TO STUDY OF ANTI-INFLAMMATORY EFFECT OF CALCIUM CHANNEL BLOCKERS IN RAT PAW EDEMA MODEL

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ABSTRACT

INTRODUCTION
The process of inflammation is one of the most fundamental responses of the vascularised living tissue to local injury.[1] Inflammation is a universal host defense process involving a complex network of cell-cell, cell-mediator & tissue interactions.[2]

AIM AND OBJECTIVES
To study of anti-inflammatory effect of calcium channel blockers in rat paw edema model.

MATERIALS AND METHODS
To evaluate the anti-inflammatory effect with different doses of calcium channel blockers nifedipine, verapamil and standard drug ibuprofen in experimentally induced acute model of inflammation in male Wister rat. Inter drug comparison of anti-inflammatory efficacy of nifedipine, verapamil with standard drug ibuprofen in rats. Divide the animals into 3 groups each comprising at least six rats. Control group inject saline, standard group inject ibuprofen 20 mg/kg. test group inject (nifedipine & verapamil) subcutaneously. After 30 min inject 0.1ml 1% (w/v) Carrageenin in the plantar region of the left paw of all rats. The left hind paw was measured plethysmograph, immediately (zero hour) and 4 hours after the sub plantar injection of Carrageenin. The difference between zero hour volume and the paw volume recorded at the end of 4 hrs. Indicated the actual volume.

RESULT
The statistical test ANOVA is applied to inter drug comparison. It is found that drug. Nifedipine 1mg/kg (86%) has more efficacy significantly as compared to standard drug Ibuprofen 20mg/kg (55%), and drug Verapamil 1mg/kg (71%) for its anti-inflammatory action. (F value is 94.27 >tab F 5.82) so this method is statistically highly significant.

CONCLUSION
Thus it was found that nifedipine has better anti-inflammatory efficacy as compared to ibuprofen, verapamil in various experimental anti-inflammatory models. The various experimental anti-inflammatory models were rat paw edema method. The calcium channel blockers included were nifedipine, verapamil and standard drug ibuprofen. Nifedipine has anti-inflammatory effect in acute inflammation, verapamil possess anti-inflammatory effect only in acute inflammation. But on inter drug comparison nifedipine has better anti-inflammatory efficacy than ibuprofen &verapamil on rat paw edema method. Thus it was found that nifedipine has better anti-inflammatory efficacy as compared to ibuprofen, verapamil in various experimental anti-inflammatory models.

KEYWORDS
Copper, Zinc Gastrointestinal cancer, Malignancy.

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INTRODUCTION: The process of inflammation is one of the most fundamental responses of the vascularised living tissue to local injury.[1] Inflammation is a universal host defense process involving a complex network of cell-cell, cell-mediator & tissue interactions.[2] The word inflammation derived from Latin "Inflammatic" and the Greek "Phlegm asia" is one might say as old as medicine it self.[3] The four cardinal signs of inflammation as Erythema (Rubor), Swelling (Tumor), Heat (Calor), Pain (Dolor), initially enunciated by Celsus. Later Galen (129-201A.D) while dealing with inflammation had included a fifth sign namely loss of sensation.[4] Normally, inflammation is the starting point of the body's self-repair process initiated by body's defense system throughout pathological assault but occasionally it runs amok, leading to physiological chaos &

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death. It occurs in response to a variety of stimuli viz, physical, chemical traumatic, antigen challenge & infectious agents. The nerves that carry the signals, set up by chemical and mechanical stimulation of sensory receptors that we perceive as pain, themselves in turn promote an increase in local blood flow through the axon reflex mechanism. The nerve fibers (axon) give off branches back to their site of origin, and these release 'substance P, a peptide that relaxes the vessel walls. Alleviation of the pain of inflammation by analgesic drugs is clearly beneficial to the sufferer; otherwise the first concern of treatment is if possible to remove the cause (such as treating infection by antibiotics, or removing foreign material). Other treatments in recent decades have been directed against inflammation itself, in conditions related to injury, 'wear-and-tear', and auto-immunity. Sodium salicylate was first used by buss in 1875 in the treatment of rheumatic fever. Currently available NSAIDs have been reported to reduce the synthesis of both prostaglandins, & leukotrienes.[5] Inflammation is an immune system response to protect the body from infection. Inflammation occurs when white blood cell migrate out of blood vessels into the infected area, where they act as phagocytes (destroyers of foreign matter).

