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Original Article

Comparison of Anti-Inflammatory Effect of Ethanolic Extract of Carica Papaya Leaves and Indomethacin in Carrageenan Induced Rat Paw Edema Animal Model

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Abstract

Background: Carica papaya is very useful vegetables for daily living. **Objective:** The purpose of the present study was to compare the anti-inflammatory effect of ethanolic extract of Carica papaya leaves and indomethacin in carrageenan induced rat paw edema animal model. **Methodology:** This was an animal study carried out in the Department of Pharmacology at Dhaka Medical College, Dhaka, Bangladesh during the period from July 2014 to June 2015 for a period of one (01) year. The leaves of *Carica Papaya* collected from Botanical garden, Mirpur, Dhaka, Bangladesh. The animals were divided into four groups. Inflammation was produced by injecting 0.1 ml of 1% carrageenan solution in normal saline in all experimental animals. Group I were served as control that received normal saline. Group II were received ethanolic extract of *Carica Papaya*. Group III were received ethanolic extract of *Carica Papaya* leaves. Group IV were received indomethacin. **Result:** The experiment was carried out on 48 Long Evan Norwegian rats. Rats were divided in 4 groups of six animals each. The mean initial antero-posterior diameter of rat's paw of group I, II, III and IV were 3.83±0.04, 2.12±0.02, 1.74±0.01 and 1.60±0.03 respectively. The inhibition of edema formation were 44.64%, 54.57% and 58.22% in group II, III and IV respectively. **Conclusion:** In conclusion the Carica papaya leaves has anti-inflammatory effect. *[Journal of Science Foundation 2018;16(2):49-53]*

Keywords: Anti-inflammatory; ethanolic extract; carica papaya leaves; indomethacin; carrageenan induced; rat paw edema; animal model

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Introduction

Inflammation is fundamentally a protective response, the ultimate goal of which is to get rid of the organism of both the initial cause of cell injury and the consequences of such injury (Owoyele et al., 2008). It is part of the complex biological response of body tissue to harmful stimuli such as pathogens, damaged cells or irritants. Inflammation involves immune cells, blood vessels and molecular mediators. It serves to destroy, dilute or wall off the injurious agents, clear out necrotic cells and tissue damaged from the original insult and the inflammatory process and to initiate tissue repair (Sagnia et al., 2014). It is a genetic response and is considered as a mechanism of innate immunity as compared to adaptive immunity, which is specific for each pathogen.

The anti-inflammatory drugs that are now available includes non-steroidal anti-inflammatory drugs, corticosteroids, gold, disease modifying anti-rheumatic drugs (DMARD) such as methotrexate, cyclosporine (Anaga and Onehi 2010). None of these are drugs have been found safe; all are known to produce mild to serious side effects. For this reason, use of herbal medicine throughout the world is increasing. Plants are still remaining the primary source of supply of many important drugs (Comalada et al., 2005). Therefore, studies are still going on in search of potent, less toxic, cheaper and easily available anti-inflammatory agents.

Carica papaya is an economically important fruit in the *Caricaceae* family as different parts of the plant can be eaten and also have been used for medicinal purposes (Krishna et al. 2008). The leaves (specially fallen onas) are used for treatment of fever, gonorrhea, syphilis, inflammation and as dressing for foul wounds[6]. Some of the scientifically validated research of *Carica papaya* include the abortifacient activity of the seeds (Nayak et al., 2007), the effects of the seeds on germinal epithelium of the seminiferous tubules (Anuar et al., 2008), the fruit juice for lowering blood pressure (Vuong et al., 2013), the wound healing effects of the leaves and several other studies (Mahmood et al., 2005). However there are only few reports on the investigation into the biological activity of the dried leaf extract. The present study was undertaken based on the observation in the local community that the leaves of *Carica papaya* are used for the treatment of inflammatory conditions such as asthma, rheumatism, arthritis and wound healing. The purpose of the present study was to compare the anti-inflammatory effect of ethanolic extract of Carica papaya leaves and indomethacin in carrageenan induced rat paw edema animal model.

