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Comparative anti-inflammatory effect of methanolic extract of *Tamarindus Indica* seeds and Indomethacin on rats

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Abstract
Anti-inflammatory drugs exhibit adverse effects due to chronic use in inflammatory conditions. Drugs from herbal sources may provide a safe alternate option. The aim of this study was to evaluate anti-inflammatory effect of Methanolic extract of Tamarindus indica seeds on experimental rats. This experimental study was carried out in Department of Pharmacology, Dhaka Medical College, Bangladesh, from July 2015 to June 2016. Fifty-six long Evan Norwegian rats of either sex, weighing 150 to 200gm, were included in this study. The study was divided into two parts, each with 28 animals. Anti-inflammatory activity of Methanolic extract of T.indica seeds were assessed in carrageenan induced paw edema and cotton pellet induced granuloma model in rats, then compared with Indomethacin. Mean increase in anteroposterior diameter in group II (2.11±0.02mm) was statistically significant (P<0.05) compared to control group I (3.45±0.04mm). Group III (1.70±0.01mm) and standard drug group IV (1.53±0.03mm) showed highly significant finding (P<0.001). Maximum percentage inhibition of edema formation was in group III (50.72%) and group II (41.74%), but lesser than group IV (55.65%). Mean increase in cotton pellet weight in Group II (28.59±0.36) and Group III (23.74±0.56) was statistically significant (P<0.05) compared to group I. Group IV showed highly significant (P<0.001) increase. Group III (54.54%) showed higher percentage of inhibition of granuloma formation than Group II (45.25%), when compared with group I, this was lesser than Group IV (62.05%). Methanolic extract of Tamarindus indica seeds of 300mg/kg body weight and 600mg/kg body weight orally produced significant reduction in paw thickness in carrageenan induced acute inflammation and cotton pellet induced chronic inflammation. The presence of tannins and flavonoids in Methanolic extract of Tamarindus Indica appears to inhibit prostaglandin synthesis and exerts the anti-inflammatory effects.

Keywords: Anti-inflammatory effect, Tamarindus indica seeds, Indomethacin.

Introduction
Inflammation is defined as the local response of living mammalian vascularized connective tissue to the injury caused by various exogenous and endogenous stimuli. It is a body defense reaction in order to eliminate or limit the spread of injurious agent as well as to remove the consequent necrosed cells and tissue.[1] Inflammation helps to clear the infections and along with the repair, it makes wound healing possible, both have considerable potential to cause harm. For example, inflammatory reactions underlie life threatening anaphylactic responses to insect bites or drugs as well as, chronic diseases such as rheumatoid arthritis and atherosclerosis. An initial inflammatory stimulus triggers the release of chemical mediators from plasma or cells which then regulate the subsequent vascular and cellular responses.[2] Inflammation is the characteristic response of mammalian tissue to injury. It is defensive to injury and arises from the resultant tissue damage.[3] Diseases of inflammation and immunity can occur when the normal inflammatory response progresses to chronic inflammation either because of long term inappropriate response to a stimulus or because the offending agent is not removed. For example, chronic infection, transplantation and autoimmunity.[4] Prolonged uses of both steroidal and non-steroidal anti-inflammatory drugs are well known to be associated with peptic ulcer formation. Hence, search for new anti-inflammatory agents that can retain therapeutic efficacy and yet are devoid of these adverse effects is justified. There