Inflammation Can Be Classified As: There are Two Basic Types of Inflammation:

1. Acute.
2. Chronic.

Acute Inflammation: Is the initial response of body to harmful stimuli and is achieved by the increased movement of plasma & leukocytes from the blood into the injured tissues.

- Acute inflammation is of short duration, which could be anything from a few minutes to a few days; such inflammation is caused by foreign substances entering the body, or by physical damage. A viral infection may also precipitate acute inflammation.
- **Chronic Inflammation:** Chronic inflammation is a pathological condition characterized by concurrent active inflammation, tissue destruction, and attempts at repair. Chronic inflammation is associated with the presence of fibroblasts, small blood vessels & connective tissue histological. Many factors modify the course & histology appearance of chronic inflammation. The most likely source of inflammatory mediatory is the plasma from the cell or the damaged tissue itself.
- Chronic inflammation is long lasting. It may persist for weeks, months or even years. Chronic inflammation may be brought on by acute inflammation or it may be the result of an auto immune disease.
- **Chemical Mediators of Inflammation:** Biochemical mediators released during inflammation of intensify & propagate the inflammatory response. These mediators are soluble, diffusible, molecules that can act locally & systemically. The most important is the influence of anti -inflammatory drugs on chemical mediators of inflammation.
    1. Vasoactive amines: histamine, serotonin.
    2. Plasma proteases.
    3. Arachidonic acid (AA) metabolites.
    4. Lysosomal constituents.
    5. Oxygen-derived free radicals.
    6. Acetylated alkyl phosphoglycerides.
    7. Lymphocyte factors: Lymphokines.

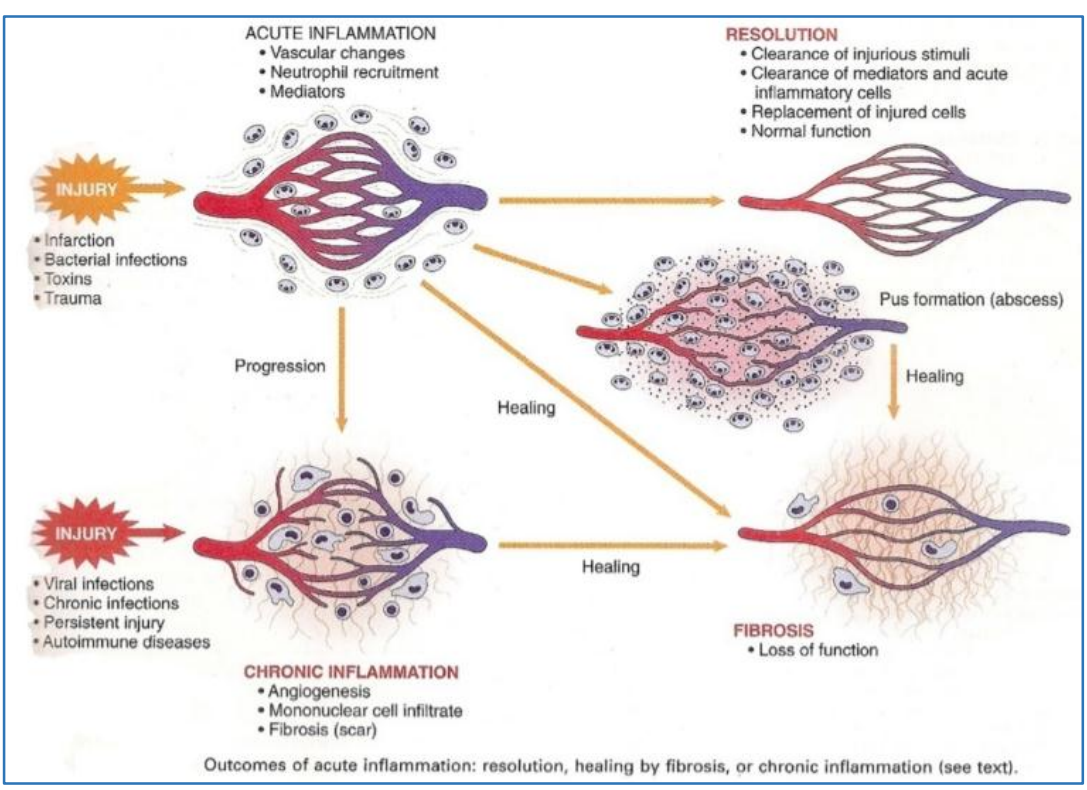

Fig. 1: Photograph of Acute inflammation

AIMS & OBJECTIVES:

