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An Experimental study to evaluate the analgesic effect of Probenecid and Allopurinol in Swiss albino mice and it's comparison with Tramadol using Eddy's Hot Plate Analgesiometer

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Abstract

Pain is an important symptom of gout patient. Allopurinol and Probenecid are used in treatment of gout. **Objective:** To evaluate the analgesic activity of Allopurinol and Probenecid in analgesic model of mouse. **Material&Methods**: the analgesic activity of Allopurinol (200 mg/kg) and Probenecid (200mg/kg) was evaluated using Eddy's Hot Plate. Both the drugs were compared with control drug normal saline and standard drug Tramadol & also among themselves. **Result**: Both Allopurinol and Probenecid should significant increase in reaction time at various periods in hot plate method. However Allopurinol has superior analgesic activity than Probenecid. **Conclusion**: Both Allopurinol and Probenecid exhibit central analgesic activity

Keywords: Allopurinol, Probenecid, Eddy's Hot Plate, Tramadol

INTRODUCTION

Pain is an unpleasant sensation but a protective mechanism of our body. Analgesics are defined as substances, which decrease pain sensation by increasing pain threshold to external stimuli without altering consciousness.[1]

Analgesics are drugs which decrease pain sensation by increasing the nociceptive threshold to external stimuli without altering consciousness.[1] 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 painful, metabolic disease that most commonly affects middle aged to elderly men and postmenopausal women. It results from an increased body pool of urate with hyperuricemia.It typically is characterized by episodic acute and

chronic arthritis caused by deposition of monosodium urate crystals in joints and connective tissue tophi.[2] The current management for acute gout include anti-inflammatory
drugs such as non-steroidal anti-inflammatory drugs (NSAIDs), corticosteroids, and for chronic gout include urate-lowering treatment such as allopurinol, febuxostat,and probenecid.[3] NSAIDs and corticosteroids have many noted serious adverse effects.[4,5] Therefore, analgesic drugs lacking the side effect as an alternative to NSAIDs and corticosteroids in the management of acute gout is on demand. Allopurinol is a potent inhibitor of xanthine oxidase. It inhibits the transformation of hypoxanthine to xanthine and uric acid thereby reducing uric acid formation and purine degradation.[6] This leads to an increase in the concentration of hypoxanthine. Hypoxanthine is converted to inosine, inosine monophosphate (IMP), and consequently to adenosine via the purine salvage pathway.[7] Adenosine is a purine nucleoside that occurs naturally in all cells of the body. Its role in the modulation of pain has been confirmed by receptor-mediated action In spinal, supraspinal sites, and peripheral sites.[8] It may be related to the inhibition of neuronal conduction by: Increase in K+ conductance; decrease in substance P and glutamate;[9] and attenuation of basal as well as N-methyl- D-aspartate (NMDA) induced nitric oxide production which is an excitatory neurotransmitter.[10]
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.[11,12,13]
Probenecid a uricosuric agent, competitively blocks acid transport processes, predominantly in the kidney. Temperature-activated transient receptor potential ion channels (thermoTRPs) are known to function as ambient temperature sensors and are also involved in peripheral pain sensation. The thermoTRPs are activated by a variety of chemicals, of which specific activators have been utilized to explore the physiology of particular channels and sensory nerve subtypes. A study observed that under inflammation, probenecid elicited nociceptive behaviors in in vivo assays. These results suggested that TRPV2 is specifically activated by probenecid.[14]
L-Kynurenine is the primary product of tryptophan metabolism. L-Kynurenine, rather than kynurenic acid, its metabolite, crosses the blood– brain barrier to reach the central nervous system. L-Kynurenine and kynurenic acid produce antinociception in acute and inflammatory pain. The L-kynurenine/probenecid combination reduces tactile allodynia in rats. Intrathecal acid reduces tactile allodynia and this effect is enhanced by probenecid since it increases kynurenic acid levels in cerebrospinal fluid.[15]
This study was done to find out the analgesic effect of the allopurinol and probenecid in Swiss albino mice and its comparison with standard drug tramadol.

MATERIAL & METHODS

Study was performed in the department of Pharmacology & Therapeutics, King George's Medical University, Lucknow after getting approval from the Institutional Animal Ethics Committee (IAEC).