is much hope of finding active anti-rheumatic compounds from indigenous plants as there are still used in therapeutics despite the progress in conventional chemistry and pharmacology in producing effective drugs. Herbal drugs are being proved as synthetic drugs with lesser side effects.[5] Tamarind or Tamarindus Indica L. of the Fabaceae, subfamily Caesalpinioideae, is an important food in the tropics. It is a multipurpose tree of which almost every part finds at least some use[6], either nutritional or medicinal. Traditionally seeds of Tamarindus indica are being used in asthma, bronchitis, leprosy, tuberculosis, wounds, ulcers, inflammation, stomach algae, diarrhea, dysentery, burning sensation, giddiness, vertigo and diabetes.[7] It has been reported that seeds of Tamarindus indica are having anti-ulcer, anti-asthmatic, anti-diabetic and anti-oxidant activity.[8,9] Moreover, it is also rich in phenolic compounds, polymeric tannins and fatty acids flavonoids, saponins, alkaloids and glycosides.[10,11] Flavonoids, tannins, saponins and alkaloids are responsible for anti-inflammatory and analgesic activity.[12] The seeds are reported to contain polymeric tannins and polyphenolic compounds like (+) catechin, procyanidin trimer, procyanidin tetramer, procyanidin pentamer, procyanidin hexamer, taxifolin, apigenin, eriodictyol, luteolin and naringenin.[13] Abnormal accumulation of elastase, a serine proteinase from human neutrophil, causes a number of acute and chronic inflammation diseases. It was found that Tamarind seeds contained serine proteinase inhibitor, with high inhibitory activities against human neutrophil elastase (HNE)[14], which probably explains the anti-inflammatory properties of Tamarind. The aim of the present study is to evaluate the anti-inflammatory effect Methanolic extract of Tamarindus indica seeds on experimental rats.

Materials and Methods

This experimental study was carried out in the Department of Pharmacology, Dhaka Medical College, Dhaka, Bangladesh from July 2015 to June 2016 for a period of one year.

Experimental Animals: The experiment was conducted on 56 long Evan Norwegian rats. They were collected from International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,b), Dhaka, Bangladesh. The rats were of either sex, weighing between 150 to 200gm. The rats were kept in the animal house of the Department of Pharmacology, Dhaka Medical College. The rats were housed in different plastic cages according to batches of various groups. 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.[15]

Drugs and Reagents: The plant material (seeds of *Tamarindus indica*) was collected from Botanical Garden, Mirpur, Dhaka. The plant was authenticated by National Herbarium, Dhaka, a voucher specimen was deposited and voucher number 42759. The seeds of *Tamarindus indica* were dried and grounded to coarse powder, then supplied to "Centre for Advanced Research in Sciences (CARS)", University of Dhaka for making Methanolic extract. The coarse powder of Tamarind seeds (100gm) were subjected to maceration for 72hours at room temperature using 500ml methanol. The extract was filtered and the solvent was evaporated under vacuum to obtain powdered residue. The dried extract was kept in air tight container in desiccator and used throughout the study.[16]

- Indomethacin- Raw material of indomethacin was collected from Novartis Pharmaceuticals.
- Carrageenan- Carrageenan was collected from the Department of Pharmacy, University of Dhaka, Bangladesh. One percent of Carrageenan suspension in normal saline was prepared.

Study design: The entire study was divided into two parts to demonstrate the anti-inflammatory effects of methanolic extract of *Tamarindus indica* seeds and compare the extract with Indomethacin on carrageenan induced paw edema and cotton pellet induced inflammation in rats to determine the acute and chronic inflammation, respectively.

Experiment-1: This part of study was carried out to determine the acute anti-inflammatory effect of methanolic extract of *Tamarindus indica* seeds in Carrageenan induced rat paw edema. Twenty-eight rats were randomly divided into four groups with seven rats in each group. Control group (Group I) of rats was given only 0.6ml normal saline solution. Methanolic extract of *Tamarindus indica* seeds was given orally by nasogastric tube at doses of 300mg/kg body weight and 600mg/kg body weight to experimental groups (Group II and III), respectively. Indomethacin was given orally at a dose of 10mg/kg body weight as standard anti-inflammatory drug (Group IV). After one hour of drug administration, 0.1 ml of 1% Carrageenan in sterile saline solution was injected into the sub-plantar surface of right hind paw of animals in all groups, for the production of acute inflammation. Antero-posterior diameters of paw was measured by slide calipers at zero hour and three hours of Carrageenan injection. Progress of inflammatory exudative lesion was assessed by measuring the maximum linear cross section of joint at zero hour and three hours. Percentage inhibition of edema formation was taken as an index of acute anti-inflammatory activity which was calculated by –

Percent inhibition of edema = 100 x (1-Vt/Vc) where,
Vc = Mean paw edema volume in control group.
Vt = Mean paw edema volume in drug treated group.

