

**EFFECT OF METHYLXANTHINES ON INFLAMMATION AND THEIR INTERACTIONS WITH NSAIDs IN WISTAR RATS.**

**S.M. Supale, <sup>1</sup>P.A. Patil\*, <sup>1</sup>Suneel. I. Majagi**

KLE College of Pharmacy and <sup>1</sup>Department of Pharmacology, J.N. Medical College,  
Nehru Nagar, Belgaum-590010, Karnataka- India.

**Summary**

In the present study, anti inflammatory activity of methylxanthines viz, theophylline and caffeine is studied in both acute and sub-acute model of inflammation in rats. The study also aimed to elicit the possible interaction of these methylxanthines with aspirin and paracetamol, in acute and sub-acute models of inflammation.

Both the methylxanthines in their therapeutic equivalent dose exerted significant anti-inflammatory activity. The sub anti-inflammatory (SAI) doses of theophylline (4mg/kg), caffeine (10mg/kg) when coadministered with SAI dose of aspirin (54mg/kg) and paracetamol (100mg/kg) showed significant anti-inflammatory activity in both acute and subacute model of inflammation.

Caffeine and theophylline in their therapeutic equivalent doses found to be toxic to gastric mucosa. Present findings indicate that SAI of theophylline coadministered with that of aspirin or paracetamol potentiates the anti-inflammatory activity and reduces gastrotoxicity of the latter NSAIDs. However, ulcerogenic potential of theophylline can be minimised by coadministering famotidine. Such an interaction of theophylline with NSAIDs like, aspirin and paracetamol worth exploiting clinically, if the present findings could be extrapolated to humans.

**Key words: Aspirin, Caffeine, Inflammation, Interaction, Paracetamol, Theophylline.**

**\*Corresponding author:** Dr. P.A.Patil, Professor,

Department of Pharmacology, J.N. Medical College,

Nehru Nagar, Belgaum-590010.

Karnataka- India.

E-mail: drpapatil@yahoo.co.in

Phone: 0831-24091828, Fax: 08312470759.

## **Introduction**

Inflammation is a common clinical condition encountered in day-to-day practice and is the central event for pathogenesis of many diseases such as asthma, allergy etc. Many such anti-inflammatory agents well established in clinical practice, include steroidal as well as non-steroidal anti-inflammatory drugs (NSAIDs). For more than 100 years NSAIDs have been in use for symptomatic relief of painful and inflammatory conditions. Steroidal drugs though not used routinely, can sometimes be useful when NSAIDs fail to produce desired relief. Unfortunately, adverse effects of both the groups of anti-inflammatory agents often limit their use.

Intensive investigations in the past years regarding the pathogenesis of inflammation have revealed a number of inflammogens and various mechanisms that contribute for the process of inflammation, indicating possibility of developing newer agents other than steroids and NSAIDs. In fact, a number of drugs unrelated to steroids and NSAIDs have been investigated for their possible anti-inflammatory activity include calcium channel blockers [1,2], catecholamines [3], tetracycline antibiotics [4], sulfonamides [5]. Recognition of cAMP as a suppressant of inflammogenic signal has opened avenue for a new class of anti-inflammatory agents. Logically, increasing cAMP levels either by promoting its formation or by its sustained preservation by inhibiting its breakdown could suppress inflammation [6]. Phosphodiesterase (PDE) inhibitors therefore could be effective anti-inflammatory agents. PDEs represent bottleneck enzymes in the regulation of cyclic nucleotide turnover [7]. PDEs occur widely in the biological systems of mammalian tissue except in red blood cells. There are at least eleven sub types of PDEs recognised in man, creatively labelled as PDE1 to PDE11. It has been reported that PDE 4 predominates at the site of inflammation [8].

Some specific PDE4 inhibitors such as quinazolinone (CP-77059) and rolipram have been already proved to be anti-inflammatory in acute and chronic models of inflammation [9]. Similarly, Zardaverine, a cAMP specific PDE3/4 inhibitor by virtue of its anti-inflammatory activity has shown to be beneficial in bronchial asthma [10].

