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ORIGINAL RESEARCH ARTICLE

Behavioral Alterations of Ethanol on Lithium Treated Methylphenidate Induced Mania-Like Phenotype and Corticosterone Induced Depression-Like Phenotype in Swiss Albino Mice

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ABSTRACT

The present study designed to evaluate the behavioral alterations of ethanol and also to compare the effect of ethanol in the manic and depressive-like behavior mice after treated with lithium chloride, when measured by actophotometer test, elevated plus maze test (EPM) and tail suspension test (TST). Male Swiss albino mice (n=72) weighing 25-30g were randomly divided into twelve groups (n=6/group). Repeated administration of MPD (2.0 mg/kg) on a 10-day, mice elicited locomotor sensitization as demonstrated by the increased in motor activities in the actophotometer test. However, LiCl (50 mg/kg) + EtOH (2.0 g/kg) groups subsequently treated in methylphenidate sensitization mice shows increased in locomotor activity. Repeated administration of CORT (20 mg/kg, i.p.) on a 16-day, mice elicited depressive-like behavior as demonstrated by the obvious increased in immobility time in the tail suspension test. However, LiCl (50 mg/kg) + EtOH (2.0 g/kg) subsequently treated in depressive-like behavior mice shows increased with immobility duration. The anxiolytic effect was potentiated by EtOH showing greater number of entries into the open arm in EPM. In contrast, LiCl failed to produce anti-anxiety like effect synergistic with EtOH exhibit increased in closed arm entries. Based on the behavioral assessment we suggested that ethanol attenuates the lithium treatment in manic and depressive-like behavior mice.

Key words: Methylphenidate, Corticosterone, Locomotor sensitization, Anxiety, Lithium chloride and Ethanol.

1. INTRODUCTION

Bipolar affective disorder is characterized by repeated episodes of mania interspersed with periods of depression. A strong association between alcohol dependence and bipolar disorder has been reported in numerous studies. Males with bipolar disorder have higher rates of substance abuse than females (59.7% vs. 37.8% for alcohol; 54.5% vs. 33.8% for other drugs) [1] . Anxiety disorder is a common co-morbidity during both manic and depressive episodes [2] . However, Lithium salts are the most effective long-term preventive treatment for bipolar disorder [3] and helping alcoholics to reduce their intake or prevent relapse.

In line with that approach, it may be a helpful strategy to attempt and develop specific models within the realm of BPD that represent components of the disorder rather than its entire scope. Since BPD includes episodes of mania and of depression, these models may represent each pole separately.

An ideal model of a ‘‘bipolar animal’’ is most likely unattainable and also an adequate animal model of a psychiatric condition must fulfil three core criteria: share pathophysiological characteristics of the human condition (face validity), have similar behavioral manifestations as the human disease (construct validity), and improve with medications that improve the

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symptoms seen in afflicted humans (predictive validity) [4,5,6] . The methylphenidate and corticosterone are the repeatedly tested drugs that fulfill the three criteria for an adequate animal model for bipolar illness [7-11].

Methylphenidate (MPD) is commonly prescribed for children who have been diagnosed with attention deficit hyperactivity disorder (ADHD), one of the most common chronic neurobehavioral diseases of childhood [12]. Methylphenidate has the potential of abuse because it has pharmacological stimulant properties similar to amphetamine and cocaine [13] .Repeated administration of MPD causes behavioral sensitization. It has been implicated as an experimental model of psychiatric illnesses, such as bipolar disorders. [14] Behavioral sensitization in rodents is characterized by a progressive increase in their locomotor activity in response to a fixed dose of a psychostimulant.

Chronically elevated glucocorticoids (i.e., primarily cortisol in human, corticosterone in rodents) are found in a number of neuropsychiatric disorders [15,16]. Repeated CORT administration in mice produces reliable behavioral and neurobiological alterations that parallel many of the core symptoms and neurobiological changes associated with human depression.[17,18] Animal models that are used to elucidate mechanisms underlying depression often display altered anxiety-related behavior. There is also evidence to suggest that repeated administration of exogenous CORT produces anxiety-like behaviors in a variety of tasks including the elevated plus maze test [19].