Experiment-2: This part of study was carried out to determine the chronic anti-inflammatory effect of drugs in cotton pellet induced granuloma on rats. Twenty-eight rats were similarly divided into four groups with seven rats in each group. A sterile cotton pellet each weighing 30mg was implanted subcutaneously in the groin region of each rat. They were fed with respective drug daily as in experiment 1, for 14 days along with free access to water and food and libitum. The animals were anesthetized on 15th day and cotton pellets with granulation tissue was removed, extraneous tissue was cleaned, then the pellets were dried in a hot air oven to a constant weight. Finally, the dry weight of cotton pellets were determined. Dry weight of the granuloma (amount of actual granulation tissue formed) was calculated by noting the difference between dry weight of cotton pellets recorded before and after implantation. Animal weight was also recorded on day 1 as initial weight and on day 15 as final weight. Percentage change of granuloma weight relative to vehicle control group was taken as an index of chronic inflammatory activity. It was

calculated by-
Percent inhibition = 100 x (1- Wt/Wc) where,
Wt = Mean dry weight of granuloma in drug treated group.
Wc = Mean dry weight of granuloma in control group.

Statistical analysis: The results are given as mean±SD for the independently performed experiments. Unpaired students' t-test was used to determine the level of significance. P value <0.05 was considered as statistically significant and P<0.01 as highly significant.

Results

Effects of Methanolic extract of *Tamarindus indica* seeds and Indomethacin on Carrageenan induced rat paw edema after 3 hours of Carrageenan injection.

Table-1 and Figure-1 displays that group II (6.18±0.03mm) and group III (5.83±0.03mm) revealed the least anteroposterior diameter (mean±SEM) of rat paw after 3 hours of Carrageenan injection compared to control group I (7.74±0.03mm). But it was more than the anteroposterior diameter in group IV (5.68±0.03mm).

Mean increase in anteroposterior diameter in group II (2.11±0.02mm) was statistically significant (P<0.05) compared to control group I (3.45±0.04mm), while group III (1.70±0.01mm) and standard drug group IV (1.53±0.03mm) showed highly significant finding (P<0.001).

Table 1: Effects of Methanolic extract of *Tamarindus indica* seed and indomethacin on rat paw edema after carrageenan injection.

Groups	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)
Group I	7	4.29 ± 0.04	7.74 ± 0.03	3.45 ± 0.04
Group II	7	4.17 ± 0.03	6.18 ± 0.03	2.11 ± 0.02*
Group III	7	4.13 ± 0.02	5.83 ± 0.03	1.70 ± 0.01**
Group IV	7	4.15 ± 0.02	5.68 ± 0.03	1.53 ± 0.03**