1. To evaluate the anti-inflammatory effect with different doses of calcium channel blockers nifedipine, verapamil and standard drug ibuprofen in experimentally induced acute & sub-acute models of inflammation in male Wister rat.
2. Inter drug comparison of anti-inflammatory efficacy of nifedipine, verapamil with standard drug ibuprofen.

MATERIAL & METHODS: PROCEDURE: The procedure adopted was method of Wilhelmi and Domenjotz. The procedure required 2 operators one for dipping the oedematous paw in mercury and the other to record the foot volume simultaneously. Weigh the animals and number them. Make a mark on both the hind paws (right &left) just beyond tibio-tarsal junction, so that every time the paw dipped in the mercury column up to the fixed mark to ensure constant paw volume. Note the initial paw volume (right &left) of each rat by mercury displacement method.

Divide the animals into 3 groups each comprising at least six rats. Control group inject saline, standard group inject ibuprofen 10, 15, 20 mg/kg. Test group inject (nifedipine &verapamil) subcutaneously. After 30 min inject 0.1ml 1% (w/v) Carrageenin in the plantar region of the left paw of all rats. The left hind paw was measured plethysmograph, immediately (zero hour) and 4 hours after the sub plantar injection of Carrageenin. The difference between zero hour volume and the paw volume recorded at the end of 4 hrs indicated the actual volume.

Percentage inhibition of oedema was calculated by the following formula:

$$\frac{Vc-Vt/Vs}{Vc} \times 100$$

Vc=Mean volume of the paw oedema in control animals.
Vt=Mean volume of paw oedema in test drug (nifedipine/verapamil) treated animals.
Vs=Mean volume of paw oedema in standard (ibuprofen) drug treated animals.

STATISTICAL ANALYSIS: Data were expressed as Mean±SEM.
The results were analysed by analysis of variance (ANOVA).

OBSERVATIONS & RESULTS: Carrageenin Induced Hind paw oedema model in rats.

No. of animals	Paw volume 0hr of carrageenin	Paw volume after 4hrs of carrageenin	Actual paw volume	Percentage inhibition
1	0.4	1.2	0.8	
2	0.2	1.1	0.9	
3	0.6	1.4	0.8	
4	0.6	1.6	1	
5	0.4	1.2	0.8	
6	0.5	1.6	1.1	
Mean paw volume ±SE0.9±				

Table 1: Group Control

No. of animals	Paw volume 0hr of carrageenin	Paw volume after 4 hrs of carrageenin	Actual paw volume	Percentage inhibition
1	0.2	1.0	0.8	
2	0.4	1.1	0.7	
3	0.6	1.2	0.6	21%
4	0.5	1.3	0.8	
5	0.4	1.1	0.7	
6	0.3	1.0	0.7	
Mean paw volume±SE0.71±0.0307				

Table 2: Group-ibuprofen 10 mg/kg.(mentioned earlier as 20 mg/kg only)

No. of animals	Paw volume 0hr of carrageenin	Paw volume after 4 hrs of carrageenin	Actual paw volume	Percentage inhibition
1	0.6	1.4	0.8	
2	0.4	1.1	0.7	
3	0.5	1.3	0.8	30%
4	0.7	1.2	0.5	
5	0.6	1.0	0.4	
6	0.4	1.0	0.6	
Mean paw volume±SE0.63±0.06				

Table 3: Group-Ibuprofen 15 mg/Kg

No. of animals	Paw volume 0hr of carrageenin	Paw volume after 4 hrs of carrageenin	Actual paw volume	Percentage inhibition
1	0.2	0.5	0.3	55%
2	0.3	0.8	0.5	
3	0.2	0.6	0.4	
4	0.1.	0.5	0.4	
5	0.1	0.5	0.4	
6	0.2	0.6	0.4	
Mean paw volume±SE0.4±0.02				

