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The study to evaluate the antidepressant effect of Probenecid and Allopurinol in Swiss albino mice and it's comparison with imipramine using forced swim test

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Abstract

Background: Depression is a prevalent psychiatric disorder with multi factorial origins, including neurochemical imbalances and oxidative stress. Current antidepressants have limitations in efficacy, onset of action, and side-effect profiles, prompting the need for novel therapeutics approaches.

Objective: This study aimed to evaluate the potential antidepressant effects of Probenecid and Allopurimol, both xanthine oxidase inhibitors, in Swiss albino mice, and to compare their efficacy with Imipramine, a standard antidepressant, using the Forced Swim Test (FST).

Methods: A total of 24 male Swiss albino mice were randomly assigned to four Groups: control (saline), Probenecid (200 mg/kg), Allopurinal (200 mg/kg), and Imipramine (10 mg/kg). The FST was conducted on days 1, 10, 20, and 30, and the duration of immobility was recorded as an index of depressive behavior. A decrease in immobility time indicates antidepressant activity.

Results: No significant differences in immobility duration were observed among the groups on days 1 and 10. However, significant reductions were noted on days 20 and 30 in the Probenecid, Allopurinol, and Imipramine groups compared to the control. The antidepressant effect was most pronounced in the Imipramine group, but both Probenecid and Allopurinol also demonstrated substantial efficacy, with statistically significant decreases in immobility time from baseline. The data suggest a role for oxidative stress modulation in depression and support the involvement of xanthine oxidase inhibition.

Conclusion: Probenecid and Allopurinol exhibit significant antidepressant-like effects in mice, comparable to imipramine. These findings highlight the potential of xanthine oxidase inhibitors as promising candidates for the treatment of depression, likely through mechanisms involving oxidative stress reduction and serotonin metabolism enhancement.

Keywords: Depression, Forced Swim Test, Allopurinol, Probenecid, Imipramine, Swiss albino mice, Xanthine oxidase, Oxidative stress

INTRODUCTION

Depression is a widespread chronic psychiatric condition which can affect the physical, emotional, and social development of adults[1]. It is an affective disorder characterized by the change in mood.
It is a complex condition with several contributing factors at different, genetic, psychological, biological and social levels[2].

Depression is a commonly encountered psychological ailment and It is the global reason of disability. Universally, a projected 264 million people are suffering with it. Preponderance of female sex is frequently found [3,4].Its characteristic feature is mood change. It is a foremost problem of community health concern in terms of prevalence, morbidity, suffering, dysfunction, illness and economic burden [5,6].

A sense of sadness, low mood, disliking in performance of routine activities, blaming oneself, troubled sleeping, loss of appetite, low energy, weariness, low self-regard, social isolation and poor concentration[4,7,8]. The patients also have several physical grievances with no known origin. It may be continuous or persistent, significantly harming patient's capability of work performance and to handle routine situations. Subsequently it may cause suicidal thoughts[4].

In general, it is basically of two types, major i.e. unipolar depression or manic-depressive illness i.e. bipolar depression. Major depression is known as depressed mood on a routine basis for a minimal period of two weeks[9] . Though this type draws more concern but the fundamental mechanism is mostly unspecified[10]. According to World Health Organization (WHO) report, it will be a most important cause of global disability by 2030 [9,10].

While the minor depression is reserved for those patients who experience at least 2 symptoms of depression for 2 weeks but do not fulfill the standards of major depression[10]. Minor depression responds to pharmacologic treatment but has notable morbidity and mortality[11].

Furthermore, presently accessible therapies have remarkable restrictions (i.e. little reaction rates, refusal to accept therapy, frequent relapses, and a time-gap of several weeks to develop a response), underlining a highest unfilled demand for a novel effective and quick acting antidepressant, especially with the excessive suicide rates in depression[12].

The "monoamine hypothesis" is an essential issue to investigate and study in the arena of depression. It recommends that a lack or disproportion in the monoamine neurotransmitters like serotonin (5-HT), dopamine (DA) and nor-epinephrine (NE) may cause depression. This hypothesis is also reinforced by the fact that the acknowledged antidepressants like monoamine oxidase inhibitors, tricyclic antidepressants and selective serotonin reuptake inhibitors have been recognized to enhance monoamine function[13].