* Group II vs Group I; ** Group III, IV vs Group I

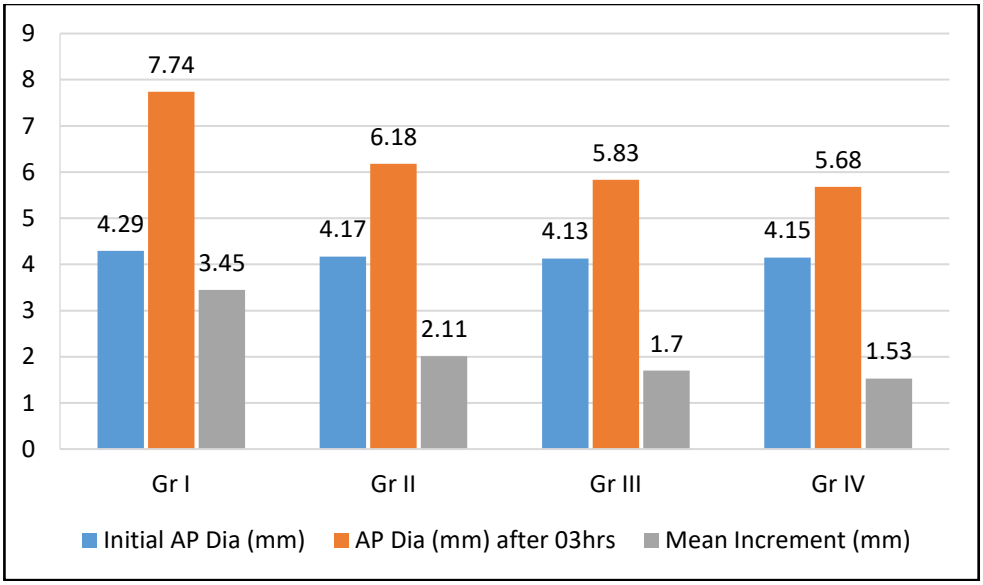

Fig. 1: Bar diagram showing change in anteroposterior diameter of rat paw before and after Carrageenan injection.

Regarding percentage inhibition of edema formation when compared to control group I, maximum inhibition was revealed by group III (50.72%) followed by group II (41.74%), which was lesser than standard drug group IV (55.65%) as displayed in Figure-2.

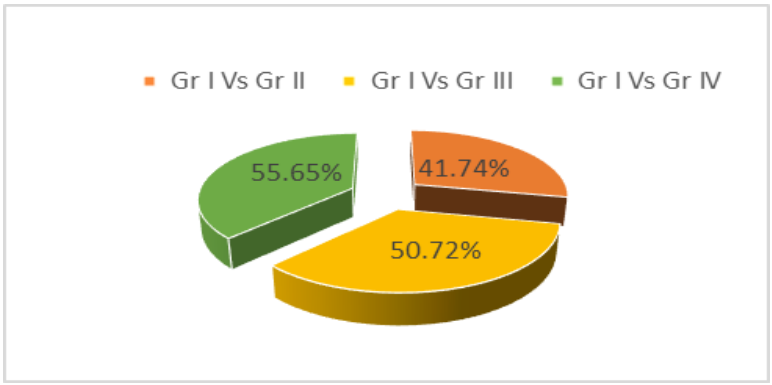

Fig. 2: Pie chart showing percentage inhibition of edema formation of different groups in comparison to control.

Effects of Methanolic extract of *Tamarindus indica* seeds and Indomethacin on cotton pellet induced granuloma

Table-2 and Figure-3 reveals that Group II (58.76±0.36) and Group III (53.86±0.36) showed minimum final weight of pellet after 15 days compared to control group I (82.30±1.01). But this finding was not less than standard drug group IV (49.87±0.30).

Mean increase in cotton pellet weight in Group II (28.59±0.36) and Group III (23.74±0.56) was statistically significant (P<0.05) compared to control group I. Group IV showed highly significant (P<0.001) increase.

Table 2: Effects of Methanolic extract of Tamarindus indica seeds and indomethacin on cotton pellet induced granuloma.

Groups	Number of rats	Initial weight of cotton pellet (mean ± SEM)	Final weight of cotton pellet (mean ± SEM)	Increase in weight of cotton pellet (mean ± SEM)
Group I	7	30.08 ± 0.02	82.30 ± 1.01	52.22 ± 1.02
Group II	7	30.17 ± 0.03	58.76 ± 0.36	28.59 ± 0.36*
Group III	7	30.12 ± 0.02	53.86 ± 0.36	23.74 ± 0.56*
Group IV	7	30.05 ± 0.02	49.87 ± 0.30	19.82 ± 0.43**