Clinically used methylxanthines, like theophylline and caffeine are known to produce their pharmacological effects by inhibiting PDE and by blocking adenosine receptors. Theophylline, a non-specific PDE inhibitor is used in the treatment of bronchial asthma while the other non-specific PDE inhibitor, caffeine is quite often combined with other drugs for the treatment of pain disorders.

It has been proposed that theophylline and newer selective PDE4 isoenzyme inhibitors can inhibit the activation of inflammatory cell types such as T-lymphocytes, eosinophils, mast cells and macrophages, in vitro [11]. This evidence suggests that beneficial effects of theophylline in bronchial asthma are not only due to bronchodilator activity but also due to its anti-inflammatory activity since this disorder is associated with an element of inflammation.

This is further supported by the studies which showed that low dose of oral theophylline attenuates airway inflammatory response to allergen inhalation in atopic asthma [12]. Recent study has shown that non-specific PDE inhibition by theophylline has a characteristic profile of action on monocyte arachidonic acid metabolism resulting in a decrease in leukotriene B4 (LTB<sub>4</sub>), a potent inflammogen.

Surprisingly, another study showed that reversal of anti-inflammatory effects of methotrexate by non-selective adenosine receptor antagonists like theophylline and caffeine [13], while another study showed potentiation of leukotriene B<sub>4</sub>-mediated inflammatory response by adenosine antagonist, 8-phenyl theophylline. These reports suggest proinflammatory activity of theophylline [14].

In view of these equivocal reports regarding anti-inflammatory activity of theophylline and paucity of information regarding its interaction with NSAIDs, the present study was planned to probe the anti-inflammatory activity of methylxanthines and also their interaction with commonly used NSAIDs viz, aspirin and paracetamol in Wistar rats.

### **Materials and methods**

**Animals:** The complete course of experiments were carried out using healthy male rats of Wistar strain, weighing between 100-150 grams. The animals were acclimatized to normal laboratory conditions with 12-hr natural light-dark cycle and were maintained on standard laboratory diet with free access to water.

**Drugs used and their doses:** The clinical doses of the drugs were converted into rat equivalent doses with the help of converting table [15]. The drugs used were theophylline 10 mg/kg (I.P. grade powder, courtesy Unicure India Pvt. Ltd), caffeine 18 mg/kg (I.P. grade powder, courtesy Nicholas Piramal India Ltd., Mumbai), aspirin 200 mg/kg (I.P. grade powder, courtesy Nicholas Piramal India Ltd., Mumbai), paracetamol 100 mg/kg (I.P. grade powder, courtesy Nicholas Piramal India Ltd., Mumbai), famotidine 3.6 mg/kg (tablet Topcid-20 of Torrent Pharmaceuticals Ltd. purchased from local market).

After confirming the anti-inflammatory activity with their therapeutic equivalent dose in carrageenan (acute) induced inflammation, a series of experiments were conducted to elicit their dose immediate next to effective dose that just failed to show anti-inflammatory activity and was taken as sub-antiinflammatory (SAI) dose. The SAI dose (mg/kg) were found to be 4, 10 and 54 for theophylline, caffeine and aspirin respectively. However, paracetamol 100 mg/kg showed weak but insignificant anti-inflammatory activity hence the same was taken as SAI dose for interaction study.

In acute studies all the treatments were administered to different groups of animals (n=6 in each) in a single dose, thirty minutes prior to subplantar injection of carrageenan while in sub acute studies the treatment was started after implanting the sterile foreign bodies and continued every 24 hours for 10 days. Control animals received equivalent volume of gum acacia suspension. All the drugs were administered orally as a suspension with 1% gum acacia.