No psychiatric disorder has a single unique model representing the entire scope of the disease. Even well established tests, such as the actophotometer test for locomotor activity, tail suspension test (TST) for antidepressant activity or Elevated plus maze test (EPM) for anxiety disorder [20,21], do not represent the entire scope of these disorders but only specific aspects. These models are considered more naturalistic in the induction of a manic and depressive-like state and are suggested to have better potential homology to the human situation.

The present paper suggests two different strategies to develop mania and depression models that may contribute to further research, both for drug screening and for research related to better understanding of the biological basis of bipolar disorder and its treatment.

2. MATERIALS AND METHODS

2.1 Animals:

In the present study, we used adult male Swiss albino mice (Purchased from Rajah Muthiah Medical College, Annamalai University). Male Swiss albino mice (n=72) weighing from 25-30g were housed in the experiment room in groups of six per cage (size: 41×34×16 cm) at an ambient temperature of 23±2°C and relative humidity of 55±10%. Animals were maintained on a 12:12 h light (07.00-19.00)/dark (19.00-07.00) cycle with ad libitum food pellets and water, and allowed to acclimate to the experimental room for a minimum of 10 days prior to experimentation. Wood chip bedding was changed once in a week.

2.2 Ethics:

The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) and care of the animals was carried out as per the guidelines of the Committee for the Purpose of Control and Supervision of Experimental Animals (CPCSEA), Rajah Muthiah Medical College and Hospital, Annamalai University (Reg. No.-160/1999/CPCSEA). All efforts were made to minimize the number of animals used and their suffering.

2.3 Drugs:

Twenty-four Swiss albino mice were treated with methylphenidate hydrochloride (MPD; Inspiral, Ipca pharmaceutical company) was dissolved in physiologic saline (0.9% NaCl), at a (dose of 2.0 mg/kg BW). Twenty-four mice were assigned to Corticosterone-treated group. CORT (Sigma-Aldrich, India) was first dissolved in 0.1% v/v ethanol at a (dose of 20 mg/kg BW) before being diluted to the mentioned concentrations in saline. This concentration of ethanol was also present in the drinking solutions of the CORT groups. Methylphenidate and corticosterone treatments were injected intraperitoneally. All injections were of equal volume (0.5 ml) and given between 12:00 h and 14:00 h. The concentrations of methylphenidate and corticosterone were individually adjusted for each mouse to ensure a similar dose in all animals (2.0 mg/kg BW MPD and 20 mg/Kg BW for CORT). This procedure was used to keep the stimulus mice uniformly active during and across test sessions. Ethanol solutions (20% v/v) were prepared from 96% ethanol dissolved in water and administered via oral gavage (dose of 2.0 g/Kg BW). Likewise

LiCl 50 mg/kg BW was also dissolved in water and administered via oral gavage. However, Lithium and ethanol were administered to methylphenidate and corticosterone groups after the expression of manic and depressive-like phenotype in mice, which will be monitored through actophotometer, TST and EPM. The dosage of the drugs was based on previous work [22,23].

2.4 Experimental Design:

Mania is induced by methylphenidate and depression is induced by corticosterone, lithium chloride positively modulates the phenotypes, whereas ethanol negatively modulates the phenotypes (**Fig 1**).