Methodology

This was an animal study. This study was carried out at the Department of Pharmacology, Dhaka Medical College, Dhaka during the period from July 2014 to June 2015 for a period of one (1) year. The experiment was carried out on 48 Long Evan Norwegian rats. They were collected from the ICDDRB, Dhaka. The rats were of either sex, weighing between 130 to 160 gm. The rats were kept in the animal house of the Department of Pharmacology at Dhaka Medical College, Dhaka, Bangladesh. Rats of different groups were kept in different metallic cages. They were allowed to feed on standard laboratory diet and to drink water ad libitum. The plant material i.e. leaves of *Carica Papaya* collected from Botanical garden, Mirpur, Dhaka, Bangladesh. The plant was authenticated by National Herbarium, Dhaka and a voucher specimen was deposited. A voucher number was obtained. The voucher number of it is 41888. The leaves of *Carica Papaya* were cut into pieces shade-dried and grounded to coarse powder and then supplied to "Centre for Advanced Research in Sciences (CARS)", University of Dhaka for making ethanolic extract. At CARS the leaves of *Carica Papaya* was soaked in Ethanol (800) ml with continuous shaking at 25.C for 3 days and filtered. The organic extract was evaporated under vacuum to obtain a semisolid residue (4.1gm). Indomethacin powder was collected from Novartis pharmaceutical. Carrageenan was collected from the department of pharmacy, Jahangirnagar University, Savar, Dhaka. 1% carrageenan solution in normal saline was prepared. Carrageenan induced rat paw edema animal model were used in this study. In this method, rats were divided in 4 groups of six animals each. The animals were pre-treated with drugs orally 1 hour before the experiment 0.1 ml of 1% carrageenan was injected aseptically into the sub planter surface of right hind paw of each rat. Progress of the local inflammatory exudative lesion was assessed by measuring the maximum linear cross section of the joint at '0' hour and at the end of '3' hours. The measurements were taken as accurately as possible by verniar scale. The difference between the 0 and 3 hours gives the actual edema. Percentage inhibition of edema formation was taken as an index of acute anti-inflammatory activity. The percent inhibition of edema = 100 X (1 – Vt / Vc) Where, Vc = mean paw edema diameter in the control group. Vt = mean paw edema diameter in the drug treated group. The animals were divided into four groups.

Inflammation was produced by injecting 0.1 ml of 1% carrageenan solution in normal saline in all experimental animals and treated as follows Group I consisted of 6 rats and were served as control that received normal saline in a volume of 0.6 ml one hour before the carrageenan injection. Group II consisted of 6 rats and were received ethanolic extract of *Carica Papaya* at a dose of 50 mg/kg body weight orally one hour before the carrageenan injection. Group III consisted of 6 rats and were received ethanolic extract of *Carica Papaya* leaves at a dose of 100 mg/kg body weight orally one hour before carrageenan injection. Group IV consisted of 6 rats and were received indomethacin at a dose of 5 mg/kg body weight orally one hour before carrageenan injection. All the results have been expressed as mean plus/minus standard error of mean (mean± SEM). Significance of difference between groups were assessed by using student's t test with $P<0.05$ considered as being significant.