Experimental Animals:

24 Adult male swiss albino mice of similar body constitution (in terms of age, body weight), weighing 20-30 gm had been used in the study. Mice were procured from animal house of Indian Institute of Toxicology Research (IITR), Lucknow. IITR is one of the certified center by Committee for the Control and Supervision of Experiments on Animals (CCSEA) for breeding and housing of animals. The animals were allowed to access food and water ad libitum and were retained in the institutional animal house of King George's Medical University (KGMU) under temperature controlled environment [25º±2ºC], humidity (60% ± 10%) with 12 hours light/12 hours dark cycle. All experiments were carried out between 09.00 and 17.00 hrs. The animals were housed for two weeks prior to the experiments to acclimatize to laboratory temperature. The care of animals was done as per CCSEA guidelines. The maintenance of the animals and the experimental procedures were in accordance with the Guide for the Care and Use of Laboratory Animals' published by the National Institute of Health (NIH Publication no. 85-23 revised 1996, Latest revision in 2011) and the guiding principles of IAEC which strictly adhered to the guidelines of CCSEA.
Swiss albino mice (male) weighing between 20-30 gm were irregularly divided into 4 groups, each group containing 6 mice.
Group 1: Mice were administrated normal saline (0.25 ml) i.p. (Intraperitonial)
Group 2: Mice were administrated probenecid (200 mg/kg BW) i.p. (Intraperitonial)
Group 3: Mice were administrated allopurinol (200 mg/kg BW) i.p. (Intraperitonial)
Group 4: Mice were administrated standard drug tramadol (20 mg/kg BW) i.p. (Intraperitonial)
Drugs were taken from the authorized medical store.

Table 1: The number of group and animals taken for the experiments.

Group		**Number of mice**
Group1	Normal saline (Control)	**6**
Group2	Probenecid (Testdrug1)	**6**

Group3	Allopurinol (Testdrug2)	**6**
Group4	Tramadol (Standard drug)	**6**

The mice were permitted to habituate to laboratory environment prior to testing .
The method use was Eddy's Hot Plate In this method, before doing the experiment the hot plate was put for a temperature 55±1ºC. Then each mouse was gently put down on the hot plate, animals which showed paw licking or jump responses within 6-8 sec were chosen for the study. This test was used for assessment of centrally acting analgesic drugs. Each animal was tried out before experiment for control reaction time (at 0 min). The animals were treated with respective drugs and reaction time was again measured at 0, 30, 60, 90, 120 min after administration of test/standard/control drug. The response time was noted as the time at which animals reacted to the pain stimulus either by jumping, withdrawal of the paws, paw licking , whichever appeared first. The cut off time for the reaction was 15 sec. to avoid damage to the paws.

The reaction time of all animals towards thermal heat was recorded. The mean reaction time for each treated group was determined and compared with that obtained for each group before treatment.
Each of above mentioned drugs were dissolved/diluted in normal saline (vehicle) just before administration. The strength of solution was adjusted in such a way that 1 ml of solution contained the desired dose that was to be administered in an individual mouse.

RESULT
Analgesic effect of drugs, reaction time on Eddy's hot plate was recorded just after administration of drug (0 min), at 30 min, 60 min, 90 min and 120 min.

Table 2: Intergroup Comparison of Analgesic Effect at different time intervals

SN	**Group**	**0min**		**30min**		**60min**		**90min**		**120min**	
		Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
1-	Group1	4.80	0.32	4.99	0.22	4.97	0.17	4.97	0.17	4.99	0.18
2-	Group2	5.18	0.52	5.17	0.52	7.92	0.58	8.37	0.40	8.89	0.30
3-	Group3	4.87	0.19	8.42	0.34	9.21	0.32	10.01	0.45	8.10	0.57
4-	Group4	4.88	0.25	12.62	0.45	13.47	0.41	14.57	0.38	8.65	0.65
ANOVA		F=1.454;p=0.257		F=480.966;p<0.001		F=468.997;p<0.001		F=712.074;p<0.001		F=89.698;p<0.001	

At 30 min, 60 min and 90 min, maximum duration of stay on Eddy's hot plate was of Group 4, followed by that of Group 3, Group 2 and least of Group 1.