2.5 Experimental groups:

After 48-72h habituation to the experimental room, the mice were randomly divided into twelve groups (n=6/group) as summarized in (**Table 1**). In the 1st day morning all the mice were received 0.9% saline (i.p.) injection. On the 2nd day onwards the drug treatments was started for all the12 groups. Methylphenidate (2.0 mg/kg) and Corticosterone (20.0 mg/kg) were given intraperitoneally whereas; lithium (50 mg/kg) and ethanol (2.0g/kg) were given by oral gavage. On days 2-7, mice in Group II received LiCl (50 mg/kg, p.o.), Group III received EtOH (2.0g/kg, p.o.) and Group IV received LiCl (50 mg/kg, p.o.) + EtOH (2.0g/kg, p.o.). However mice in Groups V to VIII received MPD (2.0 mg/kg, i.p.) followed by Groups IX to XII received CORT (20.0 mg/kg, i.p.). On days 8-9, no drug treatments were given in all twelve groups (i.e., washout period). On days 10-14, Groups II to V and Groups IX to XII received the same treatment as on days 2-7. Whereas mice in Group VI received MPD+LiCl, Group VII received MPD+EtOH, Group VIII received MPD+LiCl+EtOH treatments on a 10-day (i.e., after the expression of locomotor sensitization to methylphenidate), monitored by actophotometer test. On day 15, mice in all twelve groups received no treatment. On days 16-21, mice in Groups II to IX received the same treatment as on days 10-14. However, mice in Group X received CORT+LiCl, Group XI received CORT+EtOH and Group XII received CORT+LiCl+EtOH treatments on a 16-day (i.e., after expression of depressive-like behavior to corticosterone), monitored by tail suspension test. This protocol had been modified from previous studies on MPD-elicited behavioral sensitization and CORT-elicited depressive-like behavior in mice.[14,22,24,25]

2.6 Behavioral assessment:

The mice were acclimatized for 2-5 minutes before initiating the experimental protocol in actophotometer. It is necessary to acclimatize the animals to the test apparatus in the behavioral studies to avoid potentially confounding effects induced by the novelty of the testing apparatus that in turn reduces the variation in the experimental data. Whereas for EPM test the measurement was taken directly without acclimatize the animals. The first five minutes of the time could possibly allow for novelty induced anxiety and affect the outcome measure.

2.6.1 Actophotometer test:

The Spontaneous motor activity was measured by using actophotometer (INCO, Ambala, India), which operates in six photoelectric cells which are connected in circuit with a counter. When the beam of light falling on the photocell is cut off by the animal, a count is recorded. Therefore, the number of counts is directly related to movement of the animal inside the actophotometer chamber. An actophotometer could have a square arena (30 x 30 x 25 cm) with wire mesh bottom, in which the animal moves. The first measure of animal's activity in a novel environment progressively spend less time in movement and exploration, so the second measure was considered as the rate of spontaneous activity of the mice. The counting was started following 5 minutes of adaptation period. Increase in count was regarded as central nervous system stimulant activity. Decrease in count was regarded as central nervous system depressant activity. Locomotor activity is considered as an index of alertness and a decrease in the activity would indicate sedative activity. After the period of drug treatment (21 days), the motor activity was measured using Actophotometer. Readings were noted on the final day of drug administration. Starting thirty minutes after drug administration mobility measurement was recorded for 10 minutes for drug treated groups. After each test apparatus was wiped clean with 20% alcohol and dried with paper towels.[26]

2.6.2 Tail Suspension test:

Each group was separated in different cages. Animals are transported from the housing room to the testing area in their own cages and allowed to adapt to the new environment for 1 hour before testing. After the period of drug treatment (21 days) the passive behavior was noted by using a tail suspension test. Readings were noted on the final day of drug administration. All the drugs were administered 30 minutes before the test. For

the test the mice are suspended on the edge of a shelf 58 cm above a table top by adhesive tape placed approximately 1 cm from the tip of the tail. The duration of immobility records for a period of 5 minutes. Mice were considered immobile when they hang passively and completely motionless. The animal was acoustically isolated and about 100 cm away from the nearest object. The percentage of reduction in immobility period with respect to the control group was calculated from the tracings.[27]