* Group II, III vs Group I; ** Group IV vs Group I

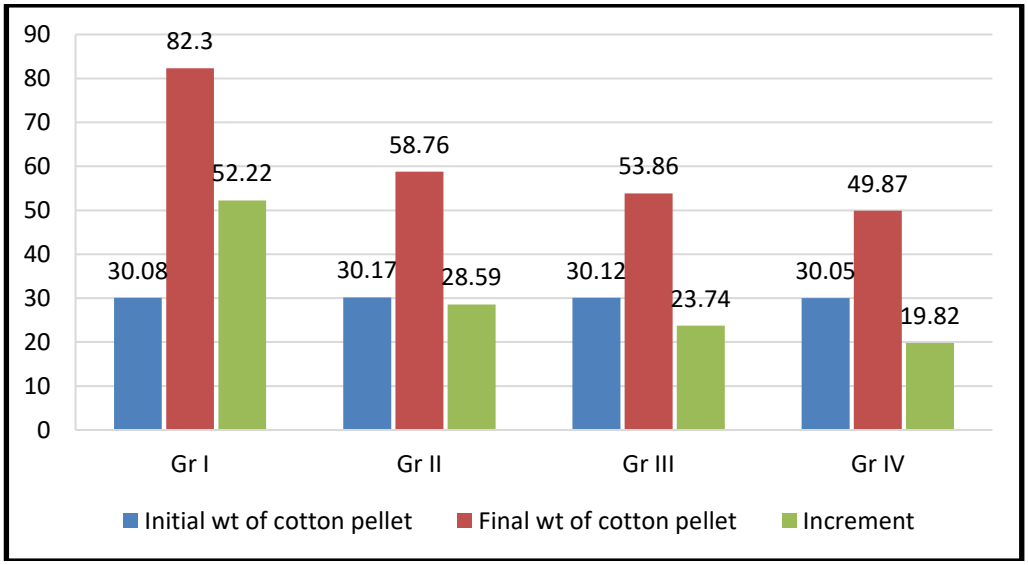

Fig. 3: Bar diagram showing comparative initial, final and increment of weight of cotton pellet.

Figure-4 displays percentage of granuloma formation where, among both experimental groups Group III (54.54%) showed higher percentage of inhibition of granuloma formation than Group II (45.25%), when compared with control group I. But this finding was lesser than percentage of inhibition by Group IV (62.05%) in comparison.

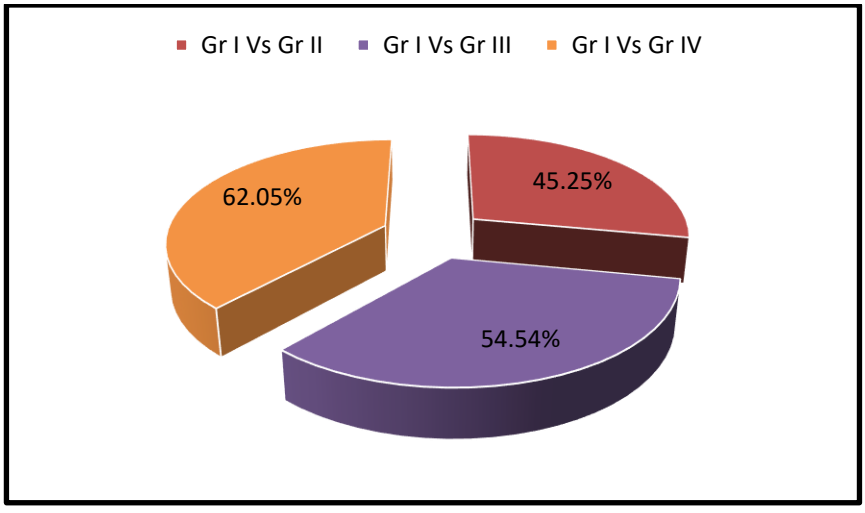

Fig. 4: Pie chart showing percentage inhibition of granuloma formation of different groups in comparison to control.

Discussion

The present study was carried out to evaluate the anti-inflammatory effect of Tamarindus indica seeds. Administration of Methanolic extract of Tamarindus indica seed a dose of 300mg/kg body weight and 600/mg/kg body weight orally produced a significant ($P<0.05$ and $P<0.001$ respectively) anti-inflammatory effect, where the percentage of inhibition of edema formation was 41.74% and 50.72%, respectively, showing a dose dependent effect. Following administration of Indomethacin, the anti-inflammatory effects were highly significant ($P<0.001$) and the percentage of inhibition of edema formation were 55.65%. These findings were similar with the work of other researchers.[16,17]

The development of edema in the paw of the rat after the injection of carrageenan is due to the release of histamine, serotonin and prostaglandins like substances. According to Deepti Bandawane et al,[15] the carrageenan induced paw edema is a biphasic event. The first phase occurs within an hour and is partly due to the trauma of injection and also attributed to the release of histamine, serotonin and kynins. The second phase is sensitive to most clinically effective anti-inflammatory drugs. The second phase of edema is due to the release of prostaglandins play a major role in the development of second phase of reaction, which is measured, after three hours of inflammation induction.