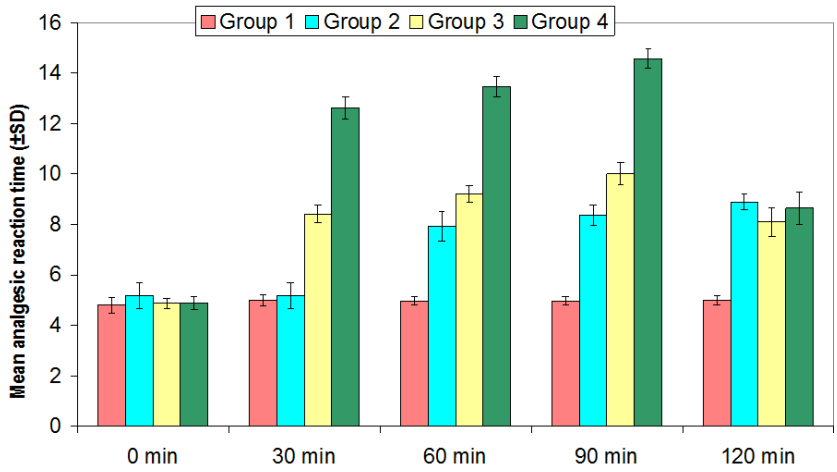
Intergroup differences were found to be statistically significant, on exploring between group differences at 30 min, Group 1 and Group 2 had comparable duration of stay at hot plate, rest of the between group differences were significant.

On 120 min, maximum duration of stay on Eddy‘s hot plate was of Group 2 (8.89±0.30 sec) followed by Group 4 (8.65±0.65 sec) while minimum stay was of Group 1 (4.99±0.18 sec) followed by Group 3 (8.10±0.57 sec). Group 1 had significantly lower duration of stay on hot plate as compared to rest of the groups, Group 2 had significantly higher duration of stay on hot plate as compared to Group 3. Group 2 and Group 3 had comparable duration of stay on Eddy‘s hot plate.

Table 3: Between Group difference in Analgesic effect at different time intervals

SN	Group	0min			30min			60min			90min			120min		
		Mean diff.	SE	‘p’	Mean diff.	SE	‘p’	Mean diff.	SE	‘p’	Mean diff.	SE	‘p’	Meain diff.	SE	‘p’
1-	1vs.2	-0.38	0.20	0.247	-0.18	0.23	0.859	-2.95	0.23	<0.001	-3.40	0.21	<0.001	-3.90	0.27	<0.001
2-	1vs.3	-0.07	0.20	0.982	-3.43	0.23	<0.001	-4.24	0.23	<0.001	-5.04	0.21	<0.001	-3.11	0.27	<0.001
3-	1vs.4	-0.09	0.20	0.970	-7.63	0.23	<0.001	-8.50	0.23	<0.001	-9.60	0.21	<0.001	-3.66	0.27	<0.001
4-	2vs.3	0.31	0.20	0.421	-3.25	0.23	<0.001	-1.29	0.23	<0.001	-1.64	0.21	<0.001	0.78	0.27	0.040
5-	2vs.4	0.30	0.20	0.463	-7.45	0.23	<0.001	-5.56	0.23	<0.001	-6.21	0.21	<0.001	0.24	0.27	0.810
6-	3vs.4	-0.02	0.20	1.000	-4.20	0.23	<0.001	-4.26	0.23	<0.001	-4.57	0.21	<0.001	-0.55	0.27	0.214

Graph 4: Intergroup Comparison of Analgesic Effect (Reaction time on Eddy’s hot plate)



According to the Table 4 at 0 min (baseline) stay on Eddy‘s hot plate was maximum for Group 2 (5.18±0.52 sec) followed by Group 4 (4.88±0.25 sec) while minimum stay was of Group 1 (4.80±0.32 sec) followed by Group 3 (4.87±0.19 sec). On comparing intergroup and between group differences, none of the differences were found to be significant i.e. stay on Eddy‘s hot plate of experimental animals of above four groups was found to be comparable.

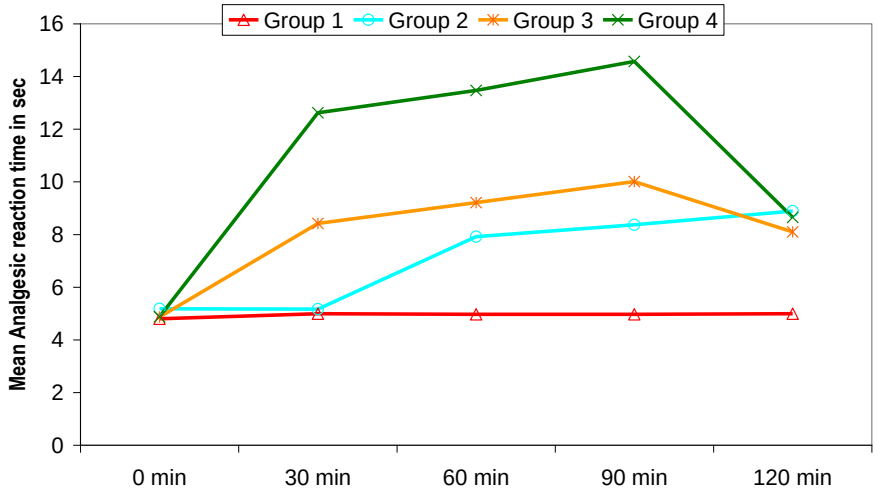
Table 5: Intragroup change in Baseline Analgesic effect (Paired ‘t’ test)

