Journal of Cardiovascular Disease Research

ISSN: 0975-3583,0976-2833 VOL13, ISSUE 10, 2022

Anti-Inflammatory activity of Cetirizine in Albino rats: An experimental study

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Received Date: 22/09/2022 Acceptance Date: 10/11/2022

Abstract

Background: Cetirizine may have anti-inflammatory activity due to its different action relating to mediators of inflammation, such as inhibition of histamine release, LTB4, Macrophage migratory inhibitory factor, kinin and interleukin. Aim of the present study is to test the hypothesis that cetirizine prevent the development of inflammation through inhibition of the inflammatory processes including proliferation of inflammatory cells. **Methods:** Albino Wistar rats of either sex weighing 150-250 grams, 4 groups consisting of 6 animals per group were used. Group I: Control: 1% Gum acacia. 2ml/kg, Group II: Standard drug: Diclofenac sodium 4.5mg/kg; Group III: Test Drug 1: Cetirizine Dihydrochloride 900 ug/kg; Group IV: Test Drugs 2: Cetirizine Dihydrochloride 900ug/kg + Diclofenac sodium 4.5mg/kg. Drugs were administered orally. For acute and chronic anti-inflammatory activity, Carrageenan induced rat paw oedema method and Rexin pellet induced granuloma method has been used. **Results:** Cetirizine Dihydrochloride, is found to have significant anti-inflammatory activity in rats (900 ug/kg dose) alone and in combination with Diclofenac Sodium. Cetirizine (0.201) per se and in combination (0.203) inhibit the increase in paw volume (ml) significantly ($p<0.001$) as compared to control (0.341). Cetirizine was 22.22% whereas percentage inhibition in combination with Diclofenac Sodium was 45.74%. Cetirizine (5.23) has shown mean gain in dry granuloma weight (mg) alone and in combination with Diclofenac Sodium (5.12) significantly lower ($p<0.001$) as compared to control (9.54). The percent inhibition of rexin pellet induced dry granulation tissue weight by Cetirizine was 45.17% and Cetirizine in combination with Diclofenac Sodium has shown 46.33%. Mean total mast cells count, inflammatory cell counts and extent of granulation tissue formation has been found to be significantly low in cetirizine group alone and in combination with diclofenac sodium. **Conclusion:** Cetirizine Dihydrochloride possess acute and chronic anti-inflammatory activity alone and in combination with Diclofenac sodium.

Keywords: Antihistaminic drugs, Diclofenac Sodium, Inflammatory Cell count, Mast cell count

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Introduction

Inflammation is defined as a series of molecular and cellular responses acquired during evolution, designed to eliminate foreign agents and promote repair of damaged tissues.[1] According to Celsus, the characteristics of inflammation are Rubor (redness), Tumor

ISSN: 0975-3583,0976-2833 VOL13, ISSUE 10, 2022

(swelling), Calor (heat), Dolor (pain), and Functio laesa (loss of function).[2] Various mediators of inflammation are histamine, bradykinins, serotonin, leukotrienes, and prostaglandins.[3] Histamine plays a major role in inflammation by increasing vascular permeability, vasodilatation, and chemotaxis.[4]

Acute inflammation is a short-term response that usually results in healing leukocytes infiltrate the damaged region, removing the stimulus, and repairing the tissue. Chronic inflammation, by contrast, is a prolonged, dysregulated, and maladaptive response that involves active inflammation, tissue destruction, and attempts at tissue repair. Such persistent inflammation is associated with many chronic human conditions and diseases, including allergy, atherosclerosis, cancer, arthritis and autoimmune diseases.[5] Various mediators of inflammation are histamine, bradykinins, serotonin, leukotrienes, and prostaglandins.[6]

The current management of inflammation involves use of nonsteroidal anti-inflammatory agents. But long-term use of these drugs are associated with serious adverse effects like gastro-intestinal damage, nephrotoxicity, hypertension, and thrombosis.[7] Hence, the search for a new, safe anti-inflammatory drug is still going on.

