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VARIED ANTI- INFLAMMATORY ACTIVITY OF INDOMETHACIN IN DIFFERENT EXPERIMENTAL ANIMAL MODELS

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ABSTRACT

OBJECTIVE: To evaluate the anti-inflammatory activity of indomethacin in different experimental anti-inflammatory animal models

METHODS: Swiss albino rats weighing around 150 g – 250 g of either sex were randomly selected and divided into 6 groups (n=6) for 3 models, each having control and Standard .

Materials used are Carrageenan, turpentine, Cotton pellets, Mercury and water plethysmographs. Methods adopted in this study are; (1) Carrageenan induced rat-paw edema (paw volume measured through mercury and water plethysmographs), (2) turpentine induced arthritis (by measuring lateral diameter of knee joint), as models of acute inflammation, (3) Cotton pellet induced granuloma (measured through dry weight of granulation tissue) as model of sub-acute inflammation.

RESULTS: The following observations were made. Indomethacin exhibited potent anti-inflammatory activity in Carrageenan induced edema model (83.34% inhibition in mercury plethysmograph) compared to control. But its anti-inflammatory activity is of lower order both in turpentine induced arthritis & cotton pellet granuloma model (48% & 40.16% inhibition respectively). All the results obtained were statistically significant with P< 0.001 in all four investigated methods

CONCLUSION: In the light of the above mentioned observations even an established anti-inflammatory agent (Indomethacin) has not shown considerably equal anti-inflammatory activity against all conventional models of experimental inflammation. So, a drug capable of inhibiting only one particular model of experimental inflammation might still be an effective anti-inflammatory agent. Hence, it may be suggested that any new drug to be evaluated for anti-inflammatory activity must be screened for more than 2-3 conventional models of inflammation.

INTRODUCTION: Pain and inflammation are disabling accompaniments of many medical illnesses. So, control of both pain and inflammation assumes top priority for the physician to reduce morbidity & mortality. Inflammation is a local response of living mammalian tissues to the injury. It is a body defence reaction in order to eliminate or limit the spread of injurious agents. There are various components of an inflammatory reaction that can contribute to the associated symptoms and tissue injury like oedema formation, leukocyte infiltration and granuloma formation [1].

Acute and chronic inflammatory diseases are still one of the most important health problems in the world like trauma, infective arthritis, acute gout and Rheumatoid arthritis, Osteoarthritis respectively. Although there are several selective & non selective drugs avilable to treat pain and inflammation in above mentioned disorders, most common agents used are the NSAID's.

But there is continuous search for newer NSAIDs with less adverse effects as the existing drugs are very efficient in controlling the inflammatory reaction but associated with many adverse effects.

Equally there are many animal experimental models of inflammation to screen the potential drug for its anti-inflammatory activity . Various models for acute and subacute inflammation being turpentine induced arthritis, ultraviolet erythema in Guinea Pigs, oxazolone induced ear edema in Mice, croton oil ear edema in Rats and Mice etc.,[2] and models for chronic inflammation include cotton pellet induced granuloma, carrageenan induced pouch model etc., [2] Each experimental model is associated with particular autocoid/s to mediate development of experimental Inflammation.

Carrageenan induced paw edema is widely used for determining the acute phase of inflammation[3]. Edema formation in the paw is the result of synergism between various inflammatory mediators that increase vascular permeability and/or the mediators that increase blood flow [4].

The initial phase results in the release of histamine and serotonin causing vasodialation and increased permeability of capillaries; whereas the release of bradykinin, prostaglandins, protease and lysosomal enzymes which regulate the process of adhesion of molecules is attributed to the second phase [5, 6, 7, 8]. Subcutaneous injection of carrageenan into the rat paw produces accumulation of plasma and fluid and plasma protein exudation also takes place along with neutrophil extravasations [9].

The early phase of inflammation begins immediately after carrageenan injection and extends up to 6 h whereas the late phase remains up to 24 h.

In turpentine oil induced joint edema, there are some sequential release of mediators i.e. histamine and serotonin in the early phase, kinin like substances in the intermediate phase and prostaglandin in the late phase [10]. Cotton pellet granuloma model is indicative of proliferative phase of inflammation involving macrophages, neutrophil, fibroblast cells and collagen formation [8].