Result

The present study was carried out to evaluate the anti-inflammatory effect of *Carica Papaya* leaves. Its anti-inflammatory effects were tested on Long Evan Norwegian rats. This work was designed for acute inflammatory model. The experiment was carried out on 48 Long Evan Norwegian rats. Rats were divided in 4 groups of six animals each. The mean initial antero-posterior diameter of rat's paw of control group (group I) was 3.28±0.04 mm. The mean antero-posterior diameter of rat's paw of control group (group1) after 3 hours of carrageenan injection was 7.12±0.02 mm. Photograph showing carrageenan induced paw edema in rat's hind paw in control group. The mean initial antero-posterior diameter of rat's paw of group II (ethanolic extract of *Carica Papaya* 50 mg/kg) was 3.14±0.02 mm, where as the mean antero-posterior diameter of rat's paw of group II (ethanolic extract of CP 50 mg/kg) after 3 hours of carrageenan injection was 5.26±0.03 mm. Photograph showing carrageenan induced paw edema in rat's hind paw in group II. The mean initial antero-posterior diameter of rat's paw of group III (ethanolic extract of CP 100 mg/kg) was 3.12±0.02 mm. The mean antero-posterior diameter of rat's paw of group III (ethanolic extract of CP 100 mg/kg) after 3 hours of carrageenan injection was 4.86±0.03 mm. Photograph showing carrageenan induced paw edema in rat's hind paw in group III. The mean initial antero-posterior diameter of rat's paw of group IV (indomethacin 5 mg/kg) was 3.16±0.01 mm. The mean antero-posterior diameter of rat's paw of group IV(indomethacin 5 mg/kg) after 3 hours of carrageenan injection was 4.76±0.03 mm. Photograph showing carrageenan induced paw edema in rat's hind paw in group IV (Table 1).

Table 1: Effects of ethanolic extract of *Carica Papaya* leaves, Indomethacin on carrageenan induced rat paw edema after 3 hours of carrageenan injection

Group	**Number of rats**	**Initial antero-posterior diameter** (mean ± SEM)	**Antero-posterior diameter after 3 hrs of carrageenan** (mean ± SEM)	**Increase in antero-posterior diameter** (mean± SEM)	**Inhibition of edema formation %**
Group I	6	3.28±0.04	7.12±0.02	3.83±0.04	
Group II	6	3.14±0.02	5.26±0.03	2.12±0.02*	44.64%
Group III	6	3.12±0.02	4.86±0.03	1.74±0.01**	54.57%
Group IV	6	3.16±0.01	4.76±0.03	1.60±0.03**	58.22%

*P<0.05 in a test of significance difference from control. **P<0.001 in a test of significance difference from control. **Group I :** 0.6 ml normal saline orally and served as control; **Group II :** Ethanolic extract of *Carica Papaya* 50mg/kg body weight orally; **Group III :** Ethanolic extract of *Carica Papaya* 100mg/kg body weight orally; **Group IV :** Indomethacin 5mg/kg body weight orally

Discussion

Inflammation is the immune response of body tissue to injury or infection (Owoyele et al., 2008). It is an important component of innate immunity. The inflammatory process involves a complex biological cascade of molecular and cellular signals that alter physiological responses and ultimately resulting in the familiar clinical symptoms of pain, swelling, heat and redness (Comalada et al., 2005). 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 lymphocytes (Mahmood et al., 2005).

