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RESEARCH ARTICLE

Antipsychotic Activity of Amiloride in Swiss Albino mice

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Abstract

Background: Psychosis is a clinical syndrome characterised by distortions in perception, disorganised thinking, and impaired reality testing, commonly manifesting as hallucinations, delusions, and thought disorganisation. Neuroinflammation and ion channel dysregulation, especially involving Acid-Sensing Ion Channels (ASICs), are increasingly recognised in psychosis pathophysiology. Activation of ASICs contributes to calcium-mediated neuroinflammation and excitotoxicity. Amiloride, a diuretic that non-selectively inhibits ASICs, shows neuroprotective and anti-inflammatory effects. Therefore, this study aims to evaluate the antipsychotic effects of Amiloride in Swiss Albino mice. **Materials and Methods**: Male Swiss Albino mice, weighing between 20-30 g, were used in this study. The Mice were randomly assigned to four groups with 4 animals in each group. Control (Normal Saline- 10 ml/Kg), Standard (Chlorpromazine 3 mg/kg), Test 1 (Amiloride 10 mg/Kg), Test 2 (Amiloride 20 mg/kg). Psychosis induced by Inj Ketamine 50 mg/kg ip for 5 days. At day 8, Locomotor activity was assessed after administration of the Test and standard drug at 0, 30 and 60 min using the Actophotometer**.** Anhedonia (negative symptom) was assessed using the Sucrose preference test. **Results**: Amiloride 20 mg/Kg demonstrated a comparable antipsychotic effect with the Standard drug in Swiss Albino mice when tested for locomotor activity and the Sucrose preference test.

Keywords: Amiloride, ASIC, Antipsychotic, Locomotor Activity, Sucrose Preference Test

1. Introduction

Psychosis is a complex, debilitating mental disorder that affects a large number of people. Usually, psychosis is categorised into three main symptoms: positive, negative and cognitive symptoms. Bizarre behaviour, delusions, and hallucinations are positive symptoms of psychosis. Anhedonia, flat affect or social withdrawal or loss of emotion are negative symptoms of psychosis[1].

Hyperactivation of the mesolimbic pathway and dysfunction of the mesocortical pathway generate an imbalance in neurotransmission, are major reason for psychosis. Other reasons for psychosis are heredity, stress, oxidative stress, NMDA receptor antagonists, drug abuse and traumatic injury[1].

Emerging evidence implicates neuroinflammation and ionic dysregulation in the pathophysiology of psychosis. Among these, Acid-Sensing Ion Channels (ASICs), particularly ASIC1a, play a role in modulating neuronal excitability, synaptic plasticity, and neuroinflammatory responses. ASICs are proton-gated cation channels activated under conditions of extracellular acidosis, which promotes calcium influx, leading to downstream excitotoxic and inflammatory signalling pathways that may contribute to neuronal dysfunction and psychiatric symptoms[2].

Amiloride, a potassium-sparing diuretic used for its effects on renal epithelial sodium channels, has been identified as a non-selective inhibitor of ASICs. Preclinical studies suggest that amiloride can attenuate neuroinflammation and glutamatergic excitotoxicity by inhibiting ASIC1a-mediated calcium entry. In animal models, amiloride has demonstrated neuroprotective and anti-inflammatory effects, including reductions in

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proinflammatory cytokine expression and microglial reactivity.

These properties suggest a potential repurposing of amiloride as an adjunctive treatment in psychosis, particularly in individuals with treatment-resistant symptoms or prominent inflammatory markers. To date, there are no studies investigating the relationship between Amiloride and psychosis. This study aims to evaluate the antipsychotic activity of Amiloride in Swiss albino mice.