Histamine, being a potential target, plays a major role in inflammation by increasing vascular permeability, vasodilatation, and chemotaxis.[4] The release of histamine from mast cells during antigen antibody reactions is well known, as is its involvement in the inflammatory response to skin injury.[8] However, the role of histamine in acute inflammation is associated with mast cell degranulation in non-rodent species including man, where as its role in chronic inflammation is yet to be established.

Anti-histaminics may play a role in decreasing the inflammation. Very few studies have evaluated anti-inflammatory activities of anti-histaminics. These studies have shown that antihistaminics inhibit multiple inflammatory mediators like histamine, interleukins, and prostaglandin involved in chemotaxis and adhesion.[4]

Differences exist in the pharmacology of individual second-generation antihistamines, and possibly, in their individual ability to suppress pro-inflammatory mediators associated with an unfolding allergic response. Some anti-inflammatory effects of antihistamines seem to require initial interaction with the histamine receptor, while, others are receptor-independent. Further studies are needed to determine whether these differences in anti-inflammatory pharmacology translate into clinically meaningful effects.

Cetirizine which is a second generation antihistaminic drug, having good pharmacokinetic properties with better safety profile.[9] However, there is not much information regarding its anti-inflammatory activity. Considering this, the present study has been undertaken to evaluate the anti-inflammatory activity of cetirizine on experimental animal models. In this study an earnest attempt is made to explore its Anti-inflammatory activity.

Hence in the light of the development cited, an attempt has been made to study the anti-inflammatory activity of Cetirizine in experimental animal models of inflammation individually and in combination with the standard diclofenac sodium.

Materials

Animals

Albino Wistar rats, of either sex, weighing 150-250 grams were used. Study was conducted after approval from the Institutional Animal Ethics Committee, the body approved by CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals). The rats were grouped in separate polypropylene cages on husk bedding with six animals in each group. Animals were fed with standard pellet diet and water ad libitum. The animals were allowed to adjust to the laboratory conditions such as light, temperature, and noise before being subjected to the experiment (acclimatization). They were kept under

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standard conditions in a colony room at ambient temperature of 25±2°C with help of air coolers and enough humidity on a 12 hour light – dark cycle. They had free access to food and water. Study was conducted during the day time (between 10.00 to 18.00 hrs).

Drugs and Chemicals

Drugs

Drugs (Cetirizine Dihydrochloride & Diclofenac Sodium) were obtained from Sigma Aldrich, Aurangabad, India. Chemicals (carrageenan & diethyl ether) were obtained from Ozone Laboratory Chemicals, Mumbai, India and Qualigens Scientific sales and services, Latur, India. Drugs were obtained from manufacturers in pure powder form. Chemicals were of analytical grade. All the drugs were dissolved in 1% Gum Acacia. Fresh solutions were prepared half an hour before the experiment. All the drugs were administered per oral.

Instruments

Digital Plethysmometer: Dolphin, Mumbai

Study Design

Grouping of animals

For each experiment 4 groups consisting of 6 animals per group were used. They were adapted to the study condition for 10 days.

Group I: Control: 1% Gum acacia (2ml/kg) [4, 10]

Group II: Standard drug: Diclofenac sodium (4.5mg/kg) [4]

Group III: Test Drug: Cetirizine Dihydrochloride (900ug/kg) [4]

Group IV: Cetirizine Dihydrochloride (900ug/kg) + Diclofenac sodium (4.5mg/kg)

The doses of the drugs under study was calculated by extrapolating human into animal dose. [4]

METHODS

The animals were subjected to the following experiments:

Acute Anti-inflammatory method

Carrageenan induced-rat paw oedema[4,7]

The method used herein is comprised of the study of inflammatory reaction induced by phlogistic agent, carrageenan injected into the sub-plantar surface of the either right/left hind paw of each rat according to the method of Winter et. al 1962 [11] with some modifications.[12] The instrument used in this study for recording the paw oedema was Digital Plethysmometer (Figure 1A). Swiss albino rats weighing between 150-250 grams were used for evaluation of anti-inflammatory activity; 4 groups consisting of 6 animals were formed to carry out the experiment. Food was withdrawn 12 hours prior to drug administration till completion of experiment. Left paw was marked with ink at the level of lateral malleolus; basal paw volume was measured plethysmographically by volume displacement method using Digital Plethysmometer by immersing the paw till the level of lateral malleolus.[13] All the drugs were administered orally. After one hour 0.1ml of 1% carrageenan (1% in 0.9% Normal Saline solution) was injected into sub-plantar region of the hind paw of the rat. (Figure 1B)