Unfortunately a promising drug under evaluation cannot be screened against all available models of Experimental Inflammation and also a promising drug/ herbal product with anti-inflammatory activity can be easily , quickly & economically screened with the help of these animal experimental models of inflammation before these drugs are subjected for characterization, mechanism of action (molecular, cellular, markers etc.), which are fairly expensive.

The present study have been undertaken to study the anti-inflammatory activity of the commonly used established NSAID Indomethacin in various acute and chronic models of inflammation for it's anti-inflammatory activity which is an established drug in treatment of many acute & chronic inflammatory conditions like ankylosing spondylitis, rheumatoid arthritis, gouty arthritis,etc.

Following are the established animal anti-inflammatory models that are used in the present study- Carrageenan induced rat paw edema, Turpentine induced arthritis and Cotton pellet induced Granuloma animal models in albino rats.

MATERIALS AND METHODS:

Drugs and Chemicals: Indomethacin 10mg/kg BW (Sigma), carrageenan (Sigma) and all other chemicals were of analytical grade.

Animals: Adult albino rats of either sex weighing between 200 to 250 gms were randomly selected from central animal facility, JSS Medical College, Mysore. Animals were housed at an ambient temperature of 25 ± 1^{o} C with ad libitum access to food and water. The care and maintenance of the animals were as per the approved guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) in India. The study protocol was approved by Institutional Animal Ethical Committee.

Grouping of animals: The animals were subjected to the following experiments:

a) Carrageenan induced-rat paw oedema,

b) Turpentine induced- arthritis and

c) Cotton pellet induced- granuloma models.

Animals were divided into two groups for each model (control and test) consisting of 6 animals in each group.

A. Carrageenan induced- rat paw oedema model: The method used herein comprised of the study of inflammatory reaction induced by phlogistic agent, carrageenin injected into the subplantar surface of the right hind paw of each rat according to the method of Winter et. al 1962[11] with some modifications. The appliance used in this study for recording the paw oedema was Mercury plethysmograph. The rats were pre-treated with the indomethacin 1 hour before injection of Carrageenan (0.1ml of 1%). The paw volume was measured at zero hour and at the end of four hours in millimeters, by dislocation of the mercury column using Mercury Plethysmograph [11]. The edema was calculated by substracting the zero-hour reading from the four-hour reading. From the mean edema volume, the percentage inhibition of the edema was calculated between the treated and control groups.

Percent inhibition of edema = 100 (1- Vt / Vc)

Where, Vc and Vt represent the average paw edema volume in the control and drug treated groups, respectively.

B. **Turpentine induced -arthritis model in rats:** The method of Hanson JM *et al.*, 1974 with some modification was adopted here to study the acute inflammatory reaction induced by turpentine oil injected into the knee joint cavity of rats. All the rats were fed orally with the indomethacin one hour prior to the injection of turpentine oil. Joint inflammation was induced by injection of 0.01 ml of turpentine oil into the synovial cavity of the right knee joint. Knee joint lateral diameter was measured by using screw gauge immediately after the injection of turpentine oil into the knee joint cavity (0 hour reading) and after four hours using the screw gauge. The mean increase in joint diameter in the control group was compared with that in the drug treated groups.

Percent anti-arthritic activity= 100(1- Dt/Dc)

Where, Dc and Dt represent the mean lateral diameter of knee joint in the control and drug treated groups respectively.

C. **Cotton wool pellet granuloma in rats :** The method of Meir R et al, 1950 [12]with some modification was adopted here to study the chronic inflammatory reaction induced by subcutaneous implantation of 10mg cotton pellets into both axilla and groins of all the rats. All the rats were fed orally with indomethacin 1hr prior to the cotton pellet implantation on the first day, and later on, once daily for six consecutive days.

The rats were anaesthetized lightly with ether and small linear incisions of about 1 cm were made in each axilla and groin under aseptic precautions. The sterile cotton wool pellets weighing 10mg each were implanted in these areas (4 pellets in each rat) and the wounds were sutured with black silk thread with aseptic precautions [13, 14]. The animals were maintained in clean cages and the food and water were allowed throughout the period of experimentation.

Later the animals were sacrificed on the 8th day and the cotton pellets with granulation tissue were removed, cleaned of the extraneous tissue and dried in a hot air oven to a constant weight. The dry weight of the granuloma (i.e. the amount of actual granulation tissue formed) was calculated by noting the difference in the dry weight of the cotton pellets recorded before and after implantation.