The presently accessible antidepressants require several weeks to months to show their effects. Moreover, they don't have a favourable safety profile. A wide variety of adverse effects are caused by them like epigastric discomfort, tiredness, drowsiness, shivers, urinary retention, postural hypotension, sexual dysfunction, hallucinations, bipolar states etc. Additionally, their metabolism mostly depends on the action of liver cytochrome enzymes that occasionally leads to clinically important drug interactions. Further, development of tolerance or physical dependence for the effects of these antidepressants is also reported[14].

Tryptophan is the aromatic amino acid precursor of serotonin which is also a monoamine neurotransmitter. Enzyme tryptophan pyrrolasemetabolises this amino acid. Xanthine oxidase is
recognized as an endogenous activator of tryptophan pyrrolase enzyme that causes its elevated destruction and reduces its level in the body [15].

There are several prevention programmes that targets children (e.g. concluding defence and emotional care subsequent to sexual abuse) and adults (e.g. concluding psychosocial help following tragedies and struggles) [4].

Moreover, depression of mild to moderate category can be efficiently cured by talking therapies, like cognitive behavioural therapies or psychotherapies. Antidepressants are used for moderate to severe depression but they are not the 1stlinetherapeutic resort for mild depression. They are not recommended for children and are not used as a 1stlineintervention in adolescents, and it should be used cautiously in them[4].

For its proper management several psychosocial factors are considered like identification of stress factors, like monetary issues, problems at workplace or sexual or psychological abuse, surrounding family members and friends. The preservation or renaissance of social networks and activities is imperative[4]

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 ,Lucknow. IITR is one of the certified center by Committee for the Purpose of Control and Supervision of Experiments on Animals 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 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.

Grouping - Swiss albino mice (male) were randomly divided into 4 groups, each group containing 6 mice.
Group 1: Mice were administrated normal saline (0.25 ml) i.p.
Group 2: Mice were administrated probenecid (200 mg/kg b.w.) i.p.
Group 3: Mice were administrated allopurinol (200 mg/kg b.w.) i.p.
Group 4: Mice were administrated standard drug imipramine (10 mg/kg b.w.) i.p.

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	Imipramine (Standarddrug)	**6**

Procedure:
Forced swimming test (FST) is the most widely used pharmacological model for assessing antidepressant activity, described by Porsolt et al. (1978) [16]. The apparatus consisted of transparent cylinder (14 cm diameter and 19 cm height) filled to 12 cm depth with water (25±2°C) so that it could not touch the bottom with its hind paws.[17,18,19] The animal showed an immediate burst of activity, try to escape, and then eventually adopted an ―immobile‖ posture, when makes only those movements necessary to keep its head above water.
Mice were administered normal saline (control group), standard treatment and test drugs at 60 min (for oral administration of drugs) before the test. One hr after administration of drugs, mice were gently dropped individually into transparent cylinder for the 6 min. Score immobility during the last 4 min of the 6-min test session by summing the total time spent immobile (i.e., the time not spent actively exploring the cylinder or trying to escape from it). Included within the time spent immobile are the short periods of slight activity where the animals just make those movements necessary to maintain their heads above water. These four groups received the respective treatment for consecutive 30 days and duration of immobility was noted against 10th, 20thand 30th day. The water was changed after each animal testing. After each test trial the mice were dried before returning to their respective home cages.
The changes in immobility duration were studied after administering drugs in separate group of animals. The mean mobility time of control and drug treated groups was compared.