The paw volume was measured plethysmometrically just before 1% carrageenan injection, that is, at "0" h and then at 1st, 2nd, 3rd, and 4th hr after carrageenan injection.[14] Same procedure was adopted for rats in all the groups.

The percent inhibition of oedema in animals of all groups was calculated by using the formula.

Percent inhibition = $\frac{V_c - V_t}{V_c} \times 100$

Where, Vc = Volume of paw oedema in control animals

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Vt = Volume of paw oedema in drug treated animals.

Chronic Anti-inflammatory Method

Rexin pellet granuloma method [4,15,16.]

This model is based on the foreign body granuloma that can be provoked by subcutaneous implantation of rexin pellets in rats. Rexin sheet has been used in this experimental model and discs of equal size were pinched out from rexin sheet. Two such discs were stitched together with thread, like their rough surface exposed outside and rexin covered surfaces facing each other. Rexin pellets were weighed (20 mg) and sterilized using 70% ethyl alcohol. Swiss albino rats weighing between 150-250 grams were used, six animals in each group were weighed and numbered appropriately.

Food was withdrawn 12 hours prior to drug administration till completion of experiment. All the drugs were administered orally. All the rats were anaesthetized with diethyl ether. The axillary skin was shaved and alcohol was applied to maintain aseptic condition. One small incision of about 1 cm length was made at axillary area on both sides. A curved forceps was passed through incisions to make subcutaneous pouch around it and sterilized rexin pellets (weighing 20 mg) were implanted into each pouch.

On the day of pellets implantation, all the rats were treated with fixed dose of drugs (as mentioned above) once in every 24 hours for seven days. The animals were provided with free access to food and water. During seven days, the rats were observed for any behavioural changes. On the 8th day, the implanted pellets along with granulation tissue were removed under anaesthesia. All the pellets were cleaned separately, extraneous tissue removed, and dried by incubating in hot air oven at 60^0C for 24 hrs. (Figure 2A & 2B)

The pellets, thus, dried along with adherent granulation tissue were weighed and the weight of the granulation tissue formed was obtained by deducting the weight of rexin pellets before implantation. Then, the mean weight of granulation tissue for each group was calculated. The difference in weight of granulation tissue of control and drug treated group was determined and percent inhibition as a measure of anti-inflammatory activity was calculated by using the following formula.

Percent inhibition= $\frac{W_c - V_t}{W_c} \times 100$

Where, Wc = Weight of pellets in control group.

Wt = Weight of pellets in drug treated group.

Study of mast cell count[4]

The mast cell was identified as a mesenchymal cell which is stained metachromatically with some blue dyes and it was recognized several years later that these cells contained in their granules the majority of the body's histamine. Histamine is not only released when the body encounters a toxic substance, it is also released when mast cells detect injury. It causes nearby blood vessels to dilate allowing more blood to reach the site of the injury or infection.[17] It is one of the principal mediator of inflammation causing vasodilation and increases vascular permeability.

From all the 4 groups of rats, subcutaneous areolar tissue near the implanted pellet was removed carefully along with surrounding granulation tissue.

Mast Cell Count In Histopathology of Granulation Tissue

This tissue was processed to make histopathology slide. Slides were fixed in 10% formalin. Toluidine Blue working solution (50ml) was made fresh by mixing Toluidine Blue (5ml) (1.0 gm + 70% alcohol 100.0 ml) with 1% Sodium Chloride (45ml). Then, the slides were deparaffinised and hydrated with distilled water. After that working Toluidine blue were used to stain for 1-2 minutes. After rinsing with water slides were dehydrated quickly through 95%

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and absolute alcohols. The microscopic study of subcutaneous granulation collected from these animals of different groups were undertaken to find out the number of mast cells in 40x high power fields at random (Figure 2C). Decrease in the mast cell count was considered as a measure of anti-inflammatory activity.