Percent anti-granuloma activity = 100(1-Wt/Wc)

Where, Wc and Wt represent the mean dry weight of granuloma in the control and drug treated groups, respectively.

Statistical Analysis: Results were expressed as mean ± Standard deviation (SD). Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Scheffe's post hoc test.

The level of statistically significant difference was defined as P<0.001. All the statistical methods were carried out through the SPSS for Windows (version 16).

RESULTS:

Carrageenan induced- rat paw oedema: The anti-inflammatory activity of indomethacin against carrageenan induced Rat paw edema model is shown in table-1. The inhibition of paw oedema by indomethacin (10 mg/kg) was statistically significant (P < 0.001) when compared to the control group. The percentage inhibition of edema by indomethacin was 83.34% compared to control (**Table 1 and Figure 1**).

Turpentine induced arthritis: The anti-arthritic activity of indomethacin (10 mg/kg) against turpentine induced arthritis is shown in table-2 and was statistically significant (P< 0.001) when compared to the control group. The percentage inhibition of arthritis by indomethacin was 48% compared to control (**Table 2 and Figure 2**).

Cotton pellet induced granuloma: The anti-granuloma activity of indomethacin (10 mg/kg) against cotton pellet induced granuloma is shown in table-3 and was statistically significant (P < 0.001) when compared to the control group. The percentage inhibition of granulation tissue by indomethacin was 40.16% (**Table 3 and Figure 3**).

TABLE 1: SHOWING THE MEAN RAT PAW VOLUME (CM) AT 0HR AND 4HR AND PERCENTAGE INHIBITION IN DIFFERENT GROUPS IN CARRAGEENAN INDUCED RAT PAW EDEMA METHOD

Groups	n	Mean Paw Edema (cms) +/- SD			% INHIBITION	% EDEMA
		0 hr	4 hr	diff		
Control	6	11.7+/- 1.457	115.0+/-4.485	103.3	0%	100%
Indomethacin (10mg/kg)	6	11.43+/-1.655	28.63+/-1.783	17.2	83.34%	16.66%

TABLE 2: SHOWING THE MEAN LATERAL KNEE DIAMETER (MM) AT 0 HR AND 4 HR AND PERCENTAGE INHIBITION IN DIFFERENT GROUPS IN TURPENTINE INDUCED ARTHRITIS MODEL

Groups	n	Lateral diameter of knee joint (mm)+/- SD			% INHIBITION	% ARTHRITIS
		0 hr	4 hr	diff		
Control	6	7.8+/-0.4733	10.38+/0.6432	2.5	0%	100%
Indomethacin 10mg/kg	6	8.65+/-0.695	9.883+/-2.043	1.3	48%	52%

TABLE 3: TABLE SHOWING THE MEAN DRY GRANULOMA WEIGHT (MG) AND PERCENTAGE INHIBITION OF DIFFERENT GROUPS IN COTTON PELLET INDUCED GRANULOMA MODEL

Groups	Dry granuloma weight initial (mg)	Dry granuloma weight final (mg)	Mean dry Granuloma Weight (mg) +/- SE	% INHIBITION	% GRANULOMA
Control	37.45	87.28	49.83+/-4.535	0%	100%
Indomethacin	38.6	68.4	29.8+/-4.070	40.16%	59.84%

