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An Experimental Study to Assess the Effect of Addition of Calcium on the Anti-Inflammatory Activity of Etoricoxib

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ABSTRACT

***Objectives**: To evaluate the effect of addition of calcium gluconate on the anti-inflammatory activity of Etoricoxib on acute and subacute inflammation in rats.*
***Material and Methods:** Five groups of rats, each group containing six rats were used. Group 1- Control 1% Gum acacia, Group 2-Etoricoxib (8 mg/kg), Group 3- Etoricoxib (5 mg/kg), Group 4- Etoricoxib (5mg/kg) and Calcium gluconate (5mg/kg), Group 5- Etoricoxib (5 mg/kg) and Calcium gluconate (50 mg/kg). Acute inflammation was induced by carrageenan and subacute inflammation by cotton pellet method. The results were analyzed by one way ANOVA & student's't' test.*
***Results**: Co-administration of Calcium gluconate (50mg/kg) and Etoricoxib (5mg/kg) showed significant anti-inflammatory activity equivalent to Etoricoxib (8mg/kg) in acute as well as subacute models of inflammation.*
***Conclusion**: Calcium gluconate (50 mg/kg) enhanced anti-inflammatory activity of Etoricoxib in rats.*

***KEY WORDS:** Calcium gluconate, Etoricoxib.*

INTRODUCTION

Inflammation is fundamentally a protective response of the body. Inflammation continues to be an area of great interest for research probably due to non availability of a safest and most effective anti-inflammatory agent. The conventional nonselective non steroidal anti-inflammatory drugs (NSAIDs) like aspirin, diclofenac etc possess side effects like gastrointestinal disturbances, ulcerations, impairment of renal functions and hypersensitivity reactions. The newer COX-2 selective agents such as celecoxib & etoricoxib etc retain the anti-inflammatory effect characteristic of NSAIDs with a marked increase in gastrointestinal tolerability as compared to classic non selective ones. But even these agents show cardiotoxic, renotoxic and hepatotoxic side effects. Hence the search for alternatives or adjuvants to these drugs is still going on in an attempt to produce an ideal anti-inflammatory drug.

In recent years increased understanding of the inflammatory mechanism and the mediators involved has led to the development of newer anti-inflammatory agents like monoclonal antibodies e.g. natalizumab and antagonists of inflammogens e.g. tumor necrosis factor-alpha (TNF-alpha) blocking agents like infliximab, etanercept etc.

Interestingly several other drugs like minocycline [1], ascorbic acid [2], calcium salts e.g. calcium dobesilate, calcium hydroxide and calcium pentosan polysulfate [3-5] have been reported to possess anti-inflammatory property.Earlier calcium chloride was advocated for the treatment of urticaria, acute edema, pruritus and erythema. Calcium carbonate & calcium gluconate were used for the treatment of insect stings[6] and calcium hydroxide to suppress periapical inflammation in dental practice. These reports indicate that calcium salts possess anti-inflammatory property. The present study was done to investigate the effect of calcium gluconate on the anti-inflammatory activity of etoricoxib.

MATERIALS & METHODS

Animals:

Wistar rats weighing (150-270 grams) of either sex, were used for this study. They were housed under standardized conditions. They had free access to standard pellet diet and water *ad libitum*. All the procedures were performed in accordance with the guidelines issued by the Institutional Animal Ethical Committee, Bharati Vidyapeeth deemed university Medical College and Hospital Sangli, constituted as per the directions of the Committee for the Purpose of Control and Supervision of Experiments on Animals. Ministry of Animal Welfare Division, Government of India.

Acute Inflammation:

Rats were divided into five groups of six each. They were starved overnight with water *ad libitum* prior to the day of experiment. The control group received 0.5 ml of 1% gum acacia suspension orally, while the other groups received different drug treatment as detailed below.

Test drugs were administered orally according to the body weight 30 minutes before injecting 0.05 ml of 1% of sterile carrageenan in normal saline into subplantar region of the left hind paw. A mark was made at the left ankle joint. Contra-lateral limb received equal volume of saline. Paw volume upto the ankle joint was measured in drug treated & untreated group. The edema volume was measured by mercury displacement with the help of a plethysmograph before carrageenan injection and 0, 30 min and 1, 3, 5 hours, 5th day and 14th day after injecting carrageenan. The difference between 0 hour & subsequent readings was considered as edema volume [7].

$$\% \text{ inhibition in edema} = \frac{\text{Mean edema in control group - Mean edema in drug treated group X 100}}{\text{Mean edema in control group}}$$

Subacute Inflammation:

Autoclaved cotton pellets (5mg) were weighed and used. Under ether anaesthesia, pellets were inserted subcutaneously through skin incision in the axilla of the animals. Aseptic precautions were taken throughout the experiment. Drugs treatment was started 2 hours after cotton pellet implantation & continued for 5 consecutive days. Control group received 1% gum acacia suspension for the same duration as the drug. On the 6th day, under anaesthesia granulomas were removed surgically and skin was sutured. The granulomas were dried for 24 hours at 60 degree Celsius and the dry weights were determined. The weight of granulomatous tissue formed was calculated by subtracting initial weight from the final dry weight of cotton pellets and percentage

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protection by the drug was calculated. Mean granuloma dry weight for the various groups were calculated and expressed as mg/100gm body weight [8].