**Acute inflammation:** Overnight fasted (with water ad lib) animals were subdivided in to a control and 8 treatment groups to receive the dose (mg/kg) of (i) Theophylline 10, (ii) Caffeine 18, (iii) Aspirin 200, (iv) Paracetamol 100. Two groups received theophylline 4 or caffeine 10 in addition to aspirin 54 and another two groups received theophylline 4 or caffeine 10 in addition to paracetamol 100. Acute inflammation was produced by subplantar injection of 0.05 ml of 1% carrageenan (from sigma co. St Louis) in left hind paw. A mark was put on the leg at the malleolus to facilitate uniform dipping at subsequent readings. The paw volume was measured with the help of plethysmograph by mercury displacement method at zero hour (immediately after injecting carrageenan). The same procedure was repeated at 0.5, 1, 2, 3, 4 and 5 hour. The difference between 0 hour and subsequent reading was taken as actual oedema volume.

**Subacute inflammation:** Subacute inflammation was produced by method D'Arcy et.al [16] with some modification. In overnight starved (with water ad lib) rats after clipping the hair in axillae and groin, under light halothane anaesthesia, two sterile cotton pellets weighing 10 mg and two sterile grass piths (25x 3 mm) were implanted subcutaneously, through a small incision. Wounds were then sutured and animals were caged individually after recovery from anaesthesia. Aseptic precautions were taken throughout the procedure. The animals were subdivided in to a control (vehicle) and 9 treatment groups (n=6 in each) to receive the dose (mg/kg) of aspirin 200, caffeine 18, paracetamol 100 individually. Other two groups received theophylline 10 alone and in combination with famotidine 3.6. While another two groups received theophylline 4 with aspirin 54 or paracetamol 100. Remaining two groups received caffeine 4 with aspirin 54 or paracetamol 100. The treatments were started after implantation and was repeated every twenty four hours, regularly for ten days.

On the eleventh day the rats were sacrificed with an overdose of anaesthesia to remove cotton pellets, grass piths and stomachs. The pellets, free from extraneous tissue were dried overnight at 60 °C to note their dry weight. Net granuloma formation was calculated by subtracting initial weights of cotton pellet (10mg) from the weights noted. Mean granuloma dry weight for various groups was calculated and expressed as mg/100 gm of body weight. The grass piths were preserved in 10% formalin for histopathological studies.

**Ulcer index:** Stomachs were cut open along the greater curvature and gently washed with normal saline. Gastric mucosa was examined for the presence of erosions, haemorrhagic spots, ulcer and perforation if any, with the help of magnifying lens. To determine the severity of the ulcer, an arbitrary scoring system as described earlier [17] was followed. Ulcer index was calculated as mean score of ulcer severity in all the treated groups and was compared with that of control.

All the procedures were performed in accordance with the CPCSEA guidelines and the study was approved by IAEC.

**Statistical Analysis:** Data were expressed as Mean  $\pm$  SEM and analysed by ANOVA followed by Dunnet's test with the help of graph pad prism software and 'p' value  $<0.05$  was considered as significant.