2. Review of Literature

Psychosis is defined by symptoms such as delusions, hallucinations, and disorganized thinking while the individual remains aware of their surroundings. Symptoms are classified as positive (*e.g.*, hallucinations and delusions), negative (*e.g.*, social withdrawal and lack of motivation), and cognitive (*e.g.*, difficulties with memory and decision-making), significantly affecting everyday functioning. The cause of psychosis involves genetic, environmental, and neurological factors. With 80% heritability, genes related to synapse function and immunity are implicated. Environmental influences include prenatal stress, childhood trauma, cannabis use, and urban living figure 1.

Additionally, disruptions in dopamine, glutamate, and GABA signalling, along with altered brain development and immune function, contribute to the disorder's complexity.

Various genes have been identified that have a potential risk for Psychosis. A computational analysis of Protein-Protein Interaction (PPI) network or the interactome of psychosis associated genes has shown that -Psychosis interactome, comprising 101 psychosis genes and 1900 PPIs, provided valuable results in the functions tied to psychosis genes through their protein interactome. A valuable result from this study showed a

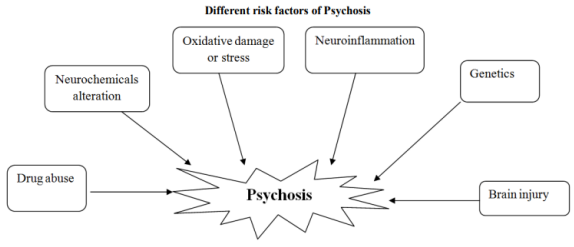

Figure 1. Risk Factor for Psychosis.

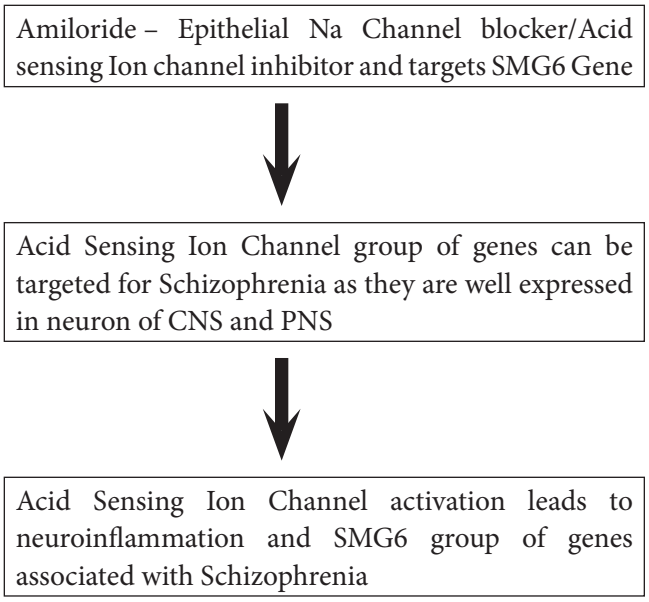

Figure 2. Study drug: amiloride.

total of 524 drugs targeting 53 proteins in the Psychosis interactome[3].

Many of these drugs were labelled for therapeutic value for the nervous system. One such drug is Amiloride. This Potassium Sparing Diuretic, which may also act on Acid-sensitive Ion Channel, belongs to the group of Epithelial Na+ Channel blockers (ENaC) family of ion channels in neurons, which has been proven to be neuroprotective. The protein targets of Amiloride are ASIC1, ASIC2, SLC9A1 and SCNN1B. The network of PPI among these targets of amiloride showed 12 genes. One such gene is SMG6, which has been associated with bipolar disorder or psychosis figure 2.

Antipsychotics are the primary treatment for psychosis spectrum disorders, typically starting with low doses that are gradually increased. Common first-generation medications include chlorpromazine, haloperidol, flupenthixol, and pimozide, while second-generation options include olanzapine, clozapine, risperidone, and aripiprazole[4].

3. Aims and Objectives

- To evaluate the antipsychotic activity of intraperitoneal amiloride with different doses in male albino mice by behavioural test (hyperactivity test in actophotometer, anhedonia- sucrose preference test).

- To compare the antipsychotic activity of intraperitoneal amiloride using chlorpromazine as a standard.