2.6.3 Anxiety behavior test:

The elevated plus maze is a widely used animal model of anxiety that is based on two conflicting tendencies (i.e., anxiolytic or anxiogenic); the rodent's drive to explore a novel environment and its aversion to heights and open spaces. The plus-maze was constructed of Plexiglas and consisted of two open and two closed arms each (10 cm wide×50 cm long, 50 cm walls for closed, 2 cm walls for open), intersected by a center platform (100 cm^2), elevated 50 cm off the floor. Readings were noted on the final day of drug administration (21 days). All the drugs were administered 30 minutes before the test. Each animal was tested for 5 min on the maze. A mouse was placed on the central platform of the maze facing the open arm. The following indices were calculated: the total number of entries into open arm and closed arm and the total time spent in each type of arm. From these values, the percentage of time spent in the open arms provided as the measures of anxiety was calculated for each animal. The number of closed arm entries provides the measure of locomotor activity in this test. Thus "anxious" animals will spend most time in the closed arms while less anxious animals will explore open areas longer. An entry was defined as the entry of all four feet into one arm and an arm exit was defined as two paws leaving the arm. Between each test maze apparatus was wiped clean with 20% alcohol and dried with paper towels. Mice were returned to their home cages after behavior testing. Percentage of open arm entries is calculated as: *[Open arm entries/(open arm entries+enclosed arm entries)] x100* and percent time spent on the open arms was determined as: *% time spent in open arm or closed arm= [Number of seconds spent on open arms or closed arm/300 total seconds (5 min observation time)] x100*.[28]

2.7 Statistical Analysis:

All data were expressed as mean (±S.D.) for each treatment group, differences between groups means were assessed by one way analysis of variance (ANOVA) followed by post-hoc analysis using Duncan's multiple range test (DMRT). A value of $P<0.05$ was considered statistically significant for analysis.

3. RESULTS

3.1 Expression of locomotor sensitization, induced by repeated administration of MPD in male mice in the actophotometer test

(**Fig 2**)summarizes after multiple methylphenidate injections, the behavior of the mice was assessed by using the actophotometer test at days 4, 7, and 10 respectively. In general, 4-day methylphenidate injections do not show any significant difference in motor activities. Whereas 7-day methylphenidate injections significantly decreased in locomotor activity compared to vehicle-control mice. On 10-day methylphenidate injection, mice elicited locomotor sensitization as demonstrated by the increased in motor activities. Duncan's multiple range test (DMRT) further showed that methylphenidate injections significantly decreased locomotor ($p<0.05$), while 10-day methylphenidate injections induced hyperlocomotion ($p<0.05$).

3.2 Expression of depressive-like behavior, induced by repeated administration of CORT in male mice in the tail suspension test

After repeated corticosterone injections, the behavior of the mice was assessed by using the tail suspension test at days 10, 13, and 16, respectively. In general, 10-day corticosterone injections induced an antidepressant-like effect, whereas 13-day no significant difference was observed between vehicle-control and 16-day corticosterone injections significantly increased depressive-like behavior in mice. Duncan's multiple range test (DMRT) further showed that 10 day corticosterone injection significantly decreased immobile time ($p<0.05$), while 16-day corticosterone injections induced an obvious increased with immobile time ($p<0.05$) (**Fig 3**).

3.3 Effect of ethanol, on lithium treated manic and depressive-like behavior mice in the actophotometer test

The results depicted in (**Fig 4**) the effect of treatment of mice with LiCl (50 mg/kg, p.o.) in vehicle + LiCl (50 mg/kg, p.o.) treated group significantly decreased the motor activity when compared with vehicle treated control groups ($p<0.05$). The post-hoc Duncan's test ($p<0.05$) indicated that vehicle + EtOH (2 g/kg, p.o.) treated groups produced a significant increase in motor activity compared with vehicle-treated

control groups. However, vehicle + LiCl (50 mg/kg, p.o.) + EtOH (2g/kg, p.o.) treated group significantly increase in locomotor activity compared to the vehicle + LiCl (50 mg/kg, p.o.) treated groups ($p<0.05$).