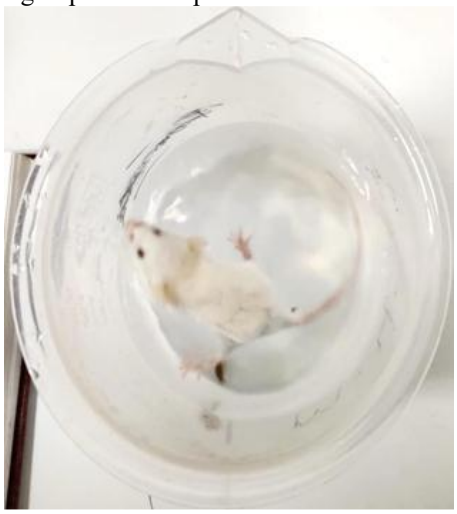


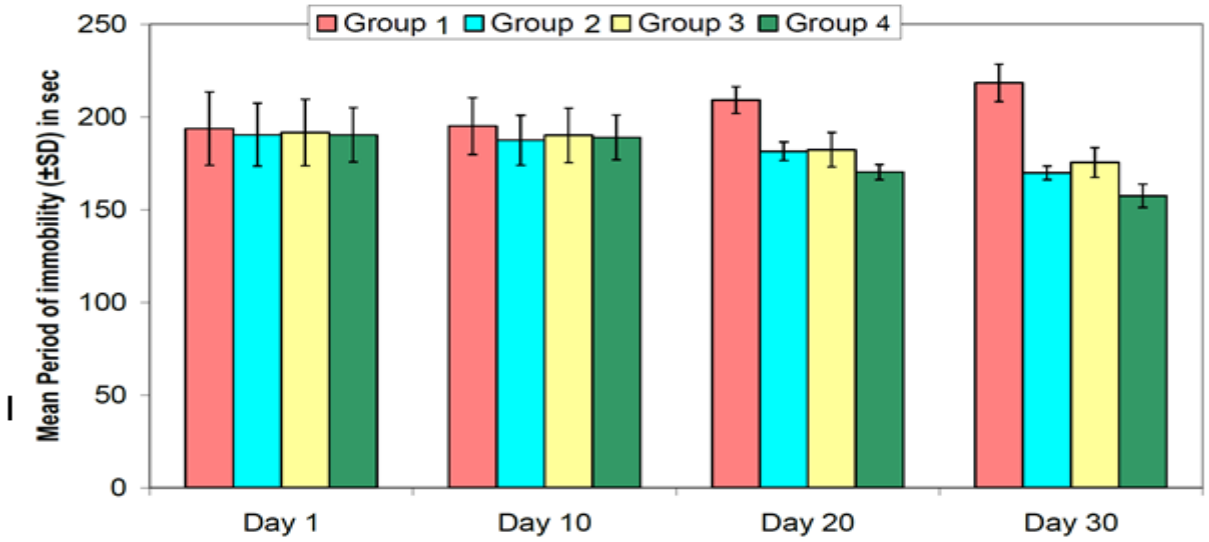
Fig. – Forced Swim Test

Table 1 (a): Intergroup Comparison of Period of Immobility at different time intervals

SN	Group	Day1		Day10		Day20		Day30	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
1-	Group1	193.68	19.78	195.02	15.28	209.01	7.23	218.32	10.02
2-	Group2	190.35	16.93	187.42	13.51	181.44	4.98	169.81	3.71
3-	Group3	191.56	17.85	190.04	14.67	182.26	9.19	175.41	7.97

4-	Group4	190.29	14.60	188.95	12.04	170.25	4.05	157.45	6.30
ANOVA		F=0.050;p=0.985		F=0.334;p=0.801		F=36.480;p<0.001		F=77.358;p<0.001	

On Day 1 (Baseline) and Day 10, period of immobility of above four groups was found to be comparable. Intergroup and between group differences were not found to be significant.



On Day 20 period of immobility was maximum for Group 1 (209.01±7.23 sec) followed by Group 3 (182.26±9.19 sec), Group 2 (181.44±4.98 sec) and minimum for Group 4 (170.25±4.05 sec). Intergroup difference was significant, on exploring between group differences Group 2 & 3 had comparable period of immobility.