Granuloma represents both the exudative and prostaglandin phase of inflammation. Proliferation of the fibroblast and formation of new blood vessels is one of the main features observed during the formation and maturation of granulation tissue. Granuloma thus represents intermingling of healing and inflammation to form a mass composed of inflammatory cells, area of granulation tissue and fibrous tissue.[18]

The healing process is modulated by a finely tuned interaction between inhibitory and growth factors. Cotton pellet granuloma method has been widely employed to assess the transudative, exudative and proliferative component of chronic inflammation. The inflammatory granuloma is typical, the repair phase of inflammation starts with proliferation of fibroblast and multiplication of small blood vessels. These proliferation cells penetrate the exudates producing a highly vascularized, red mass known as granulation tissue. The fluid absorbed by the pellet greatly influences the wet weight of the granuloma. Dry weight of the cotton pellet correlates with amount of granulation tissue formation.[15]

Treatment with Methanolic extract of Tamarindus indica seeds at doses of 300mg/kg body weight orally daily for 14 days produced significant ($P<0.05$) anti-inflammatory effect and at doses of 600mg/kg body weight orally daily for 14 days produced significant ($P<0.05$) anti-inflammatory effect and the percentage of inhibition of granuloma formation were 45.25% and 54.54% respectively. Following administration of Indomethacin for 14 days revealed highly significant ($P<0.001$) anti-inflammatory effect and the percentage of inhibition of granuloma formation was 62.05%. These were analogous with the work of Deepti Bandawane et al and MG Hivrale et al.[15,19]

In the cotton pellet granuloma model, inflammation and granuloma develops during the period of several days. This model is an indication for the proliferative phase of inflammation. Inflammation involves proliferation of macrophages, neutrophils and fibroblasts, which are basic sources of granuloma formation. Hence, the decrease in the weight of granuloma indicates that the proliferative phase was effectively suppresses by the Methanolic extract of Tamarindus indica seeds.

In this study, concomitant administration of Tamarindus indica seeds and non-steroidal anti-inflammatory drug daily for 14 days reduce weight of granulation tissue and 01 hour before Carrageenan injection reduced rat paw edema. The reduction was statistically significant in comparison to control group. But the reduction of rat paw edema and weight of granulation tissue in case of non-steroidal anti-inflammatory drugs were highly significant in comparison to Methanolic extract of Tamarindus indica seed.

The major limitation of most of the NSAIDs is the ulcerogenic activity which is due to inhibition of prostaglandin synthesis. The results of Methanolic induced ulceration in rats suggests that METI has no such side effects and can hence prove to be comparatively more effective than present synthetic preparation.

The present study provides an initial step on demonstrating the anti-inflammatory effect of methanolic extract of Tamarindus indica seed in anti-inflammatory state. The obtained data support the basis for future use of Tamarindus indica in traditional system of medicine. Thus, it could be a new agent in reducing morbidity and mortality resulting from inflammatory disease condition. The findings presented here provide a baseline for future studies designed to quantify the effects of Methanolic extract of Tamarindus indica seed.

Conclusion

Tamarindus indica seeds extract, possess significant anti-inflammatory activity in rats, where the possible mechanism of anti-inflammatory activity of polyamines may be due to their impairment of release or formation of inflammatory mediators such as histamine, 5-HT, PGs and lysosomal membrane stabilization. Studies on polyamines may be helpful in developing a new approach for better understanding of the inflammatory process and generation of new anti-inflammatory drugs. Further studies involving purification of chemical constituents of the plant and investigations in biochemical pathways may result in the development of a potent anti-inflammatory agent with low toxicity and better therapeutic index.

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