Most notably, mice treated with MPD (2 mg/kg, i.p.) during adult, exhibited persistent locomotor sensitization to MPD when tested 21 days after their last MPD injection, which was significantly greater than vehicle-treated control groups ($p<0.05$). Post-hoc analysis revealed that LiCl (50 mg/kg, p.o.) significantly attenuates motor activity in MPD + LiCl (50 mg/kg, p.o.) treated groups ($p<0.05$) compared with MPD (2mg/kg, i.p.) alone treated groups. However, MPD (2mg/kg, i.p.) + EtOH (2g/kg, p.o.) treated groups indicated that ethanol enhanced the locomotor activity in a significant manner ($p<0.05$). Post-hoc analysis revealed that EtOH (2g/kg, p.o.) significantly increased the hyperlocomotion in MPD (2 mg/kg, i.p.) + LiCl (50 mg/kg, p.o.) + EtOH (2g/kg, p.o.) treated groups when compared with MPD (2mg/kg, i.p.)+LiCl (50 mg/kg, p.o.) treated group ($p<0.05$).

Repeated administration of CORT (20mg/kg, i.p.) for 3 weeks reduced the motor activity in a significant manner ($p<0.05$) compared with vehicle-treated control groups. However, treatment with LiCl (50 mg/kg, p.o.) produced significant increased in locomotor activity in CORT (20 mg/kg, i.p.) + LiCl (50 mg/kg, p.o.) treated groups compared with CORT (20mg/kg, i.p.) alone treated groups ($p<0.05$). No significant difference was observed between CORT (20 mg/kg, i.p.) alone treated groups and CORT (20 mg/kg, i.p.) + EtOH (2g/kg, p.o.) treated groups. Post-hoc comparison indicated that CORT (20 mg/kg, i.p.) + LiCl (50 mg/kg, p.o.)+EtOH (2 g/kg, p.o.) treated groups significantly decreased locomotor activity compared to the CORT (20 mg/kg, i.p.) + LiCl (50 mg/kg, p.o.) groups ($p<0.05$).

3.4 Effect of ethanol, on lithium treated manic and depressive-like behavior mice in the tail suspension test

(**Fig 5**) shows, the vehicle + LiCl (50 mg/kg, p.o.) treated group mice significantly decrease the immobility duration and augment the antidepressant-like effect in TST. However, the results depicted in the EtOH (2 g/kg, p.o.) enhance the immobility duration in vehicle + EtOH (2 g/kg, p.o.) treated group ($p<0.05$). Post-hoc analyses ($p<0.05$) indicated that LiCl (50 mg/kg, p.o.) produced a synergistic antidepressant-like effect in vehicle + LiCl (50 mg/kg, p.o.) + EtOH (2 g/kg, p.o.) treated groups compared with vehicle treated control ($p<0.05$).

The test data demonstrated that the immobility time was significantly lower in MPD (2 mg/kg, i.p.) alone treated mice than in vehicle-treated control ($p<0.05$) in TST. Post-hoc analysis indicated that the effect of treatment of mice with LiCl (50 mg/kg, p.o.) produced a synergistic antidepressant-like effect in MPD (2 mg/kg, i.p.)+LiCl (50 mg/kg, p.o.) treated group ($p<0.05$). Interestingly MPD (2 mg/kg, i.p.) + EtOH (2 g/kg, p.o.) treated groups caused a significant elevation of immobility time in the mice TST compared with the vehicle-treated control ($P<0.05$). Our observations revealed that MPD (2 mg/kg, i.p.)+LiCl (50 mg/kg, p.o.) + EtOH (2 g/kg, p.o.) treated groups increased the immobility duration significantly with respect to MPD (2 mg/kg, i.p.)+LiCl (50 mg/kg, p.o.) treated groups ($p<0.05$).