4. Materials and Methods

The study was conducted after getting approval from the Institutional Animal Ethics Committee (IAEC). CCSEA approval number from IAEC:130/GKMC/IAEC/2023. According to the protocol, 16 male Swiss albino mice weighing 20-25 g were used. Animals were divided into 4 groups, consisting of 4 animals in each group. The study was conducted in the Department of Pharmacology, Government Kilpauk Medical College, Chennai.

Inj Ketamine diluted in 0.9% Normal Saline was given to all animals for inducing psychosis.

5. Procedure

Animals were acclimatized for 1 week under laboratory conditions. They were fed with a standard diet and water ad libitum. A 12 h light and dark cycle was maintained for 10 days prior to the experimentation. CCSEA guidelines were adhered to throughout the study. Animals were acclimatized the environment over a period of 1 week before the procedure.

5.1 Inhibition of Ketamine Induced Hyperactivity in Mice using Actophotometer

Inj. Ketamine 20 mg/kg was injected into all groups through the intraperitoneal route for 7 consecutive days. On the 8th day, Inj Ketamine 20 mg/kg was given to induce psychosis. After 20 minutes, baseline locomotor activity was checked using an actophotometer immediately figure 3.

Table 1. Grouping of animals

Group	Drug	No. of animals
Group 1 - control	Normal saline 10 ml/kg i.p	4
Group 2 - standard	Chlorpromazine 3 mg/kg ip	4
Group 3 - test-1	Amiloride 10 mg/kg i.p	4
Group 4 - test -2	Amiloride 20 mg/kg ip	4

- The control group was given 10 ml/kg Normal saline i.p.
- The standard group was given chlorpromazine 3 mg/kg i.p.
- Test groups were given amiloride 10 mg/kg i.p and 20 mg/kg i.p.

Inhibition of ketamine induced hyperactivity was checked for each group using actophotometer.

According to the groups mentioned above, the mice were placed in the actophotometer. The instrument contains infrared light beams arranged in a grid inside a closed chamber. As the animal moves, it breaks these beams. Each interruption of a beam was noted as one count of locomotor activity.

The total number of beam breaks in 5 min was noted at baseline, 30 min and 60 min.

Figure 3. Testing of Locomotor activity using Actophotometer.

5.2 Sucrose Preference Test

This is a reward-based behavioural test used as an indicator of anhedonia, a core negative symptom of psychosis. Rodents have an innate preference for sweet solutions. A reduction in sucrose preference indicates a state of anhedonia. This test was conducted 14 days after the Locomotor activity test to ensure that there was no residual effect of the test drug.

5.2.1 Pre-Treatment and Psychosis Induction

All animals were trained to consume a 1% sucrose solution to establish baseline familiarity and preference. Following this, all animals were administered ketamine (30 mg/kg, intraperitoneally) once daily for 7 consecutive days to induce psychosis-like psychotic behaviour.

5.2.2 Induction Phase – Sucrose Preference Monitoring

After ketamine administration, animals were given free access to two pre-weighed bottles - one containing 1% sucrose solution and the other plain water for a period of 5 days to assess sucrose preference and confirm anhedonia.

To avoid side bias, the left-right position of the sucrose and water bottles was alternated daily. The volume of liquid consumed from each bottle was recorded daily. The Sucrose Preference (%) was calculated using the formula:

$$\text{Sucrose Preference (\%)} = \frac{\text{Volume of sucrose consumed} \times 100}{\text{Volume of sucrose + water consumed}}$$

5.2.3 Post-Treatment Phase – Drug Intervention

At the end of the initial 5-day observation period, animals were randomly divided into four groups (4 animals in each group) and administered the following treatments intraperitoneally:

- **Group 1:** Normal Saline (10 ml/kg)
- **Group 2:** Chlorpromazine (CPZ) (3 mg/kg)
- **Group 3:** Amiloride (10 mg/kg)
- **Group 4:** Amiloride (20 mg/kg)

Following drug administration for 5 days, the Sucrose Preference test was repeated for another 5 days, using the same two-bottle choice method. Bottles were weighed daily, and the sucrose preference was calculated as above for each group figure 4.