### Results

**Table I: Effect of various treatments on carrageenan induced rat paw oedema.**

Groups (n=6)	Drugs and Dose mg/kg	Paw volume in ml (Mean $\pm$ S.E)					
		0.5 Hr	1 Hr	2 Hr	3 Hr	4 Hr	5 Hr
1	Control	1.15 $\pm 0.179$	1.55 $\pm 0.201$	2.01 $\pm 0.221$	2.17 $\pm 0.306$	1.94 $\pm 0.318$	1.31 $\pm 0.203$
2	Theophylline 10	0.79* $\pm 0.080$	0.98** $\pm 0.088$	1.06** $\pm 0.094$	1.06** $\pm 0.155$	0.92** $\pm 0.171$	0.79* $\pm 0.082$
3	Caffeine 18	0.68** $\pm 0.080$	0.96** $\pm 0.168$	1.21** $\pm 0.171$	1.46* $\pm 0.158$	1.39* $\pm 0.142$	1.21 $\pm 0.253$
4	Aspirin 200	0.48** $\pm 0.060$	0.60** $\pm 0.046$	1.00** $\pm 0.174$	1.17** $\pm 0.165$	0.96** $\pm 0.148$	0.65** $\pm 0.102$
5	Paracetamol 100	0.97 $\pm 0.072$	1.25 $\pm 0.065$	1.79 $\pm 0.060$	1.98 $\pm 0.082$	1.74 $\pm 0.075$	1.26 $\pm 0.109$
6	Theophylline 4 with Aspirin 54	0.54** $\pm 0.090$	0.70** $\pm 0.103$	1.31** $\pm 0.153$	1.71 $\pm 0.133$	1.54 $\pm 0.130$	1.24 $\pm 0.125$
7	Theophylline 4 with Paracetamol 100	0.74* $\pm 0.075$	0.89** $\pm 0.090$	1.40** $\pm 0.150$	1.82 $\pm 0.178$	1.71 $\pm 0.157$	1.43 $\pm 0.103$
8	Caffeine 10 with Aspirin 54	0.63** $\pm 0.069$	0.83** $\pm 0.082$	1.12** $\pm 0.097$	1.43* $\pm 0.109$	1.36* $\pm 0.117$	1.21 $\pm 0.128$
9	Caffeine 10 with Paracetamol 100	0.80* $\pm 0.076$	0.99** $\pm 0.133$	1.32** $\pm 0.101$	1.73 $\pm 0.166$	1.70 $\pm 0.080$	1.16 $\pm 0.124$

ANOVA followed by Dunnet's test,  $p < 0.05^*$  and  $p < 0.01^{**}$ .

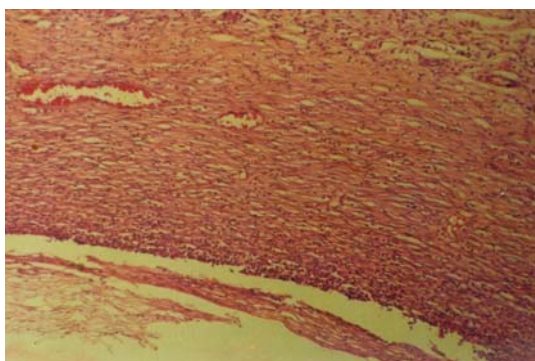
**Table II: Effect of various treatments on foreign body induced granulomas and ulcer index.**

Groups (n=6)	Drugs and Dose mg/kg	Granuloma dry weight (mg/100 g. B.W) Mean± S.E.	Ulcer Index
1	Control	21.11±0.45	8.33±4.01
2	Theophylline 10	15.88±0.73**	21.66±3.07*
3	Caffeine 18	12.28±1.00**	25.00±5.62**
4	Aspirin 200	9.78±0.60**	41.66±1.66**
5	Paracetamol 100	20.79±0.39	13.33±3.33
6	Theophylline 4 with Aspirin 54	12.00±0.68**	20.00±3.65
7	Theophylline 4 with Paracetamol 100	13.83±0.78**	13.33±3.33
8	Theophylline 10 with Famotidine 3.6	15.13±0.99**	6.66±3.33
9	Caffeine 10 with Aspirin 54	11.90±0.94**	21.66±3.07*
10	Caffeine 10 with Paracetamol 100	13.10±1.73**	16.66±3.33

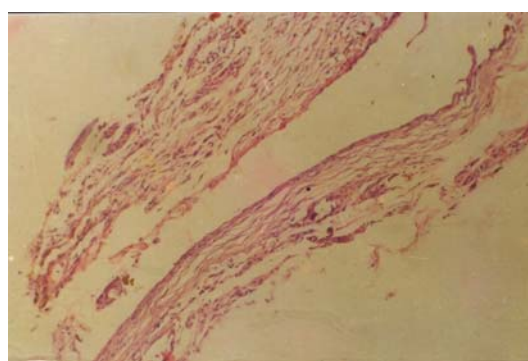
ANOVA followed by Dunnet's test,  $p < 0.05^*$  and  $p < 0.01^{**}$ .