Table 1 (b) : Between Group difference in Period of Immobility at different time intervals

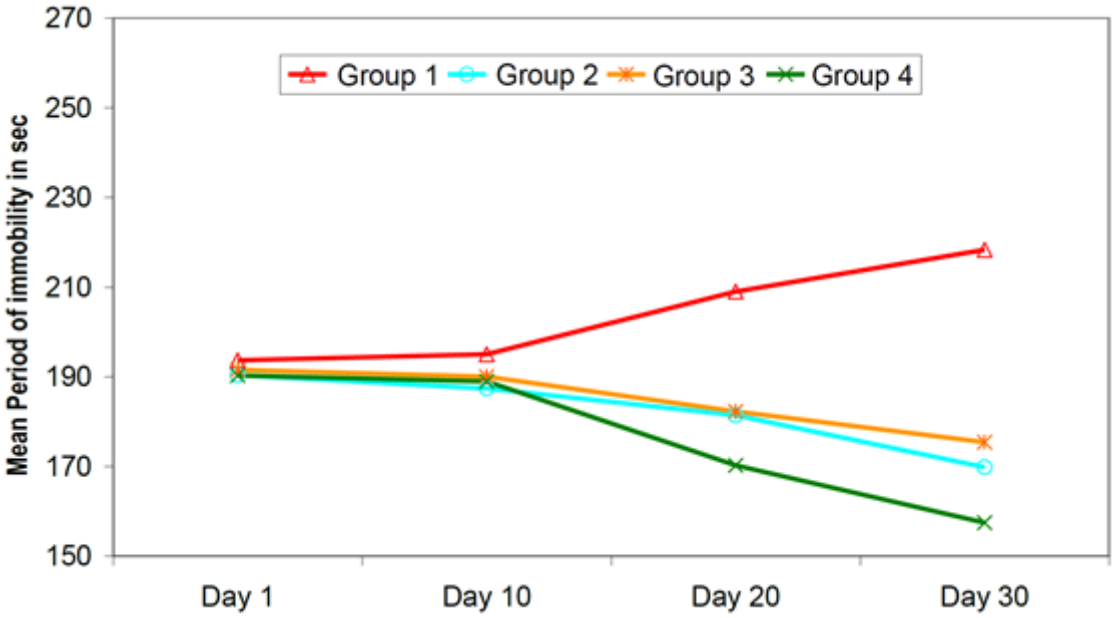
SN	Group	Day1(Baseline)			Day10			Day20			Day30		
		Meandiff.	SE	'p'	Meandiff.	SE	'p'	Meandiff.	SE	'p'	Meandiff.	SE	'p'
1-	1vs.2	3.33	10.04	0.987	7.60	8.04	0.782	27.56	3.85	<0.001	48.51	4.26	<0.001
2-	1vs.3	2.12	10.04	0.997	4.98	8.04	0.925	26.74	3.85	<0.001	42.91	4.26	<0.001
3-	1vs.4	3.39	10.04	0.986	6.07	8.04	0.874	38.76	3.85	<0.001	60.87	4.26	<0.001
4-	2vs.3	-1.21	10.04	0.999	-2.61	8.04	0.988	-0.82	3.85	0.996	-5.60	4.26	0.563
5-	2vs.4	0.06	10.04	1.000	-1.53	8.04	0.997	11.20	3.85	0.040	12.36	4.26	0.040
6-	3vs.4	1.27	10.04	0.999	1.08	8.04	0.999	12.02	3.85	0.026	17.96	4.26	0.002

Period of immobility of was higher than its baseline (Day 1) on Day 10, 20 and 30 in Group 1, while in Group 2, 3 and 4 period of immobility was lower than its baseline level on Day 10, 20 and 30.

Table 2 a : Intragroup change in Baseline period of immobility (Paired 't' test)

Group	Time	Meanch.	SD	%Change	't'	'p'
Group1	BL-Day10	1.34	4.57	0.69	0.72	0.503
	BL-Day20	15.33	12.75	7.92	2.94	0.032
	BL-Day30	24.64	10.66	12.72	5.66	0.002
Group2	BL-Day10	-2.93	3.98	-1.54	-1.80	0.131
	BL-Day20	-8.91	13.93	-4.68	-1.57	0.178
	BL-Day30	-20.54	14.11	-10.79	-3.57	0.016
Group3	BL-Day10	-1.52	4.41	-0.79	-0.85	0.436
	BL-Day20	-9.30	12.57	-4.85	-1.81	0.130
	BL-Day30	-16.15	10.27	-8.43	-3.85	0.012
Group4	BL-Day10	-1.33	2.96	-0.70	-1.10	0.319
	BL-Day20	-20.04	11.48	-10.53	-4.28	0.008
	BL-Day30	-32.84	9.99	-17.26	-8.05	<0.001