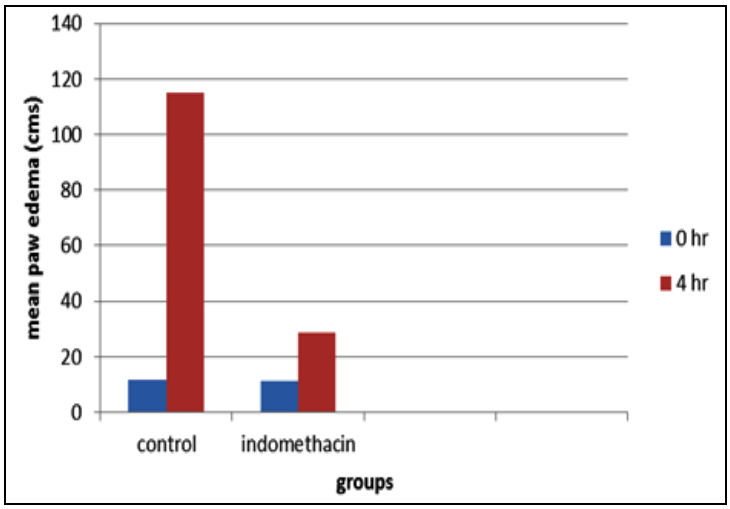

FIGURE 1: SHOWING THE MEAN PAW EDEMA (CM) IN DIFFERENT ANIMAL GROUPS IN CARRAGEENAN INDUCED PAW EDEMA MODEL

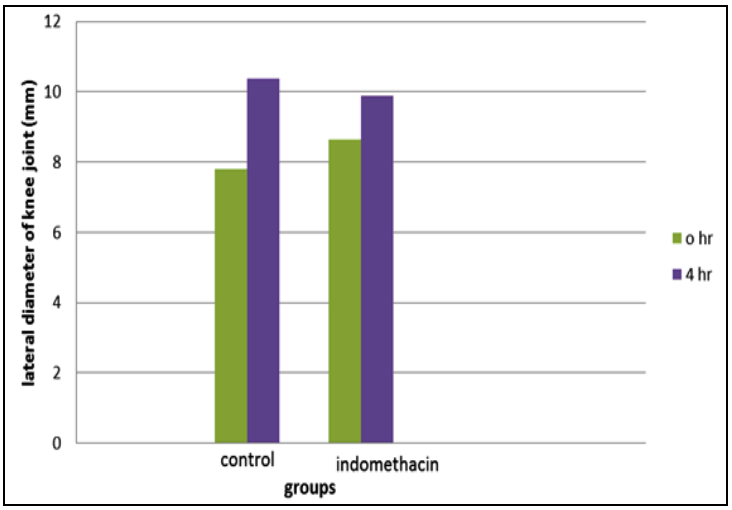

FIGURE 2 :SHOWING THE MEAN LATERAL KNEE DIAMETER (MM) AT 0HR AND 4HR BY DIFFERENT GROUPS IN TURPENTINE INDUCED ARTHRITIS MODEL

