (p=0.02) respectively, while in G3 rabbits was 94.92±2.4 % with high significant p-value (p=0.001). Moreover, the irritation inhibition percentage by standard betamethasone in both groups G1 and G2 was 98.03±2.5 % and 97.31±2.1 % respectively each with a significant p-value (p= 0.000) while for distilled water minimum irritation inhibition percentage was noted as mentioned in Table.1 with insignificant p-values in all groups. Results highlighted that dose-dependent irritation inhibition percentage by *M. indica* seed extract is comparable with standard drug betamethasone.

The results revealed that the minimum irritation inhibition percentage showed by *M. indica* seed extract 25 µg/mL was 60.21±2.9 % in the G4 group against sandpaper reducing irritation. *M. indica* 75 µg/mL showed a maximum irritation inhibition percentage of 94.92±2.4 % in the G3 group of rabbits against formalin inducer. Nevertheless, *M. indica* 50 µg/mL showed moderate inhibition as compared to former concentrations. Our results also disclosed that there is a direct relationship between the concentration of *M. indica* seed extract and percentage inhibition.

The presence of diverse elements such as flavonoids, steroids, saponins, tannins, mangiferin, alkaloids, terpenoids, glycosides, and carbohydrates was determined using conventional phytochemical identification. The anti-inflammatory response shown by the *M. indica* seed extract is associated with its phytochemical constituents, and the most active mangiferin is supposed to be involved in countering the induced inflammation and redness by various inducers used either by reducing the Ig E or suppering the anaphylactic reactions [9]. Mangiferin also shows a negative effect on released histamine to reduce the inflammation through various routes which may be associated with mast cells, basophil cells, plasma cells and prostaglandins E [10].

Table 2: Time taken to counter irritant activity by *M. indica* seed extract in rabbits

Conc.Based groups		Phenol (min)	Acetic acid (min)	Formalin (min)	Sandpaper (min)
M. indica µg/mL	25	**13**	**12**	**14**	13
M. indica µg/mL	50	**10**	**9**	**11**	10

M. indica 75 µg/mL	5	7	4	8
Betamethasone 0.08mg	2	2	1	1
Distilled water 1mL	20	25	26	30

The minimum time taken to decrease redness by 75 µg/mL against formalin was 4 minutes. While it took approximately 14 minutes for the 25 µg/mL to mitigate erythema against formalin. Response time showed by three concentrations of *M. indica* is given below:

M. indica 75 µg/mL> *M. indica* 50 µg/mL> *M. indica* 25 µg/mL

DISCUSSION

Different pharmacological constituents are responsible for anti-inflammatory activity. Mangiferin from mango seed has been identified which is demonstrated to be involved in several activities. For instance, it prevents tumor formation, extends life span and it is a possible cure for diabetes, asthma and all inflammatory responses. Owing to its richness in phenolic compounds like flavonoids, the use of the fruit byproducts, seed and peel of *M. indica* is becoming popular in research. To replace toxic synthetic compounds in the food, cosmetics and pharmaceutical industries, there is an increasing demand for bioactive compounds from fruits and vegetables [11].

More specifically, a comparison of the seed kernel and mango peel, showed that the seed exhibited more anti-inflammatory activity [12]. The phenolic compounds present in the kernel seed are highly affected by the geographic locations of the plants. It has also been demonstrated that secondary metabolites vary with the mango varieties. Flavonoids are secondary metabolites that show high anti-inflammatory activity. The most active component; Mangiferin is present in excessive amounts in mango seed and has been reported as an excellent anti-inflammatory agent Mangiferin tends to suppress inflammatory mediators like IL-6 and IL-1β and prevent inflammatory disorders by regulating NF-κB through the inhibition of phosphorylation of signalling pathway. Furthermore, Mangiferin suppresses the mRNA expression of pro-inflammatory mediators and activates the protein kinase as well as reduces total inflammatory cell infiltration and eosinophil [13].

Mangiferin possesses antibacterial activity against gram-positive bacteria, hence protecting against atopic dermatitis [14]. In addition, Mangiferin plays a major role in the reduction of triglycerides, cholesterol and free fatty acids in the heart and increases heart tissue phospholipid levels.

Preparation of phytomedicines from the active compounds of *M. indica* would be worth the while for future pharmacological uses based on these activities enumerated. Reduction of airway inflammation

in peripheral blood vessels and inhibition of IL-4 and IL-5 can be achieved with Mangiferin. Betamethasone belonging to corticosteroids; is an effective compound widely used in the treatment of inflammatory diseases like itching, erythema, scaling and crusting etc. but in the long term, topical use can cause adverse effects such as skin thinning and pigmentation [15].

Mangiferin has the advantage over betamethasone as its multiple uses do not cause any harm other than stinging and dryness as does betamethasone [16].

The xanthoid structure of mangiferin, a rich source of polyhydroxy components, contributes to its free radical scavenging ability leading to multiple biological activities [17].

The presence of Mangiferin was bound to interact with the irritated and inflamed cell membrane. Subsequently, the irritated and damaged superficial layers [18].

Furthermore, the anti-septic activity of mangiferin (flavonoids) in ethanolic extract of *M. Indica* is effective at killing microbes, bacteria and other microorganisms on the surface of damaged skin. Alcohol-based products are an important element of infection control procedures and reduce the presence of bacteria on the skin. Moreover, there is no evidence of adverse side effects of *M. Indica* ethanolic seed extract. Hitherto, Mangiferin could be a promising candidate for the development of multiple drugs to counter the effect of inflammation.