Group	Time	Mean change	SD	%Change	‘t’	‘p’
Group1	BL-30min	0.20	0.24	4.10	2.04	0.097
	BL-60min	0.17	0.22	3.58	1.89	0.117
	BL-90min	0.17	0.27	3.61	1.59	0.173

	BL-120min	0.20	0.24	4.10	2.05	0.096
Group2	BL-30min	-0.01	0.03	-0.10	-0.49	0.646
	BL-60min	2.74	0.68	52.83	9.83	<0.001
	BL-90min	3.19	0.42	61.55	18.65	<0.001
	BL-120min	3.71	0.49	71.59	18.45	<0.001
Group3	BL-30min	3.55	0.22	72.93	39.17	<0.001
	BL-60min	4.34	0.39	89.12	27.19	<0.001
	BL-90min	5.14	0.45	105.48	27.92	<0.001
	BL-120min	3.23	0.56	66.39	14.18	<0.001
Group4	BL-30min	7.74	0.47	158.54	40.19	<0.001
	BL-60min	8.59	0.43	175.96	48.70	<0.001
	BL-90min	9.69	0.40	198.53	59.23	<0.001
	BL-120min	3.76	0.64	77.12	14.51	<0.001

In Table 5. on comparing the change in baseline duration of stay, at 30, 60, 90 and 120 min, change was observed to be 4.10%, 3.58%, 3.61% and 4.10% respectively. In Group 1, change in baseline duration of stay on hot plate were not found to be significant at any of the period of observation.
In Group 2, a slight non-significant decline in baseline duration of stay on hot plate was observed at 30 min (0.10%; p=0.646). Thereafter, at 60 min, 90 min and 120 min duration of stay was significantly higher than that at baseline (52.83%, 61.55% & 71.59% respectively).
In Group 3 and Group 4, at all the periods of observation (30 m, 60 m, 90 m, 120 m) duration of stay was higher than baseline. In Group 3, range of change in baseline duration of stay was 66.39% (120 min) to 105.48% (90 min) of baseline duration. In Group 4, range of change was 77.12% (120 min) to 198.53 (90 min) of baseline duration.

Graph 6 : Change in Baseline Analgesic Effect (Reaction time on Eddy's Hot Plate)



As observed in above graph period of stay on Eddy‘s hot plate of experimental animals of Group 1 shows almost straight line, i.e. minimal change in duration of stay on Eddy‘s hot plate was observed. In group 2 it was slightly higher than that the baseline. In group 3 and group 4 group there was significant increase in the height than the baseline which was also denoted in the above description of table 5.

DISCUSSION

Pain is an uncomfortable feeling that is specific to a part of the body. It is the most widespread sign that takes a person to a doctor. This alteration is felt by the free nerve endings (pain receptors). These pain receptors are widespread and are specialized free-nerve endings that react to extremes released from damaged cells in terms of temperature, pressure, and chemicals.

In the current study, Eddy's hot plate tested the analgesic effect of Allopurinol and Probenecid. The paws of mice are heat sensitive but do not harm the skin. The responses are in the form of hopping, paw removal and paw licking. The reaction time of mice that are dropped on a heated surface and thus faced with a heat stimulus applied to the plantar surface was measured by this method. A sensitive and precise tool used to demonstrate the involvement of central mechanism nociception is pain caused by thermal stimulation of the hotplate.[16,17]. In general, centrally acting analgesics increase the pain threshold (reaction time) of animals against heat, whereas peripheral analgesics such as acetylsalicylic acid or phenyl-acetic acid do not normally affect these responses. Tramadol, the key analgesic drug, was used as the standard drug. Antinociception caused by Tramadol is mediated by opioid (mu) and nonopioid (monoamine uptake inhibition) pathways. Naloxone only partly antagonizes by its analgesic activity and, unlike most opioids, reduces norepinephrine and serotonin reuptake and therefore activates monoaminergic spinal pain inhibition [18, 19].

In terms of analgesic effect, Allopurinol is superior to Probenecid, however the best effect is present in Tramadol which was the standard drug. It can be inferred from the findings that the test drugs may exhibit anti-nociceptive behavior by both central and peripheral mechanisms

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