Table 4: Group-ibuprofen 20 mg/kg

No. of animals	Paw volume 0hr of carrageenin	Paw volume after 4 hrs of carrageenin	Actual paw volume	Percentage inhibition
1	0.2	0.9	0.7	26%
2	0.4	1.1	0.7	
3	0.6	1.2	0.6	
4	0.5	1.2	0.7	
5	0.4	1.0	0.6	
6	0.3	1.0	0.7	
Mean paw volume±SE0.66±0.020				

Table 5: Group-verapamil 0.5 mg/kg

No. of animals	Paw volume 0hr of carrageenin	Paw volume after 4 hrs of carrageenin	Actual paw volume	Percentage inhibition
1	0.5	0.9	0.4	50%
2	0.3	0.7	0.4	
3	0.6	1.0	0.4	
4	0.5	1.0	0.5	
5	0.4	1.0	0.6	
6	0.5	0.9	0.4	
Mean paw volume±SE0.45±0.033				

Table 6: Group-verapamil 0.75mg/kg

No. of animals	Paw volume 0hr of carrageenin	Paw volume after 4 hrs of carrageenin	Actual paw volume	Percentage inhibition
1	0.5	0.7	0.2	71%
2	0.6	0.8	0.2	
3	0.7	1.0	0.3	
4	0.7	1.1	0.4	
5	0.5	0.7	0.2	
6	0.4	0.7	0.3	
Mean paw volume±SE0.26±0.033				

Table 7: Group-verapamil 1 mg/kg

No. of animals	Paw volume 0hr of carrageenin	Paw volume after 4 hrs of carrageenin	Actual paw volume	Percentage inhibition
1	0.6	1.4	0.8	30%
2	0.4	1.1	0.7	
3	0.5	1.3	0.8	
4	0.7	1.2	0.5	
5	0.6	1.0	0.4	
6	0.4	1.0	0.6	
Mean paw volume±SE 0.63±0.066				

Table 8: Group-nifedipine 0.5mg/kg

No. of animals	Paw volume 0hr of carrageenin	Paw volume after 4 hrs of carrageenin	Actual paw volume	Percentage inhibition
1	0.5	0.7	0.2	71%
2	0.6	0.8	0.2	
3	0.7	1.0	0.3	
4	0.7	1.1	0.4	
5	0.5	0.7	0.2	
6	0.4	0.7	0.3	
Mean paw volume±SE0.26±0.033				

Table 9: Group-nifedipine 0.75mg/kg

No. of animals	Paw volume 0hr of carrageenin	Paw volume after 4 hrs of carrageenin	Actual paw volume	Percentage inhibition
1	0.4	0.6	0.2	86%
2	0.2	0.3	0.1	
3	0.6	0.7	0.1	
4	0.6	0.7	0.1	
5	0.5	0.6	0.1	
6	0.4	0.6	0.2	
Mean paw volume±SE0.13±0.020				

Table 10: Group-nifedipine 1mg/kg

Carrageenin Induced Hind Paw Oedema Model in Rats: The ANOVA Test:

Groups: Ibuprofen 10mg/kg, 15mg/kg, 20mg/kg**.**

Source of variation	d. f.	Sum of squares	Mean squares	F
Bss	3	0.771 6	0.2572	19.78
Wss	20	0.261	0.0130	

Bss: Variance between groups.
Wss: Variance with in groups.
d. f: Degree of freedom.
(Cal F (19.78) >Tab F at 0.5% level of significance with (3, 20) d. f. is 5.82)

Referring to the table of distribution of F against 3 d. f. for greater mean square and 20 d. f. for lesser mean square, a value 5.82 at 0.5% level of significance was found. Since the experimental value of 19.78 is far greater than recorded value we concluded that the difference between the groups are highly significant (p<0.005).

Groups: Verapamil 0.5mg/kg, 0.75mg/kg, 1mg/kg.

Source of variation	d. f.	Sum of squares	Mean Squares	F
Bss	3	1.3632	0.4544	56.80
Wss	20	0.161	0.0080	

Bss: Variance between groups.
Wss: Variance with in groups.
d. f.: Degree of freedom.
(Cal F (56.80) >Tab F at 0.5% level of significance with (3, 20) d. f. is 5.82.).

Referring to the table of distribution of F against 3 d.f for greater mean square and 20 d. f. for lesser mean square, a value 5.82. At 0.5% level of significance was found. Since the experimental value of 56.80 is far greater than the recorded value we concluded that the difference between the groups are highly significant (p <0.005).