Figure 4. Sucrose Preference Test.

6. Statistical Analysis

The collected data were entered into Microsoft Excel 2016 and analysed with IBM SPSS Statistics. The antipsychotic activity among all the groups was compared using.

- One-way ANOVA method followed by Post-hoc Tukey HSD (Honestly Significant Difference) test for multiple comparisons, pvalue (<0.05) was considered statistically significant.

7. Results

In this study, 16 male Swiss albino mice were selected and evaluated for antipsychotic activity by using an actophotometer for hyperactivity (at 0, 30 min and 60 min) and the Sucrose Preference Test (based on daily sucrose intake).

7.1 Inhibition of Ketamine Induced Hyperactivity in Mice using Actophotometer

Inhibition of ketamine induced hyperactivity is indicative of antipsychotic activity.

Table 2 shows the mean and standard deviation of the decrease in hyperactivity among four groups. At 0 min, no significant difference in mean locomotor activity among the 4 groups was noted. However, at 30 and 60 min, one-way ANOVA showed a highly significant difference among the groups in mean reduction in hyperactivity with pvalue <0.001, figure 5.

Table 2. Inhibition of ketamine induced hyperactivity after drug administration

Group	0 min (Mean ± SD)	30 min (Mean ± SD)	60 min (Mean ± SD)
Control (normal saline)	299 ± 5.48	296.2 ± 5.25	294.5 ± 2.65
Standard (chlorpromazine)	303.2 ± 6.65	247.2 ± 3.86	215.5 ± 5.32
Test 1 (amiloride 10 mg/Kg)	304± 4.76	283.5 ± 4.93	261.5 ± 6.61
Test 2 (amiloride 20 mg/Kg)	303 ± 6.38	249.7 ± 2.22	220.5 ± 6.14
pvalue	0.634	<0.001 **	<0.001**

**Highly significant

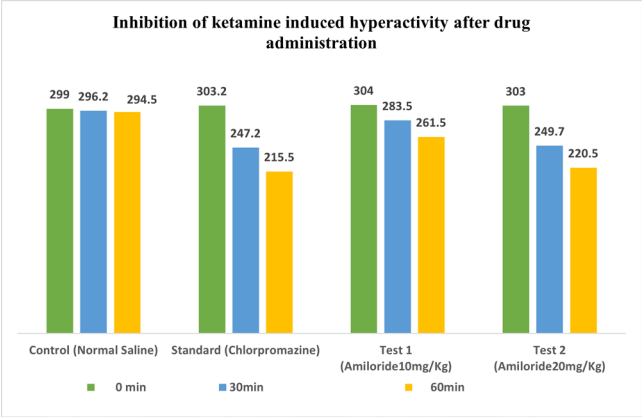

Figure 5. Inhibition of ketamine induced hyperactivity after drug administration.

Table 3. Comparison between groups-inhibition of ketamine induced hyperactivity

S. No.	Groups	pvalue - 30 min	pvalue - 60 min
1	Control vs chlorpromazine	<0.001	<0.001
2	Control vs amiloride 10 mg/kg	<0.05	<0.001
3	Control vs amiloride 20 mg/kg	<0.001	<0.001
4	Chlorpromazine vs amiloride 10 mg/kg	<0.001	<0.001
5	Chlorpromazine vs amiloride 20 mg /Kg	0.837	0.574
6	Amiloride 10 mg/kg vs 20 mg/kg	<0.05	<0.05

7.1.1 Comparison between Groups

Post-hoc Tukey (HSD) test was used for multiple comparisons at significant time points. pvalue <0.001 is highly significant; pvalue of <0.05 was considered statistically significant. At 30 and 60 minutes after drug administration, both Chlorpromazine and Amiloride at 20 mg/kg produced a statistically significant reduction in locomotor activity compared to the control group at both time points ($p<0.001$), indicating central nervous system depressant effects. Amiloride at 10 mg/kg also reduced activity significantly, though to a lesser extent ($p<0.05$ at 30 min; $p<0.001$ at 60 min).