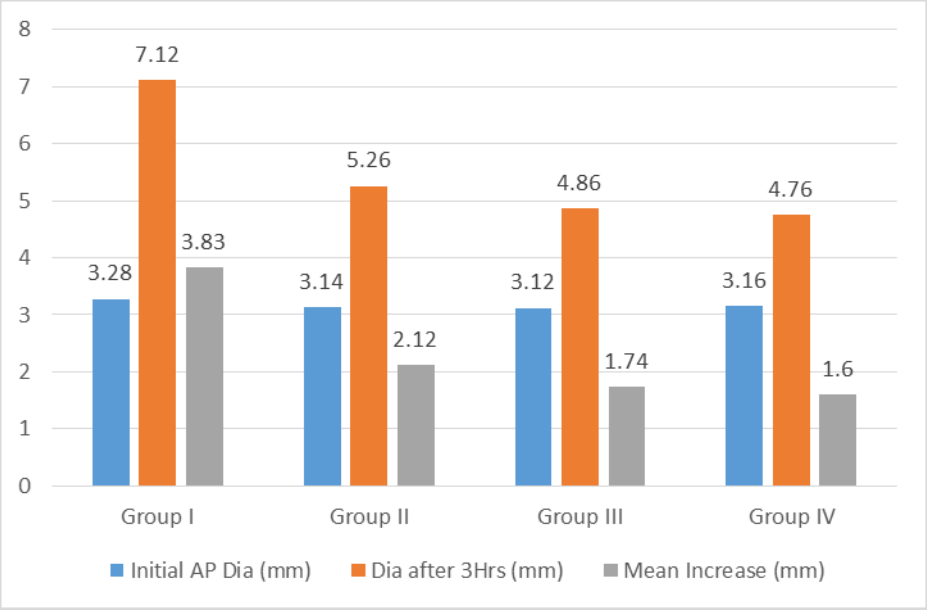

Figure I: Showing the AP diameter of rat paw edema

In the present work acute inflammation was induced. Acute inflammation was induced by injecting 0.1 ml of 1.0% carrageenan solution in normal saline into the subplanter surface of rats. The day of implantation was counted as day 1 and on the 15th day the animals were anesthetized and cotton pellets with granulation tissues were dissected out. In this study, concomitant administration of ethanolic extract of *Carica Papaya* leaves and non-steroidal anti-inflammatory drug one hour before carrageenan injection and daily for 14 days reduced rat paw edema and weight of granulation tissue. The reduction of rat paw edema and weight of granulation tissue was statistically significant in comparison to control group.

Papaya is a common man's fruit which is reasonably priced and has a high nutritive value. It is low in calories and rich in natural vitamins and minerals (Indran et al., 2008). Papaya is the fruit for vitamin C, vitamin A, riboflavin, folate, calcium, thiamin, iron, niacin, potassium and fibre. The comparative low calories content (32 kcal/100g of ripe papaya) makes this fruit favourable for obese people who are in weight reducing regime (Yogiraj et al., 2014). Papaya has more carotene compared to other fruits such as apples, guavas etc which help to prevent damage by free radicals. Unripe green papaya is used as vegetables though it does not contain carotene but all other nutrients (Ranasinghe et al., 2012). It is also used in salads, pies, juice and confections.

Carrageenan is a generic name of a family of gel forming and viscosifying polysaccharides which are obtained by extraction from certain species of red seaweeds (Subenthiran et al., 2013). It contains a considerable amount of cellulose and is used in food preparation for its gelling, thickening and emulsifying properties. It is also used in pharmaceutical application and in experimental medicine for the evaluation of anti-inflammatory agents. Carrageenan induced rat paw edema is a widely used test to determine anti-inflammatory activity and constitutes a simple and routine animal model (Imaga et al., 2010). It is used to test new anti-inflammatory drugs as well as to study the mechanism involved in inflammation. The development of edema in the rat hind paw following injection of carrageenan has been described as a biphasic, age-weight dependent event in which various mediators operate to produce the inflammatory response (Anuar et al., 2008).

Carrageenan-induced paw edema is widely used for determining the acute phase of inflammation (Sancho et al., 2011). Histamine, 5- hydroxytryptamine and bradykinin are the first detectable mediators in the early phase of carrageenan-induced inflammation whereas prostaglandins are detectable in the late phase of inflammation (Amazu et al., 2010). Although, these two phases have been identified, it is generally accepted that carrageenan functions maximally after 3 hours of its administration (Comalada et al., 2005). Therefore, the result of the carrageenan test shows that the ethanolic extract of *Carica papaya* leaves can inhibit prostaglandin mediated inflammation since the extract produced marked reduction in the carrageenan induced edema after 3 and 5 hour of carrageenan injection.

Conclusion

In conclusion, this study has established the anti-inflammatory effect of ethanolic extract of *Carica Papaya* leaves. Further basic and clinical studies are required in order to identify the exact active ingredient, determine the precise mechanism of action and to examine the toxicity of the extract.