Drug and doses:

The clinical doses for various drugs were converted to rat equivalent dose [9].

1) Calcium gluconate dissolved in water was used in 5 and 50 mg/kg doses.
2) Etoricoxib in 1 % gum acacia suspension was administered at 5 and 8 mg/kg doses.

Statistical analysis:

Statistical analysis was carried out using one way ANOVA for significance between groups for percentage inhibition of carrageenan induced edema. Data was expressed as mean ±S.D. The level of significance between individual groups was detected using student's "t" test for percentage inhibition of edema. For all tests, effects with a probability of P < 0.05 were considered to be significant.

RESULTS

Acute studies:

According to **Table 1**, Carrageenan-induced paw edema in Gr 5 (Calcium gluconate 50mg/kg+ Etoricoxib 5mg/kg) did not differ from the corresponding values in the Gr 2 (Etoricoxib 8 mg/kg), indicating the effective dose of Calcium gluconate 50 mg/kg. There was no significant difference in the means of paw volume at 1 hr, 3 hr and 5 hr in Gr 2 & Gr 5 indicating that Calcium gluconate 50 mg/kg enhanced anti-inflammatory activity of Etoricoxib (5mg/kg).

Carrageenan-induced paw edema in Gr 4 (Calcium gluconate 5mg/kg + Etoricoxib 5mg/kg) did not differ from the corresponding values in Gr 3 (Etoricoxib 5 mg/kg), indicating that Calcium gluconate 5mg/kg did not enhance anti-inflammatory activity of Etoricoxib 5mg/kg. There was no significant difference in the means of paw volume at 1 hr, 3 hr and 5th day in Gr 3 & Gr 4. Carrageenan-induced paw edema in Gr 4 was more than the corresponding values in the Gr 2 indicating that Calcium gluconate 5mg/kg combined with Etoricoxib 5mg/kg did not have anti-inflammatory effect comparable to Etoricoxib 8mg/kg. There was significant (P<0.05) difference in the means of paw volume at 3hr and 5 hr.

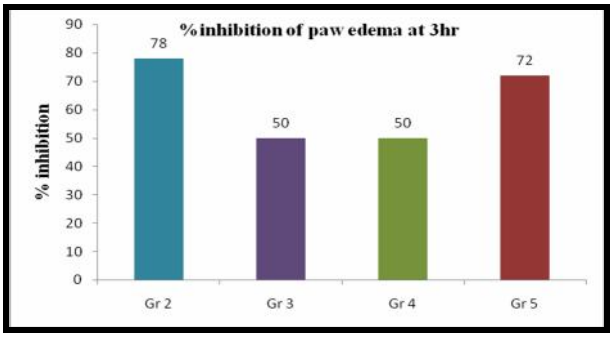
Subacute studies:

According to **Table 2**, Mean granuloma dry weights in drugs treated groups were significantly lower than the control group indicating significant (P<0.05) anti-inflammatory property of Etoricoxib and Etoricoxib combined with Calcium gluconate. Gr 5 (Etoricoxib 5mg/kg & Calcium gluconate 50 mg/kg) did not show significant difference in granuloma dry weight when compared to Gr 2 (Etoricoxib 8mg/kg). Gr 5 (Etoricoxib 5mg/kg + Calcium gluconate 50 mg/kg) showed significant (P <0.05) difference in granuloma dry weight when compared to Gr 3 (Etoricoxib 5mg/kg). Gr 4 (Etoricoxib 5mg/kg + Calcium gluconate 5 mg/kg showed significant (P<0.05) difference in granuloma dry weight when compared to Gr 2 (Etoricoxib 8mg/kg). Gr 4 (Etoricoxib 5mg/kg + Calcium gluconate 5 mg/kg) did not show significant difference in granuloma dry weight when compared to Gr 3 (Etoricoxib 5mg/kg).