When compared to the standard, Amiloride 20 mg/kg showed no significant difference in effect at either time point ($p=0.837$ at 30 min; $p=0.574$ at 60 min), suggesting comparable efficacy to Chlorpromazine. In contrast, Amiloride 10 mg/kg was significantly less effective than both the standard and the higher dose ($p<0.001$).

7.2 Sucrose Preference Test

Table 4 shows Mean Sucrose Preference (SP%), which was analysed using one-way ANOVA to assess inter-group differences at each experimental phase. In the pre-induction phase, no statistically significant difference was observed between groups ($p=0.067$), indicating comparable baseline behaviour across all groups.

Following ketamine-induced psychosis, all groups showed a reduction in sucrose preference, indicative of anhedonia. However, inter-group differences were not statistically significant at this phase ($p=0.832$), suggesting a uniform effect of ketamine across treatment arms.

In the post-intervention phase, one-way ANOVA revealed a statistically significant difference in sucrose preference between groups ($p<0.001$). The standard group (Chlorpromazine) and Amiloride 20 mg/kg (Test 2) showed increased sucrose preference compared to the control group. In contrast, Amiloride 10 mg/kg (Test 1) did not elicit any improvement in sucrose preference, as reflected by unchanged sucrose preference and remained comparable to the group figure 6.

Table 5 is the Post-hoc (Tukey's test) following one-way ANOVA, revealed that both the standard group (CPZ) and the Amiloride group (20 mg/kg) showed significantly higher sucrose preference compared to the control group ($p<0.001$ for both). The Amiloride (10 mg/kg) group did not differ significantly from the control group ($p=0.932$), suggesting no therapeutic effect at this dose. There was no significant difference

Table 4. Mean sucrose preference percentage (SP%)

Group/Time	Control (NS)	Std (CPZ)	Test1 (Amiloride 10 mg/Kg)	Test2 (Amiloride 20 mg/Kg)	pvalue
Pre-induction (SP%)	55.4 ± 1.95	53.8 ± 2.17	54.6 ± 1.82	51.6 ± 2.88	0.067
Post-induction(SP%)	45.1 ± 1.34	45.6 ± 2.30	45.8 ± 1.92	45.2 ± 2.59	0.832
Post-Intervention (SP%)	43.2 ± 1.30	48.6 ± 2.51	43.8 ± 2.41	48.4 ± 3.36	<0.001

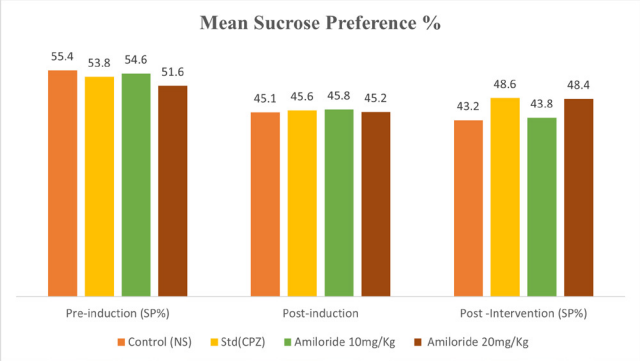

Figure 6. Mean sucrose preference %.

Table 5. Comparison between groups - mean Sucrose Preference percentage (SP%)

Comparison of the post-intervention period	95% CI	pvalue
Control (NS) vs standard (CPZ)	[3.2, 8.4]	<0.001
Control (NS) vs Test1 (10 mg/kg)	[-1.9, 3.1]	0.932
Control (NS) vs Test2 (20 mg/kg)	[2.8, 8.4]	<0.001
Standard (CPZ) vs Test1 (10 mg/kg)	[-8.1, -2.3]	0.001
Standard (CPZ) vs Test2 (20 mg/kg)	[-3.6, 3.2]	0.999
Test1 (10 mg/kg) vs Test2 (20 mg/kg)	[2.0, 8.0]	0.002

between CPZ and Amiloride (20 mg/kg) (p=0.999), indicating comparable efficacy of the two treatments.