**Figure 1 . Microphotographs of granulation tissues stained with H&E (100X)**

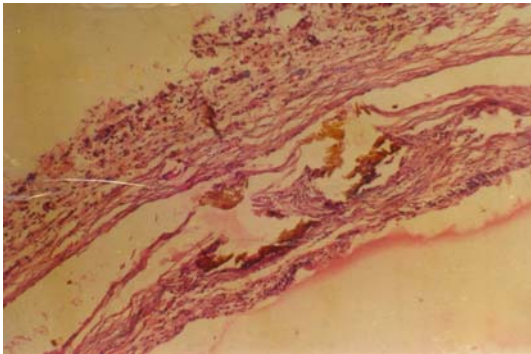
a. Control (vehicle)



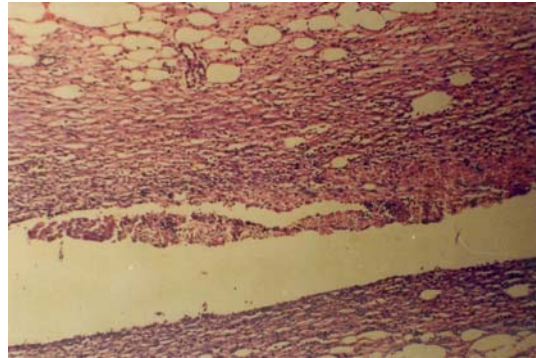
b. Theophylline (10 mg/kg)



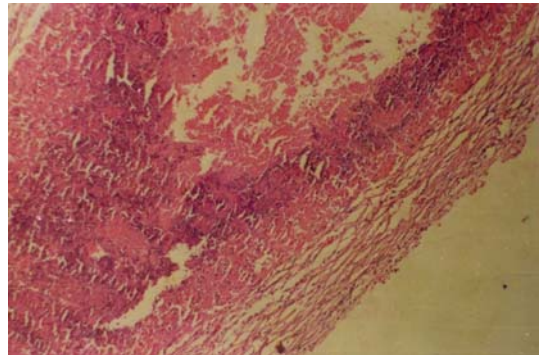
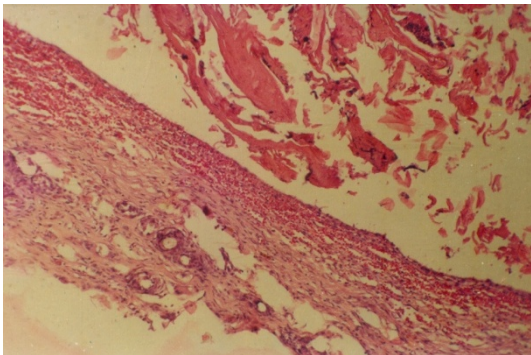
c. Caffeine (18 mg/kg)



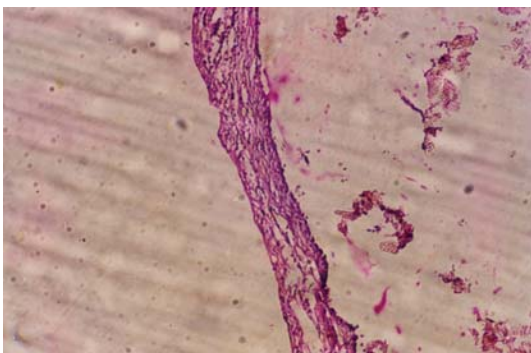
d. Paracetamol (100 mg/kg)



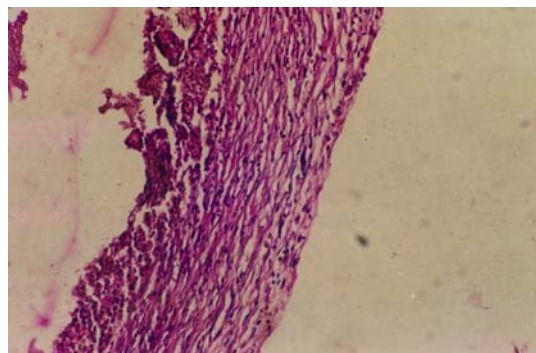
e. Theophylline(4 mg/kg) with Paracetamol (100 mg/kg) f. Caffeine (10 mg/kg) with Paracetamol (100 mg/kg)



