

**Analgesic activity of allopurinol and febuxostat in experimental animals****Yajnesh P. Sahu, Sachchidanand Pandey\*, Sabita Mohapatra**

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**ABSTRACT**

**Background:** Currently, two classes of analgesics, nonsteroidal anti-inflammatory drugs (NSAIDs) and opioid analgesics are used to manage pain in different clinical situations. Chronic uses of these drugs have various adverse effects like gastric ulceration/bleeding, analgesic nephropathy and respiratory depression, physical dependence, addiction, respectively. Xanthine oxidase inhibitors, used for chronic gout, might have a role in alleviation of pain, as per literature survey. Hence, the present study was carried out to evaluate the potential analgesic activity of allopurinol and febuxostat in different experimental models.

**Methods:** The analgesic activity of allopurinol and febuxostat was assessed by employing two different experimental pain models-tail flick latency model in rats for central analgesia and acetic acid induced writhing model in mice for peripheral analgesia and was compared with tramadol and aspirin.

**Results:** Allopurinol and febuxostat produced significant central and peripheral analgesic effects as is evident from increase in reaction time in tail flick test and inhibition in number of writhes in acetic acid induced writhing test.

**Conclusions:** The results of the present study demonstrate marked analgesic effect of allopurinol and febuxostat.

**Keywords:** Acetic acid induced writhing, Tail flick latency, Xanthine oxidase inhibitor

**INTRODUCTION**

Pain is the most common ailment for which patients seek medical advice. Chronic pain is very distressing where appropriate pain assessments together with adequate pain management are the standard of care in this situation.

Analgesics are drugs which decrease pain sensation by increasing the nociceptive threshold to external stimuli without altering consciousness.<sup>1</sup> Two classes of analgesics are currently available, the nonsteroidal anti-inflammatory drugs (NSAIDs) and Opioid analgesics, but they have significant side-effect like gastric ulceration/bleeding, analgesic nephropathy, increased risk of myocardial infarction, stroke and respiratory depression, constipation, physical dependence, addiction, respectively.

Gout is a chronic, painful, metabolic inflammatory disease, caused due to increased serum uric acid levels in which NSAIDs and corticosteroid are used for acute gout, whereas urate-lowering drugs like Allopurinol, Febuxostat (by inhibition of enzyme Xanthine Oxidase (XO), both synthesis inhibitors) and Probenecid (uricosuric drug) are used for chronic gout.<sup>2-4</sup> Conventionally they do not have any role in reducing pain of acute gout.

The enzyme Xanthine Oxidase (XO) is also responsible for catalyzing other biochemical reactions in the body, like conversion of NADH to NAD where reactive oxygen species (ROS) is released as a by-product, which have been implicated in producing pain and inflammation.<sup>5</sup> Also, XO Inhibitors, by increasing hypoxanthine levels, allow its increased conversion to inosine and inosine monophosphate which gets converted to adenosine

(through purine salvage pathway). Adenosine is found to have a role in the modulation of pain.<sup>6</sup> Recently, chronic inhibition of XO-generated ROS by Allopurinol has been suggested to inhibit symptoms of inflammation, painful diabetic neuropathy in rats and to produce acute antinociceptive activity against a variety of noxious stimuli in mice.<sup>7,8</sup> Febuxostat is a newer orally administered nonpurine XO inhibitor that has been recently approved for the treatment of chronic hyperuricemia in patients with gout.<sup>9</sup> Three randomized controlled clinical trials comparing Febuxostat with Allopurinol showed that 40mg/day Febuxostat lowered serum uric acid to similar levels as 300mg/day of Allopurinol.<sup>10</sup> It also may possess analgesic activity like Allopurinol through the aforementioned mechanisms. These reports lead to the possibility of XO Inhibitors like Allopurinol and Febuxostat to possess analgesic activity of which there is paucity of data. Hence, this study attempts to explore the analgesic activity of Allopurinol and Febuxostat in selected animal models.

## METHODS

The study was conducted in the P.G. Department of Pharmacology, V. S. S. Medical College, Burla after obtaining due approval from Institutional Animal Ethics Committee, VIMSAR, Burla. All the animals were handled carefully as per the CPCSEA guidelines.