8. Discussion

Psychosis, characterised by hallucinations, delusions, and impaired reality, is a neuropsychiatric condition with multifactorial aetiology involving dopaminergic, glutamatergic, and inflammatory pathways. Current antipsychotics, while effective in controlling positive and negative symptoms and cognitive deficits, are often associated with extrapyramidal side effects and metabolic burden[4].

In this context, Amiloride, through its modulation of Acid-Sensing Ion Channels (ASICs) and subsequent regulation of downstream inflammatory signalling, emerges as a potential drug for addressing the unmet therapeutic needs in psychosis[2].

The present study investigated the neurobehavioral effects of Amiloride by inducing Psychosis with a subanaesthetic dose of Ketamine 20-30 mg/kg for 7 days. The effects were assessed by locomotor activity test- indicative of general psychomotor function, and the Sucrose Preference Test (SPT), a measure of anhedonia- a negative symptom of psychosis.

In this study locomotor activity was assessed using Actophotometer, the procedure was done in similar to the studies done by Bakshi *et al.*[5] and Yadav *et al.*[6]. Both Chlorpromazine and Amiloride at 20 mg/kg produced a statistically significant reduction in locomotor activity at 30 and 60 min compared to the control group and low-dose Amiloride (10 mg/kg) (p<0.001), indicating a pronounced central nervous system depressant effect. Although Amiloride 10 mg/kg also showed a significant reduction in locomotor activity versus control, the effect was markedly less than that observed with either chlorpromazine or the higher dose of Amiloride (20mg/kg).

Post-hoc Tukey's HSD test revealed no significant difference between Amiloride 20 mg/kg and Chlorpromazine at both time points (p=0.837 at 30 min; p=0.574 at 60 min), suggesting comparable efficacy in reducing locomotor activity. These results support a dose-dependent depressant effect of amiloride 20 mg/kg on locomotor activity, showing antipsychotic-like properties like those of the standard drug Chlorpromazine.

In this study, Anhedonia, a negative symptom of Psychosis, was assessed using the Sucrose preference test and the procedure was done in similar to the studies done by Ntombifuthi P. Ngubane *et al.*[7] and Tsvetan *et al.*[8]. Animals subjected to the anhedonia model

exhibited a significant decline in sucrose preference after inducing psychosis with Ketamine 30mg/kg, indicative of reduced reward sensitivity.

Administration of the standard drug chlorpromazine significantly improved sucrose preference. Similarly, amiloride 20 mg/kg produced a statistically significant improvement in sucrose preference compared to the control group ($p<0.001$), suggesting a potential role in ameliorating anhedonic behaviour. While amiloride at 10 mg/kg showed a mild increase in sucrose preference, the effect did not show statistical significance, indicating a dose-dependent response.

Post-intervention comparisons revealed that Amiloride 20 mg/kg significantly improved sucrose preference, with efficacy comparable to Chlorpromazine ($p=0.999$). In contrast, Amiloride 10 mg/kg showed no significant effect compared to control ($p=0.932$). These findings indicate the potential of Amiloride 20 mg/kg in alleviating anhedonia and negative symptoms of psychosis.

9. Conclusion

The study drug, Amiloride (20 mg/kg), demonstrated significant antipsychotic activity, as evidenced by a reduction in ketamine-induced hyperactivity and an improvement in sucrose preference percentage (a measure of anhedonia) following the induction of psychosis. These effects were comparable to the standard antipsychotic drug, Chlorpromazine (3 mg/kg). Further studies are warranted to assess the clinical potential of Amiloride in the treatment of psychosis.

10. References

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