Groups: Nifedipine 0.5mg/kg, 0.75mg/kg, 1mg/kg.

Source of variation	d. f.	Sum of squares	Mean Squares	F
Bss	3	1.6572	0.5524	42.82
Wss	20	0.2595	0.0129	

Bss: Variance between groups.
Wss: Variance with in groups.
d. f.: Degree of freedom.
Cal F (42.82) >Tab F at 0.5 % level of significance with (3, 20) d.f is 5.82.

Referring to the table of distribution of F against 3 d.f for greater mean square and 20 d. f. for lesser mean square, a value 5.82 at 0.5% level of significance was found. Since the experimental value of 42.82. Is far greater than the recorded value we concluded that the difference between the groups are highly significant (p <0.005).

Groups: Ibuprofen 20 mg /kg, verapamil 1mg/kg, nifedipine 1 mg /kg.

Source of variation	d. f.	Sum of squares	Mean squares	F
Bss	3	**2.0646**	**0.6882**	**94.27**
Wss	20	**0.147**	**0.0073**	

(Cal F (94.27) >Tab F at 0.5% level of significance with (3, 20) d. f. is 5.82)

Referring to the table of distribution of against 3 d. f. for greater mean square and 20 d. f. for lesser mean square a value 5.82 at 0.5% level of significance was found. Since the experimental value of 94.27 is far greater than recorded value we concluded that the difference between the groups are highly significant (p<0.005).