Cytology of Granulation tissue

From the control and treated groups of rats subcutaneous areolar tissue near the implanted pellet was carefully removed and thinly spread on a clean slide avoiding over stretching. The spread was fixed for 2 minutes in absolute alcohol and stained for 1 min in 0.1% aqueous solution of toluidine blue. The microscopic study of subcutaneous spread collected from these animals of different groups is undertaken to find out the number of mast cells in 10x high power fields at random (Figure 2D).

Inflammatory Cells Count In
PAP Smear

This tissue was processed to make PAP smear and Neutrophils, Lymphocytes, Eosinophils, and Monocyte count was done (Figure 2E).

Extent Of Granulation Tissue In Histopathology (H & E) [18]

Tissue surrounding the rexin pellet was processed to make histopathology slide and was stained with H & E. Grading of granulation tissue was done on the basis of extent of granulation seen in the respective slide (Figure 3A-3D).

Statistical Analysis

Data was analysed by using Graph pad Prism software version 5 (Manufacturer details). How was data represented? Comparison between different groups was done by one way ANOVA followed by post-hoc analysis by Tukey's test. The 'p' value less than 0.05 was considered as statistically significant.

Results

At the end of 3 hours, we observed that Cetirizine (0.201) per se and in combination (0.203) was able to inhibit the increase in paw volume (ml) significantly ($p<0.001$), as compared to control (0.341). The percentage inhibition of carrageenan induced rat paw oedema by Diclofenac Sodium was 43.69%, while that of Cetirizine alone was 41.05%. However, the percentage inhibition in combination with Diclofenac Sodium was 40.46% (TABLE I).

Cetirizine (5.23mg) has shown mean gain in dry granuloma weight alone and in combination with Diclofenac Sodium (5.12mg) significantly lower ($p<0.001$) as compared to control (9.54mg). The percent inhibition of rexin pellet induced dry granulation tissue weight by diclofenac sodium compared with control was 50.41% and that of the Cetirizine was 45.17% and Cetirizine in combination with Diclofenac Sodium has shown 46.33% inhibition of granulation tissue (TABLE I).

Number of mast cells present in granuloma tissues in cetirizine group (7.00) has shown significantly ($p<0.05$) less compared to control group (12.90). Whereas both Diclofenac Sodium and Cetirizine when given in combination (5.90) mast cell count was very less ($p<0.01$) in number significantly compared to control group also lower than Diclofenac sodium (6.20). This states that Cetirizine might have increased the anti-inflammatory activity of diclofenac sodium, when given in combination (TABLE II).

Number of mast cells present in granuloma tissues in cetirizine group (5.00) has shown significantly ($p<0.05$) less compared to control group (9.00). Whereas both Diclofenac Sodium and Cetirizine when given in combination (4.00) mast cell count was very less ($p<0.01$) in number significantly compared to control group also lower than Diclofenac sodium (4.8). This states that Cetirizine might have increased anti-inflammatory activity of

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diclofenac sodium when given in combination. It indicates, there is significant decrease in all inflammatory cell counts in diclofenac group and its combination with cetirizine having grade I inflammation. Cetirizine group itself has shown grade II inflammation with significant decrease in inflammatory cells as compared to control group.(TABLE III)

To support these findings grading of granulation tissue has also been done in histopathology slides and it has been observed that cetirizine (grade III) has decreased the extent of granulation tissue as compared to control (grade IV). Similarly that of its combination with diclofenac sodium (grade II-III) has decreased granulation tissue as compared to control group. Diclofenac (grade II) decreased the granulation tissue significantly among all.(TABLE III)

Original Tables

Table I: Effect of different drugs on carrageenan induced rat paw oedema and rexin pellet induced granuloma.