Repeated administration of CORT (20 mg/kg, i.p.) for 21 days caused a significant elevation of immobility time in the mice compared with vehicle-treated control ($p<0.05$). Post-hoc analysis indicated that the treatment with LiCl (50 mg/kg, p.o.) produced a synergistic antidepressant-like effect in CORT (20 mg/kg, i.p.) + LiCl (50 mg/kg, p.o.) treated groups ($p<0.05$). The results depicted that CORT (20 mg/kg, i.p.) + EtOH (2 g/kg, p.o.) treated group mice significantly enhance the immobility time compared with CORT (20 mg/kg, i.p.) treated group ($p<0.05$). However, no significant difference was found between the CORT (20mg/kg, i.p.) alone groups and CORT (20 mg/kg, i.p.) + LiCl (50 mg/kg, p.o.) + EtOH (2 g/kg, p.o.) treated groups ($p<0.05$).

3.5 Effect of ethanol, on lithium treated manic and depressive-like behavior mice in the elevated plus maze test

A 5 min exposure to the EPM, post hoc analyses ($p<0.05$) revealed an increase in the number of entries into the closed arm and decrease in the percentage of time spent in the open arm in the groups injected with MPD (2 mg/kg, i.p.) and CORT (20 mg/kg, i.p.) alone when compared to vehicle-treated control in the test session (**Fig 6B & 6C**). However MPD (2 mg/kg, i.p.) and CORT (20 mg/kg, i.p.) altered time in elevated plus maze and spent more time in the closed arm compared with vehicle control groups ($p<0.05$) (**Fig 6D**).

We suggested that repeated exposure to MPD and CORT developed an anxiogenic-like effect.

Post hoc analyses ($p<0.05$) revealed that vehicle + EtOH (2 g/kg, p.o.), MPD (2 mg/kg, i.p.) + EtOH (2 g/kg, p.o.) and CORT (20 mg/kg, i.p.) + EtOH (2 g/kg, p.o.) treated groups significantly increased in the number of entries into the open arm and showed a greater exploration compared to vehicle-treated control groups (**Fig 6A**). However, MPD (2 mg/kg, i.p.)+EtOH (2 g/kg, p.o.) treated groups showed increased in the percentage of time spent in the closed arm compared to vehicle-treated control groups (Fig 6D). However, no significant differences were found between the vehicle + EtOH (2 g/kg, p.o.) treated groups and CORT (20 mg/kg, i.p.) + EtOH (2 g/kg, p.o.) treated groups in the percentage of time spent in the closed arm (Fig 6D).

The post-hoc Duncan's test revealed that vehicle+ LiCl (50 mg/kg, p.o.) + EtOH (2 g/kg, p.o.), MPD (2 mg/kg, i.p.) + LiCl (50mg/kg, p.o.)+EtOH (2 g/kg, p.o.), CORT (20 mg/kg, i.p.)+LiCl (50 mg/kg, p.o.) + EtOH (2g/kg, p.o.) treated groups significantly increased in CAE and decreased in % OAE (Fig 6A & 6C) were observed when compared with vehicle-treated control ($p<0.05$). Post hoc comparison ($p<0.05$) revealed that ethanol acts like an "anxiolytic-like" effects in MPD (2 mg/kg, i.p.) + EtOH (2 g/kg, p.o.) and CORT (20 mg/kg, i.p.) + EtOH (2 g/kg, p.o.) treated groups whereas EtOH (2 g/kg, p.o.) combined with lithium treated groups shows the anxiogenic effect and exploratory behavior also reduced significantly.