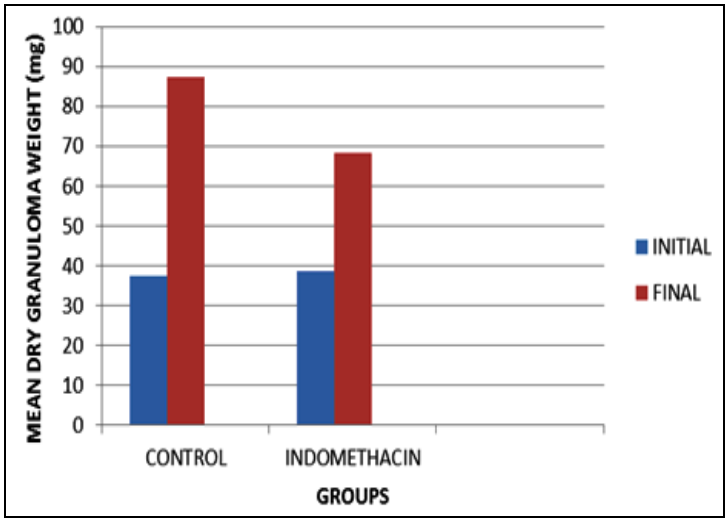

FIGURE 3: SHOWING MEAN DRY GRANULOMA WEIGHT (MG) OF DIFFERENT GROUPS IN COTTON PELLET INDUCED GRANULOMA MODEL

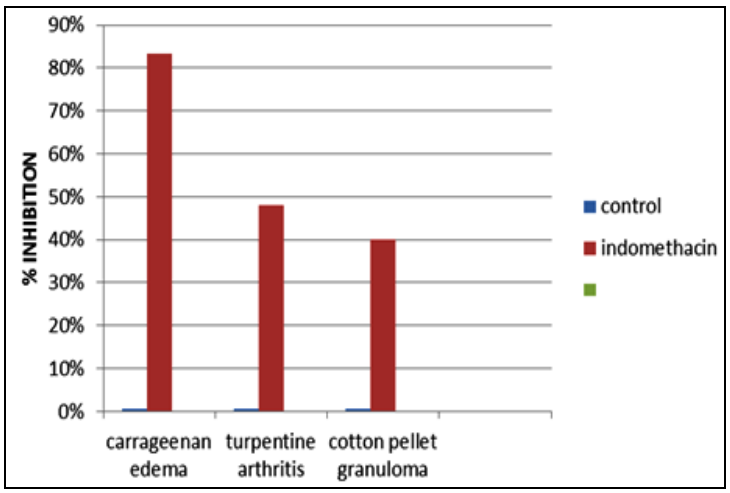

FIGURE 4: SHOWING COMPARISON BETWEEN CONTROL AND INDOMETHACIN GROUPS IN DIFFERENT ANTI-INFLAMMATORY MODELS

DISCUSSION: Inflammation is the integral part of body's defence mechanism. Acute inflammation is characterized by vasodilatation, exudation of plasma, release of various inflammatory mediators, cytokines, growth factors and emigration of leukocytes. While the features of chronic inflammation includes infiltration of mononuclear cells, proliferation of fibroblasts, blood vessels and increased connective tissue formation. Anti-inflammatory drugs inhibit different stages of inflammation [15].

Indomethacin being a nonselective inhibitor of cyclooxygenase (COX) 1 and 2, enzymes that participate in prostaglandin synthesis from arachidonic acid. Prostaglandins are hormone-like molecules normally found in the body, where they have a wide variety of effects, some of which lead to pain, fever, and inflammation. Prostaglandins also cause uterine contractions in pregnant women. Indomethacin is an effective tocolytic agent, able to delay premature labour by reducing uterine contractions through inhibition of PG synthesis in the uterus and possibly through calcium channel blockade.

Indomethacin has two additional modes of actions with clinical importance:

- It inhibits motility of polymorphonuclear leukocytes, similar to colchicine.
- It uncouples oxidative phosphorylation in cartilagenous (and hepatic) mitochondria, like salicylates.

These additional effects also account for its analgesic and the anti-inflammatory properties.

Carrageenan is regarded as an established phlogistic agent and edema induced by subplantar injection of Carrageenan in the rat hind paw is reported to have been inhibited by a number of steroidal and non-steroidal anti-inflammatory drugs [1].

In our study, it is shown that Indomethacin significantly reduced edema induced by Carrageenan. The percentage inhibition of paw edema being 83.34% compared to control. The mechanism of acute anti-inflammatory activity being inhibition of release of mediators like prostaglandins (prostaglandins being one of the mediators responsible for mediating inflammation induced by Carrageenan).

Effect of Indomethacin against turpentine oil-induced joint edema in rats showed that it inhibited joint edema gradually at 1, 2, 3 and 4 hr after treatment compared to control , the percentage of inhibition being 48%. As there are some sequential release of the mediators in turpentine oil-induced joint edema apart from prostaglandins like histamine and serotonin, kinin like substances, indomethacin has shown lesser percentage of inhibition than that of the Carrageenan induced edema model.

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 [16].

Hence, the decrease in the weight of granuloma indicates that the proliferative phase was effectively suppressed by Indomethacin the percentage of inhibition being 40.16%. This model is a widely used model for chronic inflammation which occurs by means of development of proliferated cells in the form of granuloma. Indomethacin inhibits the granuloma formation by preventing granulocyte infiltration, generation of collagen fibres, fibroblasts and suppressing mucopolysaccharides [8].

Hence the study demonstrated that Indomethacin has not shown equal anti-inflammatory activity against all the experimental models of inflammation. The percentage of inhibition in Carrageenan induced rat paw edema being 83.34%, it is 48% in turpentine induced arthritis model and 40.16% in cotton pellet induced granuloma model, thus showing that the anti-inflammatory activity is different in different models of inflammation.

CONCLUSION: In the light of the above mentioned observations, even an established anti-inflammatory agent (Indomethacin) has not shown considerably equal anti-inflammatory activity against all conventional models of experimental inflammation. So, a drug capable of inhibiting only one particular model of experimental inflammation might still be an effective anti-inflammatory agent.

Hence, it may be suggested that any new drug to be evaluated for anti-inflammatory activity must be screened for more than 2-3 conventional models of inflammation and even if a drug shows minimal anti-inflammatory activity in any one of the models of experimental inflammation, it can still be very effective antiinflammatory drugs.

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