Groups	**Dose of drugs**	**Mean Paw Volume (ml)**				**Weight in mg**	
		At 0 hours	**At the End of 3 hours**	**Mean difference at the end of 3 hours**	**% inhibition calculation at the end of 3 hours**	**Mean gain in dry granuloma weight**	**% inhibition of granulation tissue**
I Control Gum Acacia	1% 2 ml/kg	1.097±0.006	1.438±0.089	0.341±0.008	-	9.54+0.21	-
II Diclofenac Sodium	4.5 mg/kg	0.681±0.027	0.873±0.038	0.192±0.001***	43.69%	4.73+0.18***	50.41%
III Cetirizine	900 ug/kg	0.966±0.006	1.167±0.053	0.201±0.003***#	41.05%	5.23+0.09***#	45.17%
IV Cetirizine + Diclofenac Sodium	900 ug/kg + 4.5 mg/kg	0.663±0.022	0.866±0.043	0.203±0.002***#	40.46%	5.12+0.04***#	46.33%

[Values are expressed in Mean ± S.E.M. (Standard error of mean), n=6 in each group, df=5,30. *** p value < 0.001 as compared to control. # p>0.05 as compared with Diclofenac sodium]

Mean paw volume increase, is considered as a measure of inflammation and the ability to control this increase as compared to control suggests anti-inflammatory activity. Mean gain in dry granuloma weight is considered as a measure of inflammation and an ability to decrease this mean gain in dry granuloma weight as compared to control suggests anti-inflammatory activity.

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Table II: Effect of different drugs on Mast cell count in rexin pellet induced granuloma method by HISTOPATHOLOGY AND CYTOLOGY.

GROUPS		Mast cell Count in each field										
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**Mean**
I CONTROL 1% Gum Acacia 2 ml/kg	H	10	14	10	20	11	25	11	22	2	4	$12.90^{\pm}2.36$
	C	11	8	10	6	8	8	6	9	12	12	9.0
II DICLOFENAC SODIUM 4.5 mg/kg	H	10	9	5	6	7	4	8	8	6	11	$6.20^{\pm}0.55$**
	C	10	4	8	2	2	6	4	2	6	4	4.8**
III CETIRIZINE 900 ug/kg	H	3	1	5	4	8	1	5	6	9	8	$7.00^{\pm}0.63$*#
	C	12	2	8	4	2	6	4	2	6	4	5.0**#
IV CETIRIZINE 900 ug/kg + DICLOFENAC SODIUM 4.5 mg/kg	H	8	5	2	9	8	5	6	4	6	6	$5.90^{\pm}0.65$**#
	C	3	3	3	6	4	3	3	6	6	3	4.0***#

H: Histopathology C: Cytology

[Values are expressed in Mean ±S.E.M, n=6 in each group, df=5,13. *p value <0.05 as compared to control. **p value < 0.01 as compared to control. # p>0.05 as compared with Diclofenac sodium]

Mean total mast cells count is considered as a measure of inflammation and an ability to decrease this total mast cells count as compared to control suggests anti-inflammatory activity.

Table III: Effect of different drugs on inflammatory cells count in rexin pellet induced granuloma method in PAP smear and granulation tissue grades in histopathoogy

GROUPS	Mean Inflammatory cell Counts in rexin pellet induced method in PAP smear					Extent of granulation tissue in each group histopathology
	Neutrophils	**Lymphocytes**	**Monocytes**	**Giant cells**	**Grading**	**Grading**
I CONTROL 1% Gum Acacia	5.33	16.50	3.3	2.66	Grade III	Grade IV

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2 ml/kg						
II DICLOFENAC SODIUM 4.5 mg/kg	1.83**	5.00*	1.0**	0.83**	Grade I	Grade II
III CETIRIZINE 900 ug/kg	2.33**#	7.00*#	1.16*#	1.00*#	Grade II	Grade III
IV CETIRIZINE 900 ug/kg + DICLOFENAC SODIUM 4.5 mg/kg	2.16**#	1.16*#	0.83**#	1.00*#	Grade I	Grade II-III

[Values are expressed in Mean ±S.E.M, n=6 in each group, df=5,13. *p value <0.05 as compared to control. **p value < 0.01 as compared to control. # p>0.05 as compared with Diclofenac sodium]

Mean total Neutrophils, Lymphocyte, Monocytes, Giant cells count is considered as a measure of inflammation and an ability to decrease these cells count as compared to control suggests anti-inflammatory activity. The grading of granulation tissue formation has been measured by extent of granulation tissue formation in each group.