### Experimental animals

Adult healthy Wistar albino rats weighing between 150-200gms and Swiss albino mice weighing between 15-20gms were selected for the study. They were kept in polypropylene cages in 12:12 hours light:dark cycle, provided with standard laboratory diet and had water ad libitum. Food was withdrawn 12 hours before and during experimental hours.<sup>11</sup>

### Tail Flick Latency (TFL) test

#### Animals

48 Wistar albino rats were taken for this study.

#### Drugs and reagents

Tramadol (ACME Laboratories Ltd)- Standard drug, Allopurinol (Glaxo-Smith Kline Pharma.), Febuxostat (Zydus Cadila Pharma Ltd.) and 0.5% Carboxy Methyl Cellulose (CMC) (E-Merck India) were used for the study.

The rats were divided into 8 groups of 6 animals in each and were administered with test and control drugs orally through intragastric tube. Group I was used as control receiving 0.5% CMC. Group II was given standard drug Tramadol 10mg/kg. Group III, IV and V received Allopurinol in doses of 50, 100 and 200mg/kg respectively. Group VI, VII and VIII received Febuxostat

in doses of 5, 10 and 20mg/kg respectively. Pre-drug and post-drug TFL was assessed at 0hr, 0.5hr, 1 hr, 2hr and 3hr.

Each rat was placed in the rat holder and the base of the protruding out was placed on the heated nichrome wire (6 Amp) of Analgesimeter (INCO, India). The time taken from the application of heat to the flicking of the tail (TFL) was recorded. The cut-off time was fixed at 10sec to avoid tissue damage. The rats which showed a TFL of 5 to 6 seconds at baseline were included for the study.

### Statistical analysis

One-way ANOVA was used to test statistical significance of the post-drug values from the pre-drug values. The percentage increase / decrease in reaction time (as index of analgesia) at each time interval was calculated.

$$\% \text{ Analgesia (Maximum Possible Effect=MPE)} = (\text{TL-BL} / \text{ML-BL}) \times 100$$

Where ML = Maximum Latency (10sec), TL = Test Latency, BL = Basal Latency

### Acetic acid induced writhing test

#### Animals

48 Swiss albino mice were taken for this study.

#### Drugs and reagents

Aspirin (ACME Laboratories Ltd)-Standard drug and 0.6% Acetic Acid (E-Merck India) were used.

#### Procedure

Mice were intragastrically administered with test and control drugs in a similar fashion as the TFL test, 30 min before Intraperitoneal injection of acetic acid (0.6%). Then mice were placed in separate bell shaped transparent glass jars and numbers of abdominal constrictions (writhes) were counted over a period of 10 minutes commencing 10 min after injection of acetic acid. The difference in number of writhes in test group was compared with standard treated and control treated groups.

### Statistical analysis

One-way ANOVA was used to test statistical significance of the test drug values from the control values. The percentage increase/decrease in number of writhing (as index of analgesia) was calculated. The Percentage Inhibition was calculated by formula:

$$\% \text{ Inhibition} = [(Wc-Wt) \times 100] / Wc$$

Where Wc= No. of writhes in control group, Wt = No of writhes in test group.

## RESULTS

### Tail flick latency test

In this test, three doses each of Allopurinol and Febuxostat were compared with the standard drug Tramadol and Control drug (0.5% CMC). The results of analgesic activity of drugs by Tail Flick Test are shown in the Table 1.

The Basal latency (predrug TFL) in all groups was comparable. Allopurinol 100 and 200mg/kg and febuxostat 20mg/kg showed significant increase in TFL, in

comparison with predrug TFL value, from 30 min to 3 hour of observation. Tramadol 10mg/kg showed similar effect. However, the effect shown by the test drugs i.e. Allopurinol and Febuxostat was significantly lower than tramadol 10mg/kg. The effect shown with allopurinol 200mg/kg was significantly higher than allopurinol 100mg/kg depicting a dose dependent response. The effect seen with febuxostat 20mg/kg dose was significantly greater than allopurinol 100mg/kg but lower than allopurinol 200 mg/kg. Allopurinol 50mg/kg, febuxostat 5mg/kg and febuxostat 10mg/kg did not show significant increase in TFL. Percentage Analgesia with Allopurinol and Febuxostat in TFL Test in Rats is shown in Table 2.