References

Amazu LU, Azikiwe CC, Njoku CJ, Osuala FN, Nwosu PJ, Ajugwo AO, Enye JC. Antiinflammatory activity of the methanolic extract of the seeds of Carica papaya in experimental animals. Asian Pacific Journal of Tropical Medicine. 2010;3(11):884-6

Anaga AO, Onehi EV. Antinociceptive and anti-inflammatory effects of the methanol seed extract of Carica papaya in mice and rats. African Journal of Pharmacy and Pharmacology. 2010;4(4):140-4

Anuar NS, Zahari SS, Taib IA, Rahman MT. Effect of green and ripe Carica papaya epicarp extracts on wound healing and during pregnancy. Food and Chemical Toxicology. 2008;46(7):2384-9

Comalada M, Camuesco D, Sierra S, Ballester I, Xaus J, Gálvez J, Zarzuelo A. In vivo quercitrin anti-inflammatory effect involves release of quercetin, which inhibits inflammation through down-regulation of the NF-κB pathway. European journal of immunology. 2005;35(2):584-92

Gammulle A, Ratnasooriya WD, Jayakody JR, Fernando C, Kanatiwela C, Udagama PV. Thrombocytosis and anti-inflammatory properties and toxicological evaluation of Carica papaya mature leaf concentrate in a murine model. International Journal of Medicinal Plants Research. 2012;1(2):21-30

Imaga NA, Gbenle GO, Okochi VI, Adenekan S, Duro-Emmanuel T, Oyeniyi B, Dokai PN, Oyenuga M, Otumara A, Ekeh FC. Phytochemical and antioxidant nutrient constituents of Carica papaya and Parquetina nigrescens extracts. Scientific research and essays. 2010;5(16):2201-5

Indran M, Mahmood AA, Kuppusamy UR. Protective effect of Carica papaya L leaf extract against alcohol induced acute gastric damage and blood oxidative stress in rats. West Indian medical journal. 2008;57(4):323-6

Krishna KL, Paridhavi M, Patel JA. Review on nutritional, medicinal and pharmacological properties of Papaya (Carica papaya Linn.). NPR 2008;7(4):364-373

Mahmood AA, Sidik K, Salmah I. Wound healing activity of Carica papaya L. aqueous leaf extract in rats. International Journal of Molecular Medicine and Advance Sciences. 2005;1(4):398-401.

Nayak BS, Pereira LP, Maharaj D. Wound healing activity of Carica papaya L. in experimentally induced diabetic rats. IJEB 2007;45(8):739-743

Owoyele BV, Adebukola OM, Funmilayo AA, Soladoye AO. Anti-inflammatory activities of ethanolic extract of Carica papaya leaves. Inflammopharmacology. 2008;16(4):168-73

Ranasinghe P, Ranasinghe P, Abeysekera WK, Premakumara GS, Perera YS, Gurugama P, Gunatilake SB. In vitro erythrocyte membrane stabilization properties of Carica papaya L. leaf extracts. Pharmacognosy research. 2012;4(4):196

Sagnia B, Fedeli D, Casetti R, Montesano C, Falcioni G, Colizzi V. Antioxidant and anti-inflammatory activities of extracts from Cassia alata, Eleusine indica, Eremomastax speciosa, Carica papaya and Polyscias fulva medicinal plants collected in Cameroon. PloS one. 2014;9(8):e103999

Sancho LE, Yahia EM, González-Aguilar GA. Identification and quantification of phenols, carotenoids, and vitamin C from papaya (Carica papaya L., cv. Maradol) fruit determined by HPLC-DAD-MS/MS-ESI. Food Research International. 2011;44(5):1284-91.

Subenthiran S, Choon TC, Cheong KC, Thayan R, Teck MB, Muniandy PK, Afzan A, Abdullah NR, Ismail Z. Carica papaya leaves juice significantly accelerates the rate of increase in platelet count among patients with dengue fever and dengue haemorrhagic fever. Evidence-Based Complementary and Alternative Medicine. 2013;2013

Vuong QV, Hirun S, Roach PD, Bowyer MC, Phillips PA, Scarlett CJ. Effect of extraction conditions on total phenolic compounds and antioxidant activities of Carica papaya leaf aqueous extracts. Journal of Herbal Medicine. 2013;3(3):104-11

Yogiraj V, Goyal PK, Chauhan CS, Goyal A, Vyas B. Carica papaya Linn: an overview. International Journal of Herbal Medicine. 2014;2(5):01-8