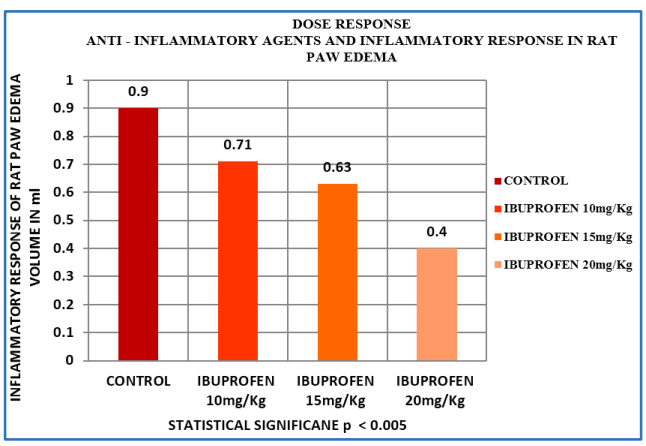

Fig. 1

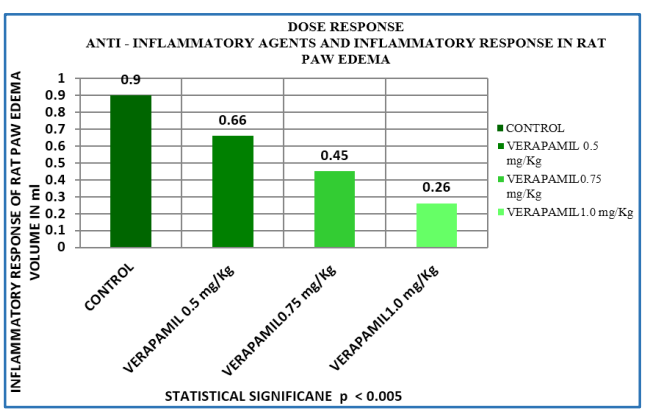

Fig. 2

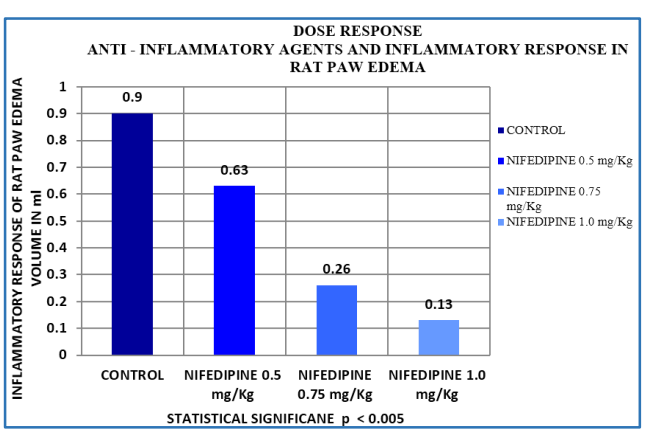

Fig. 3

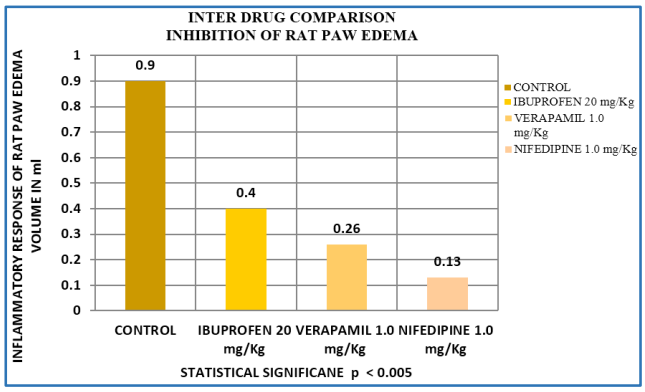

Fig. 4

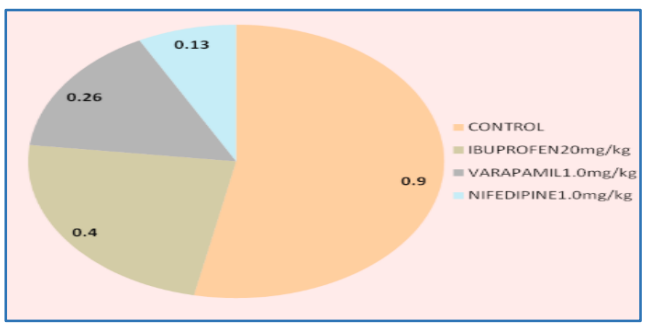

Fig. 5: Inter Drug Comparison Inhibition of Rat Paw Oedema

RESULTS: The anti-inflammatory effect of nifedipine, verapamil on paw oedema in rats is compared with that of the standard drug ibuprofen simultaneously both the test & standard drugs were compared with the control drug normal saline.

Carrageenin Induced Rat Paw Oedema: The doses selected for acute rat paw oedema were 0.5mg/kg, 0.75mg/kg, 1mg/kg.

On sub-plantar injection of 0.1 ml of 1% Carrageenin into right hind paw of rat maximum oedema was seen after 4 hrs (Table 1)

When the dose response was observed, Ibuprofen has inhibited the oedema, 21%, 30%, 55% with 10mg/kg, 15mg/kg, 20mg/kg (Table 2, 3, 4) respectively.

When the p value was calculated it was <0.005. So this method is statistically highly significant.

When the dose response was observed Verapamil has inhibited the oedema 26%, 50%,71% With 0.5mg/kg, 0.75mg/kg, 1mg/kg (Table 5, 6, 7) respectively.

When the p value was calculated, it was <0.005. So this method is statistically highly significant.

When the dose response was observed Nifedipine has inhibited the oedema 30%, 71%, 86% With 0.5mg/kg, 0.75mg/kg, 1mg/kg (Table 8, 9, 10) respectively

When the p value was calculated it was <0.005. So this method is statistically highly significant.

ANOVA Test: rat paw oedema model inter drug comparison. Ibuprofen 20mg/kg, Verapamil 1mg/kg, Nifedipine 1mg/kg.

The statistical test ANOVA is applied to inter drug comparison. It is found that drug. Nifedipine 1mg/kg (86%) has more efficacy significantly as compared to standard drug Ibuprofen 20mg/kg (55%), and drug Verapamil 1mg/kg (71%) for its anti-inflammatory action.

(F value is 94.27 >tab F 5.82) so this method is statistically highly significant.