**Table 1: Effect of allopurinol and febuxostat on tail flick latency of rats.**

Group	Drug / dose (mg/kg)	Mean TFL (in sec±SEM)				
		Basal	30min	1 hr	2 hr	3 hr
I	CMC (0.5%)	3.41±0.08	4±0.08	3.12±0.08	3.69±0.04	3.53 ±0.06
II	Tramadol 10	3.65±0.06	8.13±0.05*#@\$	8.3±0.05*#@\$	8.51±0.05*#@\$	8.38±0.05*#@\$
III	Allopurinol 50	3.19±0.02	3.10±0.02	3.25±0.06	3.27±0.04	3.21±0.06
IV	Allopurinol 100	3.56±0.10	6.05±0.05*	6.20±0.07*	6.44±0.05*	6.28±0.04*
V	Allopurinol 200	3.85±0.04	7.71±0.04*#@\$	7.76±0.03*#@\$	7.84±0.05*#@\$	7.86±0.03*#@\$
VI	Febuxostat 5	3.57±0.09	3.60±0.04	3.39±0.08	3.38±0.08	3.37±0.09
VII	Febuxostat 10	3.59±0.09	3.84±0.12	3.22±0.03	3.22± 0.02	3.11±0.00
VIII	Febuxostat 20	3.5±0.07	6.47±0.06*#	6.91±0.04*#	7.22±0.04*#	7.01±0.05*#

Data were analyzed by ANOVA followed by Post hoc – Tukey Test. Each value is expressed as Mean ± SEM. n=6. \* is p value <0.001 compared to control, # is p value <0.05 compared to Allopurinol 100, @ is p value <0.05 compared to Allopurinol 200, \$ is p value <0.05 compared to Febuxostat 20.

**Table 2: Percentage analgesia with allopurinol and febuxostat in TFL test in rats.**

Drug and dose (mg/kg)	Percentage maximum possible effect (% Analgesia) in Rats			
	30min	1hr	2hr	3hr
Tramadol - 10	70.55	73.22	76.53	74.48
Allopurinol - 100	38.66	40.99	44.72	42.23
Allopurinol - 200	62.76	63.57	64.87	65.20
Febuxostat- 20	45.69	52.46	57.23	54

### Acetic acid induced writhing test

The analgesic effect of Allopurinol and Febuxostat was evaluated with the help of Acetic Acid Induced Writhing Test. The number of writhes in mice was recorded for both the drugs and was compared with that of control (0.5% CMC) and standard (Aspirin). The results of analgesic activity by Acetic Acid Induced Writhing Test are shown in the Table 3.

On comparison with control, allopurinol 100 and 200mg/kg and febuxostat 20mg/kg dose showed significant reduction in number of writhes in mice. Aspirin showed similar effect. However, the effect shown by the test drugs i.e. allopurinol and febuxostat was significantly lower than aspirin 100mg/kg.

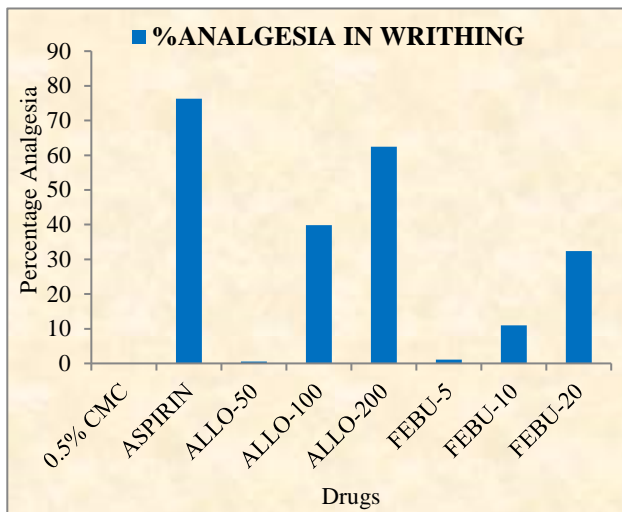
**Table 3: Effect of allopurinol and febuxostat on acetic acid induced writhing response in mice.**