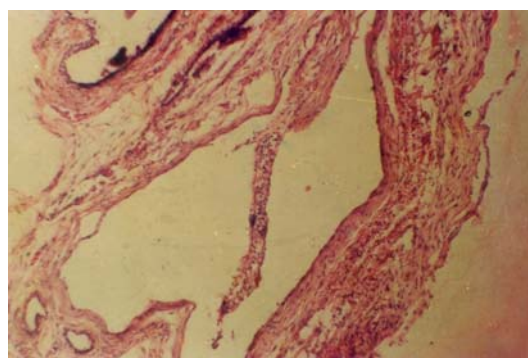
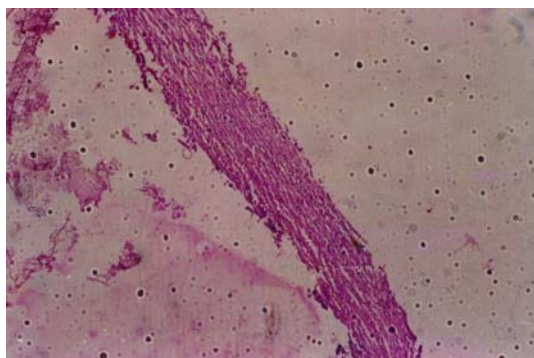
g. Aspirin (200mg/kg)



h. Theophylline(4 mg/kg) with Aspirin (54mg/kg)



i. Caffeine (10mg/kg) with Aspirin (54 mg/kg). j. Theophylline (10mg/kg) with Famotidine (3.6 mg/kg)



**Note:** All the treatment groups b to j except d (paracetamol group) decreased the amount of granulation tissue, fibroblast number and collagen content when compared with that of control a.

### **Carrageenan induced acute inflammation:**

Effects of therapeutic equivalent doses of theophylline, caffeine, aspirin, paracetamol individually and combinations of SAI dose of theophylline or caffeine with that of aspirin or paracetamol are shown in Table I. The results showed that therapeutic doses of theophylline, caffeine (except at 5<sup>th</sup> hr) and aspirin showed significant ( $p < 0.05$  and  $p < 0.01$ ) reduction in the paw volume at 0.5, 1, 2, 3, 4 and 5 hours when compared with that of control value. Whereas combinations of SAI dose of theophylline with that of aspirin or paracetamol and SAI dose of caffeine with that of paracetamol showed significant ( $p < 0.05$  and  $p < 0.01$ ) reduction in the paw volume only at 0.5, 1 and 2 hours when compared with that of control value. Combination of SAI dose of caffeine with that of aspirin showed significant ( $p < 0.05$  and  $p < 0.01$ ) reduction in the paw volume at 0.5, 1, 2, 3 and 4 hours when compared with that of control value. Whereas therapeutic equivalent dose of paracetamol failed to show any significant reduction in the paw volume when compared with that of control.

### **Sub acute inflammation (foreign body induced granulomas):**

The results showed that therapeutic equivalent doses of theophylline, caffeine, aspirin individually and combinations of SAI dose of theophylline or caffeine with that of aspirin or paracetamol, as well as therapeutic equivalent dose of theophylline with that of famotidine decreased mean granuloma dry weight significantly ( $p < 0.01$ ) when compared with that of control. Whereas therapeutic equivalent dose of paracetamol failed to produce any significant reduction in mean granuloma dry weight (Table II).