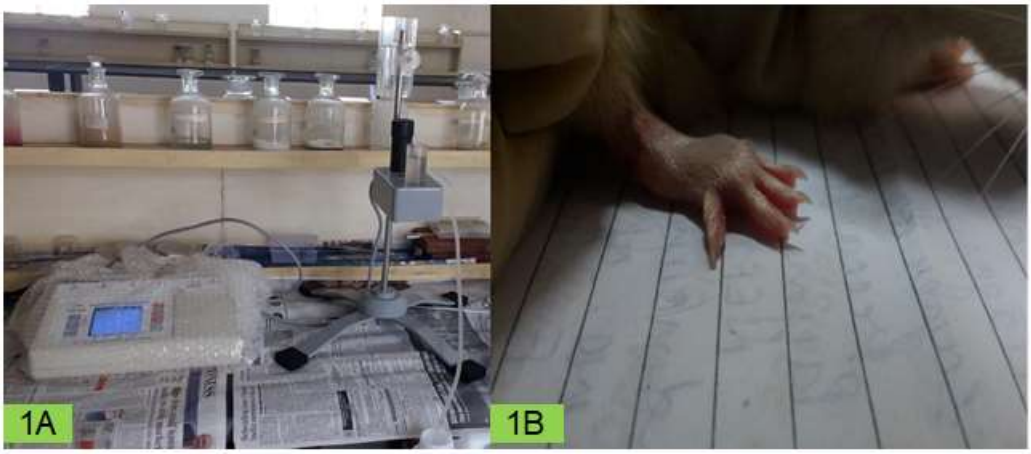

Figure 1: Carrageenan induced-rat paw oedema, 1A: Digital Plethysmometer, 1B: Rat paw oedema

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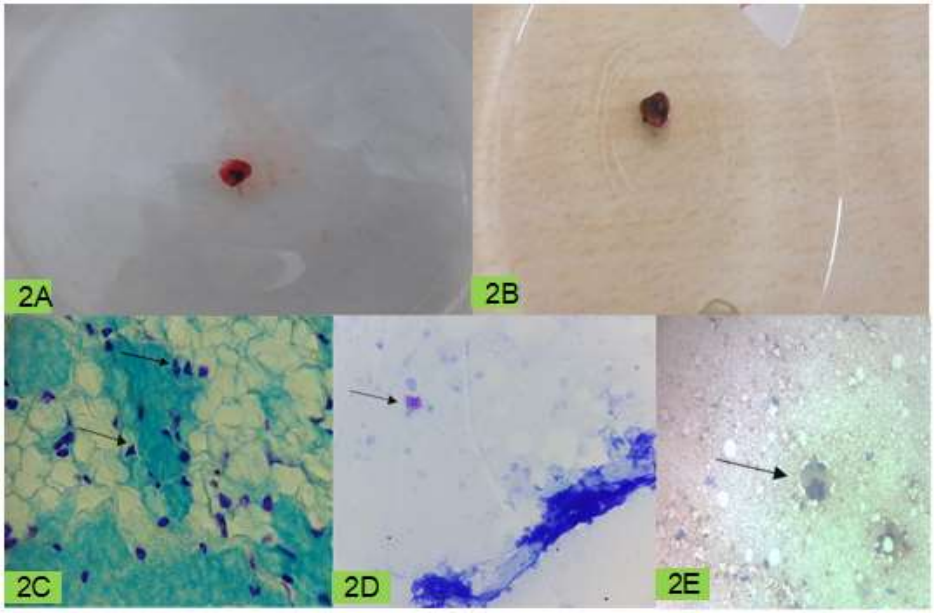