Group	Drug and dose (mg/kg)	No of writhes Mean±SEM	% Protection
I	Control (0.5% CMC)	28.83±1.01	-
II	Aspirin 100	6.83±0.30*#@\$	76.3
III	Allopurinol 50	28.67 ±0.71	0.5
IV	Allopurinol 100	17.33 ±0.33*	39.88
V	Allopurinol 200	10.83±0.47*#@\$	62.43
VI	Febuxostat 5	28.50±0.42	1.14
VII	Febuxostat 10	25.67±1.43	10.96
VIII	Febuxostat 20	19.50±0.50*	32.36

Data were analyzed by ANOVA followed by Post hoc – Tukey Test. Each value is expressed as Mean ± SEM. n=6. \* is p value <0.001 compared to control, # is p value <0.05 compared to Allopurinol-100, @ is p value <0.05 compared to Allopurinol-200, \$ is p value <0.05 compared to Febuxostat 20.

The effect shown with allopurinol 200mg/kg was significantly higher than allopurinol 100mg/kg depicting a

dose dependent response. Allopurinol 50mg/kg, febuxostat 5mg/kg and febuxostat 10mg/kg did not show any change in number of writhes in mice as compared to control. Percentage Analgesia of Allopurinol and Febuxostat in Acetic Induced Writhing Method is shown in Figure 1.



**Figure 1: Bar diagram showing percentage analgesia of allopurinol and febuxostat in acetic induced writhing method.**

## DISCUSSION

There is paucity of data available evaluating the analgesic activity of Allopurinol and Febuxostat. These drugs are already in use since long for the management of chronic painful inflammatory disease like Gout for decreasing uric acid levels. Their additional analgesic activity, if ascertained, can significantly be useful in the treatment of chronic painful conditions as XO Inhibitors are known to be safer than the available NSAIDs and Opioids in terms of having negligible adverse effects.

Allopurinol and febuxostat showed analgesic activity against both experimental models of pain. In TFL test, the 100mg/kg and 200mg/kg dose of allopurinol and 20mg/kg febuxostat showed significant analgesic effect from 30 min to 3 hours of observation time. The peak effect was observed at 2 hours with all the drugs.

However, these effects were significantly less than the standard dose (10mg/kg) of tramadol. Allopurinol produced a dose dependent analgesic effect. Percentage analgesia or MPE was found to be 44.72 and 65.2 for allopurinol 100mg/kg and 200mg/kg dose respectively during their peak effect while that of tramadol was 76.53. Percentage analgesia or MPE of 20mg/kg febuxostat was found to be 57.23 which was significantly greater than 100mg/kg of allopurinol whereas significantly lower than 200mg/kg allopurinol. In this test 50mg/kg allopurinol, 5 and 10mg/kg febuxostat did not show any analgesic activity. These effects corroborate with the findings of

other workers.<sup>12,13</sup> However febuxostat was not included in their study.

In acetic acid induced writhing test in mice, the two high doses of allopurinol i.e. 100 and 200mg/kg and highest dose of Febuxostat i.e. 20mg/kg exhibited significant analgesic effect. The percentage analgesia seen with allopurinol 100 and 200mg/kg and febuxostat 20mg/kg were 39.88, 62.43 and 32.36 respectively which were significantly lower than that of aspirin (76.3). Here also dose dependent analgesic effect of allopurinol was observed. In this test too, 50mg/kg Allopurinol, 5 and 10mg/kg Febuxostat did not show any analgesic activity. Similar effects were observed in Acetic acid induced writhing method by some other workers.<sup>12-14</sup>

Allopurinol and febuxostat possess significant analgesic activity. These drugs may be useful in chronic painful conditions like inflammatory arthritis, sickle cell disease, diabetic neuropathy, fibromyalgia and cancer.

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