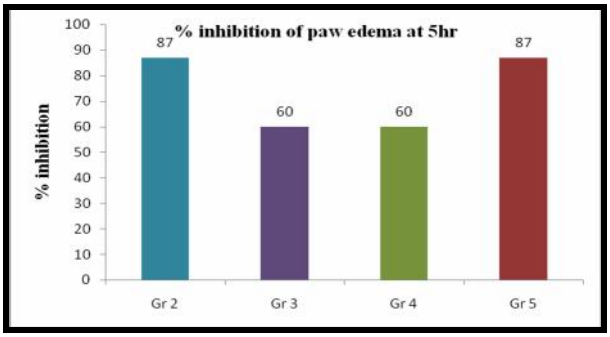
Table No. 1: Effect of various treatments in rats on carrageenan-induced paw edema

Drugs (mg/kg)	Paw volume in ml					
	0.5 h	1h	3h	5h	5th day	14th day
Gr 1-Control (1%gum acacia)	0.41±0.08	0.91±0.08	1.16±0.16	1.41±0.08	0.66±0.10	0.33±0.10
Gr 2-Etoricoxib (8mg/kg)	0.5±0	0.5±0	0.25±0.11**	0.16±0.10**	0**	0**
Gr 3-Etoricoxib (5mg/kg)	0.5±0	0.58±0.08	0.58±0.08	0.5±0	0.08±0.08	0**
Gr 4-Etoricoxib (5mg/kg) + Calcium Gluconate (5mg/kg)	0.5±0	0.5±0	0.58±0.08	0.5±0	0.08±0.08	0**
Gr 5-Etoricoxib (5mg/kg) + Calcium gluconate (50mg/kg)	0.5±0	0.66±0.10	0.33±0.16**	0.16±0.10**	0**	0**
One-way ANOVA F	1.000	5.972	7.839	45	15.972	10
One-way ANOVA P	0.426	0.002	0	0	0	0

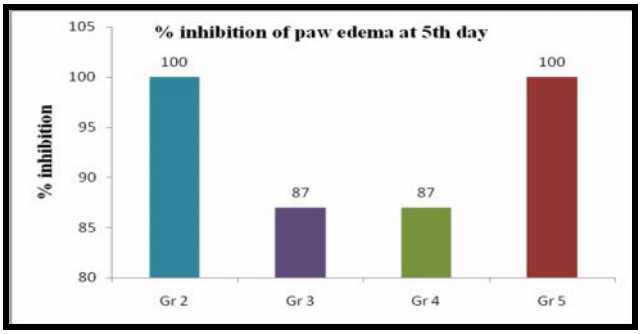
Values are mean ± SEM, n=6 in each group. All drugs are given orally 30 min prior to carrageenan. *P< 0.05,significant, **P<0.01,highly significant, when compared to control.



Graph 1: Percentage inhibition of Paw edema at 3 hr



Graph 2: Percentage inhibition of Paw edema at 5 hr

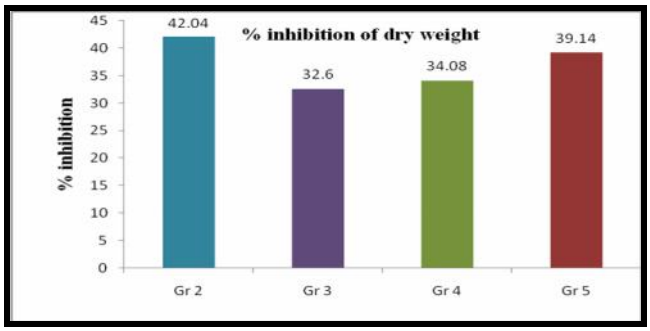


Graph 3: Percentage inhibition of Paw edema at 5th day

Table No. 2: Effects of various treatments on granuloma dry weight

Drug(mg/kg)	Granuloma –dry wt (mg% of body weight)
Control (1% gum acacia)	23.00±0.36
Etoricoxib (8mg/kg)	13.33±0.21**
Etoricoxib (5mg/kg)	15.50±0.22**
Etoricoxib (5mg/kg) + Calcium gluconate(5mg/kg)	15.17±0.3**
Etoricoxib (5mg/kg) + Calcium gluconate (50 mg/kg)	14.17±0.3**
One-way ANOVA F	180.433
One-way ANOVA P	0.00

Values are mean ±SEM, n=6 in each group. All the drugs were administered p.o. once daily, for 5 days.*P<0.05, significant, **P<0.01, highly significant, when compared to control.



Graph 4: Percentage inhibition of dry weight of granuloma

DISCUSSION

Results of present study indicate that calcium gluconate (50 mg/kg) combined with etoricoxib (5 mg/kg) possesses significant anti-inflammatory property both in acute and subacute models of inflammation which was comparable to that of etoricoxib (8 mg/kg).