Figure 2: Rexin pellet granuloma method, Mast cell count and foreign body induced granuloma. 2A: Wet Granuloma, 2B: Dry Granuloma, 2C: Mast cell count in Toluidine Blue. 2D: Mast cell count in cytology 2D: Foreign body gaint cell and other inflammatory cells in PAP smear

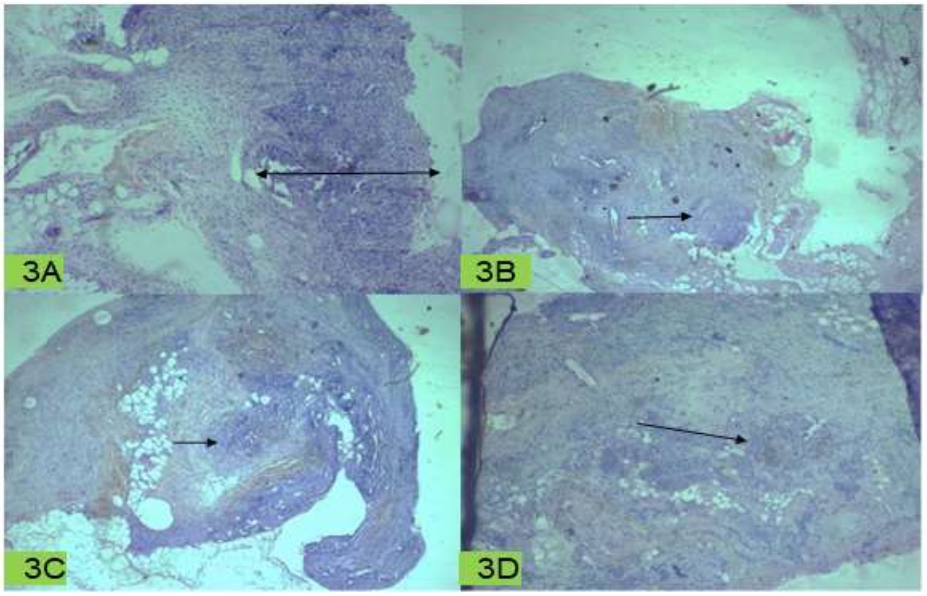

Figure 3: Granulation Tissue (Histopathology H & E) 3A: Control Group , 3B: Diclofenac Group, 3C : Cetirizine Group, 3D : Cetirizine+Diclofenac Group

Discussion

This study was designed to explore the effects of Cetirizine and Diclofenac sodium by two known experimental models of inflammation i.e., Carrageenan induced rat paw oedema and Rexin pellet induced granuloma method, including mast cell count and inflammatory cell count of granulation tissue.

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Anti-inflammatory drugs inhibit different stages of inflammation.[12] Results has shown that Cetirizine has an anti-inflammatory activity. Also, Cetirizine in combination with Diclofenac Sodium has more anti-inflammatory activity than Cetirizine alone but less anti-inflammatory activity than Diclofenac sodium. Similarly, it was observed that decrease in paw oedema by Cetirizine alone and in combination comparing with Diclofenac Sodium was not significantly different.

Carrageenan induced rat paw oedema has been a popular inflammatory model to investigate anti-inflammatory effect of compounds. Carrageenan is polysaccharides of sulfated galactose units and is derived from Irish Sea moss Chondrous crispus, which initially releases histamine and serotonin followed by prostaglandins, protease, and lysosomes.[13] It has a biphasic effect.[22] The first phase is due to release of histamine and serotonin (5HT) (02 hr), plateau phase is maintained by a kinin like substance (3hr) and second accelerating phase of swelling is attributed to PG release (>4hr).