DISCUSSION: A number of experimental procedures and animal models are available to evaluate the anti-inflammatory activity of a new compound. Every method has its own limitations and a single experimental method is not enough for determine the anti-inflammatory activity, consequently a number of tests is essential. The methods for evaluation of anti-inflammatory activity of a compound can be broadly classified into two categories i.e. acute and chronic. As is evident acute methods are required for quick evaluation of a compound for it's possible anti-inflammatory activity. Despite the appearance of various anti-inflammatory agents we have been interested in the evaluation of newer non-steroid anti-inflammatory agents for the past few years. In the present study, calcium channel blockers were tested and compared for its anti-inflammatory activity with a standard drug such as Ibuprofen. One acute methods, i.e. rat paw oedema and one sub-acute method, foreign body granuloma method were selected for the present study. In our study Carrageenin induced hind paw oedema is the standard experimental model of acute inflammation. Carrageenin is the phlogistic agent of choice for testing anti-inflammatory drugs as it is not known to be antigenic and is devoid of apparent systemic effects. Moreover, the experimental model exhibits a high degree of reproducibility. Carrageenin-induced oedema is a biphasic response. The first phase is mediated through the release of histamine, serotonin and kinins whereas the second phase is related to the release of prostaglandin and slow reacting substances which peak at 3 hrs The results of the present study indicate that CCBs (verapamil and nifedipine), That selectively block L-type calcium channel, exerted a potent anti-oedema effect in Rat paw. These effects are similar to the report of De Vries et al (1995), though they used different topical CCBs and a different inflammatory model. The possible mechanisms involved in the anti-inflammatory activity of CCBs may be through (1) A reduction of the ca concentration in blood, causing a decrease in the vessel resistance, and consequent reduction of hydrostatic pressure in the capillaries. (2) Inhibition of the release of pro-inflammatory mediators, (3) Reduction of ca leading to inhibition of the activity of PLA2 and or PLC, the enzymes responsible for the synthesis of eicosanoids and leukotrienes, and (4) Stabilization of the cell membrane integrity (by inhibiting ca influx), thus preventing tissue injury and inflammation. Rodler et al have reported that verapamil can increase the inflammatory cytokines like IL-6 in high dose but not in a low dose. When the percentage inhibition of inflammation by these methods was compared it is seen that the test compounds (verapamil, nifedipine) at a dose of 1mg/kg were more potent than ibuprofen given at a dose of 20mg/kg in the rat paw oedema method.

When a dose response was tested in rat paw oedema method, the test compounds (verapamil 71%, nifedipine 86%) at a dose of 1mg/kg was showed better response than 0.5mg/kg, 0.75mg/kg. When a dose response was tested in paper disc granuloma method, the test compound (nifedipine 53.58%) at a dose of 1mg/kg has showed better response than at a dose of 0.5mg/kg, 0.75mg/kg. Another test compound (verapamil 8.72%) at a dose of 1mg/kg has showed lesser response than compared to 0.5mg/kg, (10.26%), 0.75mg/kg (11.21%). But in Inter drug comparison in rat paw oedema method dihydropyridine group of calcium channel blocker nifedipine has showed better anti-inflammatory action as compared to standard drug ibuprofen and drug verapamil. In paper disc pellet induced granuloma nifedipine has better anti-inflammatory action than standard drug ibuprofen & phenylalkylamine group of calcium channel blockers verapamil. Thus dihydropyridine group of calcium channel blockers are better anti-inflammatory agents as compared to other calcium channel blockers when tested by acute.

CONCLUSIONS: The present study was carried out to evaluate the anti-inflammatory effect of various calcium channel blockers in experimental rat models. The various experimental anti-inflammatory models were rat paw oedema method. The calcium channel blockers included were nifedipine, verapamil and standard drug ibuprofen. Nifedipine has anti-inflammatory effect in acute inflammation, verapamil possess anti-inflammatory effect only in acute inflammation. But on inter drug comparison nifedipine has better anti-inflammatory efficacy than ibuprofen &verapamil on rat paw oedema method. Thus it was found that nifedipine has better anti-inflammatory efficacy as compared to ibuprofen, verapamil in various experimental anti-inflammatory models. However, the above preclinical experiments only give us an idea about anti-inflammatory property large scale clinical trials will be needed for final assessment.

SUMMARY: This study was carried out to evaluate the anti-inflammatory property of the drugs nifedipine, verapamil and compared with standard drug ibuprofen to evaluate this property rat paw oedema. Paper disc granuloma inhibition were used in rats presented in detail. Chemical mediators of inflammation were discussed. Types of inflammation acute and chronic were briefly presented. The drugs were evaluated at 3 dose levels in rat paw oedema inhibition and paper disc granuloma inhibition results were represented in tables. It was found that the test drug nifedipine showed better anti-inflammatory efficacy than other drugs verapamil & standard drug ibuprofen.

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