Graph 2 b: Change in Baseline Period of Immobility



subsequent increase in baseline period of immobility was observed in Group 1 on Day 10, Day 20 and Day 30. Change in baseline period of immobility was not found to be significant at Day 10. Range of percentage change in baseline period of immobility in Group 1 was 0.69% (Day 10) to 12.72% (Day 30).

In Group 2, 3 and 4 subsequent decline in baseline period of immobility were observed on Day 10, 20 and 30. Significant change in baseline period of immobility in Group 2 and 3 were on Day 30 only, while in Group 4 significant decline was observed on Day 20 and Day 30.

Range of percentage change in baseline period of immobility in Group 2, Group 3 and Group 4 were 1.54-10.79%, 0.79-8.43% and 0.70-17.26%.

DISCUSSION

The Forced Swim Test (FST) is the most widely used pharmacological model for assessing antidepressant activity in rodents. In this test, the animal is placed in a cylinder filled with water deep enough to prevent it from touching the bottom with its hind paws. Initially, the animal exhibits vigorous activity in an attempt to escape, followed by a phase of immobility, during which it makes only the minimal movements needed to keep its head above water. This immobile posture is considered to reflect a state of behavioral despair, akin to depressive symptoms in humans. The FST is highly sensitive and relatively specific to all major classes of antidepressant agents, making it a widely accepted method for predicting the antidepressant potential of various drugs[20].

In our study, mice were administered the test drugs—either allopurinol or probenecid—60 minutes prior via oral route and 30 minutes prior via intra peritoneal injection. The FST was conducted 24 hours after administration and then repeated on days 10, 20, and 30. During each session, animals were observed for duration of 6 minutes. The primary measure was the duration of immobility, with a decrease indicating potential antidepressant activity. Both allopurinol and probenecid significantly reduced immobility time compared to the control group, suggesting antidepressant-like effects. However, their efficacy was found to be lower than that of the standard antidepressant drug imipramine.

Our findings are consistent with those of Salim et al., who reported that xanthine oxidase and its substrate xanthine can induce mood swings and depressive symptoms, likely due to oxidative stress in the brain. Xanthine oxidase, a key enzyme involved in free radical production, appears to play a role in the pathophysiology of depression. This is supported by studies showing that inhibition of xanthine oxidase can alleviate depressive-like symptoms.

Brain-Derived Neurotrophic Factor (BDNF) expression in the brain is another important marker for depression. Reduced BDNF levels are associated with stress and depressive symptoms, while increased BDNF is linked to mood improvement and antidepressant effects[21]. Yamagata et al. (1999) demonstrated that BDNF has protective effects against oxidative stress in TrkB-expressing PC12h cells. As xanthine oxidase is a major source of oxidative stress, its inhibition by allopurinol may lead to elevated BDNF levels. In our study, higher doses of allopurinol correlated with increased serum BDNF and improved behavioral outcomes in the FST, further supporting this mechanism.

Additionally, other studies have shown that xanthine oxidase inhibitors like allopurinol reduce tryptophan 2,3-dioxygenase activity, resulting in increased tryptophan availability and elevated serotonin levels in the brain—another pathway contributing to their antidepressant effect[20]. This mechanism was also reflected in our findings, where allopurinol and probenecid not only showed behavioral improvements but also elevated serum BDNF levels. Consistent with this, Karve et al. and Kessing et al. (2019)[22] also demonstrated that allopurinol and febuxostat exhibit robust antidepressant effects in FST models.

Together, these findings suggest that xanthine oxidase inhibitors may exert their antidepressant effects through a dual mechanism: reducing oxidative stress and enhancing serotonergic neurotransmission, both of which contribute to increased BDNF levels and alleviation of depressive-like behaviors.

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