Although the majority of research involving H1-antihistamines has been focused on the histamine dependent early phase symptoms of the allergic response, it is now becoming clear that these drugs have anti-inflammatory effects. This follows the observation by Bakker and colleagues that histamine can activate NF-B, a transcription factor involved in the synthesis of many pro-inflammatory cytokines and adhesion molecules involved in the initiation and maintenance of allergic inflammation.[19]

Most cells involved in inflammatory reactions express H1, H2, and H4 receptor subtypes, with the H1-receptor playing a major role in potentiation of pro-inflammatory immune cell activity and effector responses fundamental to an allergic reaction; the H2-receptor, in contrast, appears to suppress inflammatory and effector functions. It is the H1 receptor antagonist mechanism responsible for its anti-inflammatory action, and could possibly be due to inhibition of histamine. Our results revealed that simultaneous administration of Cetirizine and Diclofenac Sodium inhibit the oedema starting from the first hour and all phases of inflammation, which is probably inhibition of different aspects and chemical mediators of inflammation like Histamine. So, in this study, Cetirizine in its therapeutically permissible dose showed promising results in acute models of experimental inflammation.

We have studied Rexin Pellet induced granuloma as Chronic Inflammatory experimental model which represents the exudative and proliferative phase of inflammation.[20]

The rexin pellet granuloma is widely used to evaluate the transudative and proliferative components of the chronic inflammation. The moist weight of the pellets correlates with transudes, the dry weight of the pellet correlate with the amount of granulomatous tissues. Chronic inflammation occurs by means of the development of proliferate cells. These cells can be either spread or in granuloma form. Non-steroidal anti-inflammatory drugs decrease the size of granuloma which results from cellular reaction by inhibiting granulocyte infiltration, preventing generation of collagen fibers, and suppressing mucopolysaccharides.[21]

The cetirizine dihydrochloride in combination with diclofenac sodium showed better anti-inflammatory activity in rexin pellet induced granuloma and found to be more effective in chronic inflammatory conditions, which reflected in its efficacy in inhibiting the increase in the number of fibroblasts, and synthesis of collagen and mucopolysaccharides during the granuloma tissue formation.[21] Kinin is said to be the main mediator of granuloma, as it both vasodilates and increases vascular permeability in early stages of inflammation.[20] Cetirizine Hydrochloride might have decreased Kinin levels too.

Thus, Cetirizine showed significantly anti-inflammatory effect in rexin pellet induced granuloma model alone and in combination with Diclofenac Sodium (TABLE I). Also,

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Cetirizine in combination with Diclofenac Sodium has more anti-inflammatory activity than Cetirizine alone but less anti-inflammatory activity than Diclofenac Sodium significantly.
The anti-inflammatory activity of the Cetirizine and Diclofenac Sodium was very good in Carrageenan induced rat paw oedema model & equally good in both rexin pellets induced granuloma model and carrageenan induced rat paw oedema model compare to control.
Our study was also focussed on the effects of anti-inflammatory activity of Cetirizine alone and in combination with Diclofenac sodium by counting the mast cells and inflammatory cells in granulation tissue produced in rexin pellet granuloma.
Grading of inflammation on the basis of mean inflammatory cell counts of granulation tissue in PAP smear has been done which includes neutrophils, lymphocytes, monocytes and also foreign body giant cells was identified (Figure 2E) .
The increase in the number of mast cells and the enhanced secretion at sites of inflammation, can accelerate the elimination of the cause of tissue injury, or paradoxically, may lead to a chronic inflammatory response. Some study shows that Mast cells are located in connective tissue, including the lung, skin, linings of stomach and intestine, and other sites. They play an important role in helping defend these tissues from diseases. By releasing chemical such as histamine, mast cells attract other key players of the immune defence system to areas of the body where they are needed.[22] Since, mast cells plays a significant role in chronic inflammation, its count in rexin pellet induced granuloma histopathology reveals the anti-inflammatory activity of Cetirizine and Diclofenac sodium also both in combination.

Conclusion

Cetirizine has shown good anti-inflammatory activity in acute and chronic model of experimental inflammation in comparison to the potent Diclofenac sodium standard drug. This may be due to its ability to prevent production of pro-inflammatory mediators like histamine, bradykinin, interleukins, etc. Also, it significantly decreased the inflammatory cells of chronic inflammation. However, further studies are required to establish and elaborate the molecular mechanism for proper clinical utility. Also, the development of other experimental methods prior to the treatment of various inflammatory diseases should be encouraged. Once its important practical consequences are explored in detail, it should yield more effective anti-inflammatory therapeutic and prophylactic strategies.

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