### **Histopathological studies:**

The grass piths of all the ten groups were subjected to histopathological studies. Sections stained with haematoxylin and eosin when observed under light microscope (100 X) revealed a decrease in the thickness of granulation tissue, collagen content and the fibroblast number in all the treated groups except paracetamol treated group, when compared to that of control group (Fig I).



**Ulcer Index:**

Therapeutic equivalent doses of theophylline, caffeine, aspirin and combination of SAI dose of caffeine with that of aspirin showed significant ( $p < 0.05, p < 0.01$ ) increase in ulcer index, where as in other groups no significant change was observed.

**Discussion**

The results of the present study, clearly indicate that theophylline and caffeine, non-specific PDE inhibitors, in their therapeutic equivalent doses significantly suppressed acute inflammation. These findings agree with earlier report in which xanthenes like theophylline, complamin and diprophylline significantly suppressed paw edema [18].

Similarly, in subacute studies both theophylline and caffeine significantly suppressed cotton pellet induced granuloma formation. There is paucity of information regarding the anti-inflammatory activity of methylxanthines in sub acute inflammation.

Sub anti-inflammatory (SAI) dose of methylxanthines used in the present study (theophylline 4mg/kg and caffeine 10 mg/kg) potentiated anti-inflammatory activity of aspirin (54mg/kg) and paracetamol (100 mg/kg) in acute as well as sub acute studies. The histopathological studies of granulation tissue in various treated groups confirmed the observed anti-inflammatory activity. There appears to be poor documentation of such interaction in the literature. However a study has indicated a synergistic interaction between caffeine and acetylsalicylic acid on the inhibition of nociception [19]. Though the plan of present study, does not permit us to elucidate the nature of interaction, it could be probably of pharmacodynamic nature rather than pharmacokinetic, since there are no reported pharmacokinetic interactions of methylxanthines with aspirin and paracetamol.

Based on the findings of the present study, it is not possible to comment on the mechanism of anti-inflammatory activity of methylxanthines. However in earlier reports various anti-inflammatory mechanisms proposed include: Inhibitory action of theophylline on oxidative metabolite release [20] and also of 3-isobutyl-1-methylxanthine on hydrogen peroxide synthesis [21]. Several PDEs have been shown to exist in inflammatory cells and are responsible for mediator release [22]. Methylxanthines by inhibiting PDEs cause accumulation of cAMP, and agents, elevating cAMP have been proved to be anti-inflammatory [9,23,24]. Caffeine, theophylline and dimethylxanthines have been shown to increase the endogenous serum glucocorticoid level in mice and this may partly be contributing for their observed anti-inflammatory activity [25]. Theophylline has been reported to suppress TNF- $\alpha$ , which is supposed to be a powerful inflammogen [26]. Inhibition of nuclear transcription factor NF-Kappa B, a factor essential for the expression of pro-inflammatory cytokines in human monocytes and T-cells [27], Theophylline has also been shown induction of IL-10, which suppressed pro-inflammatory cytokines including IL-1B, TNF-  $\alpha$ , IL-6, IL-12 and INF- $\gamma$  [28]. Suppression of LTB<sub>4</sub>, a well known inflammogen [29] also partly be responsible for anti-inflammatory activity of theophylline.

In gastric mucosal studies, aspirin as expected produced severe gastric mucosal damage, so also did the methylxanthines.

Present findings indicate that methylxanthines if administered concurrently with NSAIDs like aspirin and paracetamol, the dose requirement of NSAIDs could be reduced, without compromising their anti-inflammatory activity. Findings of the present study also indicate that, co-administered famotidine suppressed ulcerogenicity of theophylline without affecting its anti-inflammatory activity. Similarly SAI dose of both theophylline and caffeine increased anti-inflammatory of aspirin as well as ulcer index. However increased ulcer index in theophylline and aspirin treated group was statistically insignificant.

If the findings of present experimental studies are true to humans, coadministration of low dose of theophylline with that of aspirin along with famotidine could exert better anti-inflammatory action without gastrotoxicity. However clinical studies need to confirm the efficacy and safety of such combination.

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