CONCLUSION

In summary, the anti-inflammatory activity of the ethanolic extract of the seed of *Mangifera indica* has been demonstrated and points to the effect that mango seed is a rich source of secondary metabolites probably responsible for its anti-inflammatory effect. In addition, at the three different concentrations used; the one that had more flavonoids expressed better anti-inflammatory results. Thus, the higher the number of flavonoids, the more remarkable the anti-inflammatory as well as counter-irritancy and anti-septic properties. Hence, mango seed kernel; a by-product of mango processing industries, might be used to develop potential value-added ingredients.

DECLARATIONS

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Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Tanzila Rehman conceived and designed the study. Mehnaz Bibi and Amina Ramzan collected and analyzed the data. Acquisition

of experiment done by Ameen Abbas. Mashhud-ul-Hasan Abid supervised the study and Ameen Abbas wrote the manuscript.

REFERENCES

1. Willis AP and Leffler CW. NO and prostanoids: age dependence of hypercapniaand histamine-induced dilations of pig pial arterioles. The American physiological society. 2020. 277(1): 299-307
2. Cowden JM, Zhang M, Dunford PJ, Thurmond RL. The Histamine H4 Receptor Mediates Inflammation and Pruritus in Th2-Dependent Dermal Inflammation. J invest Dermatol. 2010. 130(4).1023-33
3. Jyotshna, Khare P, Shanker K. Mangiferin: A review of sources and interventions for biological activities. BioFactors 2016. 42(5). 504-514
4. Stohs SJ, Swaroop A, Moriyama H, Bagchi M, Ahmad T and Bagchi D. A Review on Antioxidant, Anti-Inflammatory and Gastroprotective Abilities of Mango (Magnifera indica) Leaf Extract and Mangiferin. Journal of Nutrition and Health Sciences .2018.5(3): 303-305
5. Kuganesan A, Thiripuranathar G, Navaratne AN, Paranagama PA. Antioxidant and anti-inflammatory activities of peels, pulps and seed kernels of three common mango (Mangifera indical L.) varieties in Sri Lanka. International Journal of Pharmaceutical Sciences and Research. 2017(8):70.-78
6. Rivera GD, Balmaseda IH, Leon AA, Hernandez BC, Montiel LM, Garrido GG. Anti-allergic properties of Mangifera indica L. extract (Vimang) and contribution of its glucosylxanthone Mangiferin. Journal of Pharmacy and Pharmacology. 2006. 58(3): 385-92.
7. Rivera DG, Balmaseda IH, Leon AA, Hernandez BC, Montiel LM, Garrido GG. Anti-allergic properties of Mangifera indica L. extract (Vimang) and contribution of its glucosylxanthone Mangiferin. Journal of Pharmacy and Pharmacology. 2006. 58(3): 385-92.
8. Manzoor A, Khan IA, Kousar S, Iqbal MO, Munawar SH, Manzoor Z, et al. Evaluation of cardioprotective potential of hydroalcohol peel extract of Citrullus colocynthis Linn. (Cucurbitaceae). Tropical Journal of Pharmaceutical Research 2022(21) :105–10.
9. Ahmad IK, Aziz A, Shaukat Hussain M, Zahid M, Muhammad Asif R. Dermatological evaluation of counter irritant potential of human urine in rabbits. 2015.65(1). 9-11
10. Singh A, Raju R, Munch G. Potential anti-inflammatory compounds from Australian plants. Neurochemistry international. 2021. 142(2): 117-121
11. Kuganesan A, Thiripuranathar G, Navaratne A, Paranagama P. Anti-oxidant and anti-inflammatory activities of peels and pulps and seed kernels of three common mango (M Indica L.) varieties in Sri Lanka. International Journal of Pharmaceutical Sciences and Research 2017(8):70–8.

12. Imran M, Arshad MS, Butt MS, Kwon J-H, Arshad MU, Sultan MT. Mangiferin: a natural miracle bioactive compound against lifestyle related disorders. Lipids in Health and Disease 2017.16(1): 84.
13. Mazlan N, Azman S, Ghazali N, Sofea PZ, Yusri S, Idi H, et al. Synergistic antibacterial activity of mangiferin with antibiotics against Staphylococcus aureus. Drug Invention Today 2019.12(1).14–17.
14. Zöller NN, Kippenberger S, Thaçi D, Mewes K, Spiegel M, Sättler A, et al. Evaluation of beneficial and adverse effects of glucocorticoids on a newly developed full-thickness skin model. Toxicology in Vitro 2008.22(1).747–59.
15. Kumaran MS, Kaur I, Kumar B. Effect of topical calcipotriol, betamethasone dipropionate and their combination in the treatment of localized vitiligo. Journal of the European Academy of Dermatology and Venereology. 2006.20(3). 269-73
16. Rivera DG, Balmaseda IH, León AÁ, Hernández BC, Montiel LM, Garrido GG, et al. Anti-allergic properties of Mangifera indica L. extract (Vimang) and contribution of its glucosylxanthone mangiferin. Journal of Pharmacy and Pharmacology .2006.58(3).385–92.
17. Mei S, Ma H, Chen X. Anticancer and anti-inflammatory properties of mangiferin: A review of its molecular mechanisms. Food Chem Toxicol. 2021. 149(1). 112-113
18. Vassal S, Taamma R, Marty N, Sardet A, d'Athis P, Brémont F, et al. Microbiologic contamination study of nebulizers after aerosol therapy in patients with cystic fibrosis. American Journal of Infection Control 2000.28(1).347–51.