A review of literature reveals that in different models of inflammation, calcium salts like calcium dobesilate [3], calcium hydroxide [4] and calcium pentosan polysulfate [5] possess anti-inflammatory property. Calcium pentosan sulfate suppresses neutrophil accumulation, NO activity and IL-6 activity in inflammed rat subcutaneous air-pouch model. Calcium chloride is recommended for the treatment of urticaria, acute edema, pruritus and erythema. Calcium carbonate and calcium gluconate have been used to treat insect stings [6]. Calcium hydroxide has been used in dental practice to suppress periapical inflammation [10, 11].

The mechanism of anti-inflammatory action of calcium cannot be proposed on the basis of present findings. However several mechanisms have been proposed in earlier reports. Piller, has speculated that calcium dobesilate reduced the number of circulating monocytes and also blocked the action of macrophage in order to suppress inflammation [3]. It has also been shown that calcium dobesilate can suppress platelet aggregating-factor production in endothelial cells in a dose-dependent manner [12]. The above mechanisms may play a role in the anti-inflammatory activity of calcium gluconate too. The other proposed anti-inflammatory mechanisms of calcium salts include the precipitant action of calcium on a cement substance and enhanced superoxide anions scavenging through increased activity of superoxide dismutase, peroxidase,glutathione peroxidase and glutathione reductase which are reported to be increased by calcium glubionate [13]. These enzymes suppress inflammation. In some smooth muscles, calcium through the calcium-sensitive potassium channels, can lead to hyperpolarization [13]. If this is also true for the vascular smooth muscles, then calcium can produce vasodilatation. In fact, calcium has been reported to produce vasodilatation by stabilizing the cell membrane [14, 15]. Due to vasodilatation the interendothelial cell gaps may be reduced, leading to decreased effusion, which is one of the events of inflammation.

In another study, it is shown that calcium is required for post-engulfment responses of phagocytes such as the anti-inflammatory response that normally accompanies engulfment of apoptotic cells [16]. One more study has proved anti-inflammatory effect of calcium carbonate [17]. The reduction of oxidative stress may explain the anti-inflammatory effect. Zemel and Sun [18] studied the effect of high calcium diet in the form of calcium carbonate and dairy products on inflammation markers. It was found that calcium carbonate markedly decreased inflammatory cytokine gene expression in adipose tissue with significant suppression of TNF-α, IL-6 and MCP-1(monocyte chemoattractant protein-1). Calcium gluconate, in particular, has been used to treat injuries stemming from pro-inflammatory cytokines, interleukin-6 and tumour necrosis factor-alpha after chemical burns in rats. Calcium gluconate has shown to enhance the anti-inflammatory activities of aspirin [13, 19]. Calcium gluconate effectively inhibited ligature placement-induced periodontitis and related alveolar bone loss. Experimental periodontitis (EPD) induced increases in MPO,IL-1 beta and TNF-α were significantly and dose dependently inhibited by calcium gluconate, suggesting that calcium gluconate inhibited the cytotoxic effects of polymorphonuclear neutrophils (PMNs) [20].

On the contrary, in earlier reports; locally applied calcium chloride neither inhibited edema formation nor reduced inflammatory signs when injected along with the inflammogen in rats [21]. In the study of Cates et al. [22] the intravenous infusion of Calcium gluconate in cats enhanced inflammation and increased the permeability of the pancreatic duct to large dextran molecules that had been perfused through it and were detected in the portal venous blood.

Karnad et al. [13], reported that calcium gluconate at a dose of 5mg/kg orally showed a significant pro-inflammatory activity and calcium carbonate at a dose of 10 mg/kg orally failed to produce anti-inflammatory activity in rats, while increasing the dose of both to 50mg/kg produced significant anti-inflammatory effect.

The discrepancy of the results of different studies as regards the anti-inflammatory or pro-inflammatory activity of calcium salts could be explained on the basis of the difference in animal models of inflammation used, the dose, the time and the route of administration of calcium salts. With previous known studies, calcium interaction with etoricoxib appears to be of a pharmacodynamic nature.

Human equivalent dose of etoricoxib 8mg/kg shows adverse effect like prothrombotic events on long term administration. Combination of lower dose, like etoricoxib 5mg/kg with calcium 50 mg/kg can prevent the complications of long term use of etoricoxib 8 mg/kg.

The results of the present study favour the combined use of etoricoxib and calcium gluconate, if the present findings could be extrapolated to clinical situations. The advantages of such combined preparations are obviously a reduction in the dose of etoricoxib that could still produce significant anti-inflammatory action and a possible protection against COX -2 inhibitor induced cardiotoxic, renotoxic and hepatotoxic events.

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