It can be seen from (Fig 6A & 6B) the vehicle+LiCl (50 mg/kg, p.o.) treated groups significantly decreased in percentage of open arm entries and percentage of time spent on the open arm when compared with vehicle-treated control groups, indicating an anxiogenic effect ($p<0.05$) whereas, MPD (2 mg/kg, i.p.) + LiCl (50 mg/kg, p.o.) and CORT (20 mg/kg, i.p.) + LiCl (50mg/kg, p.o.) treated groups showed a significant increase in open arm entries when compared with vehicle-treated control and also increased in percentage of time spent on the opened arm groups when compared to the vehicle+LiCl treated groups.

Therefore the results revealed that lithium significantly increased the open arm entries, induced anti-anxiety like effect and improved exploratory activity in MPD and CORT groups.

4. DISCUSSION

The present study shows psychopharmacological changes in behavior of experimental animals when it is exposed to methylphenidate and corticosterone treatment for a period of 21 days. We evaluated the behavioral alterations of ethanol and also compared the effect of lithium in the manic and depressive-like behavior mice after administered the ethanol by using actophotometer, TST and EPM tests.

Regarding the voluntary activity, when it was measured through actophotometer the methylphenidate treated animals showed hyperactivity when compared to the vehicle-treated control, whereas corticosterone treated animals showed decreased in the activity. The present study confirms previous findings in mice that chronic administration of methylphenidate produced significant increases in behavioral locomotor sensitization [29,30].

However, it must be noted that multiple oral administration of lithium significantly decreases the motor activity in vehicle+LiCl treated groups[31] and also attenuate the expression of locomotor sensitization induced by the MPD.[14,32] Whereas, lithium reverses its effect on CORT+LiCl treated groups and produced a significant increased in locomotor activity [33].

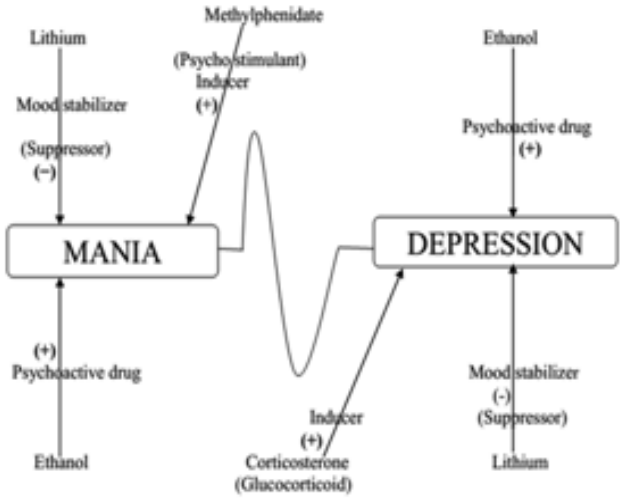


Fig 1: Schematic representation of experimental design

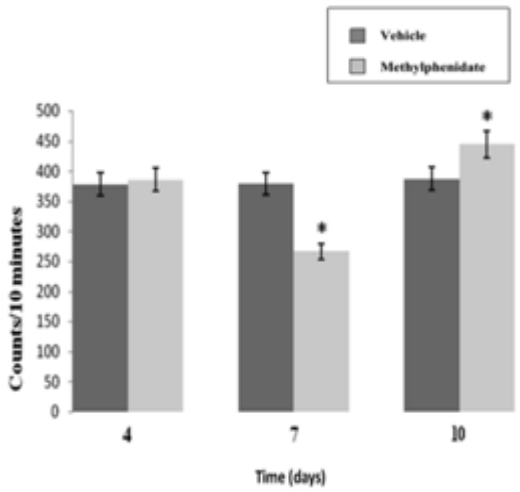


Fig 2: Effects of repeated MPD injections induce locomotor sensitization in male mice in the actophotometer test (n=6).
The mean (±S.D.) of locomotor activity during the 10 min of actophotometer test is shown. Data was analyzed by using a one-way analysis of variance (ANOVA) with treatment and time as factors. *p<0.05, for the methylphenidate vs. vehicle control, Duncan's multiple range test (DMRT).

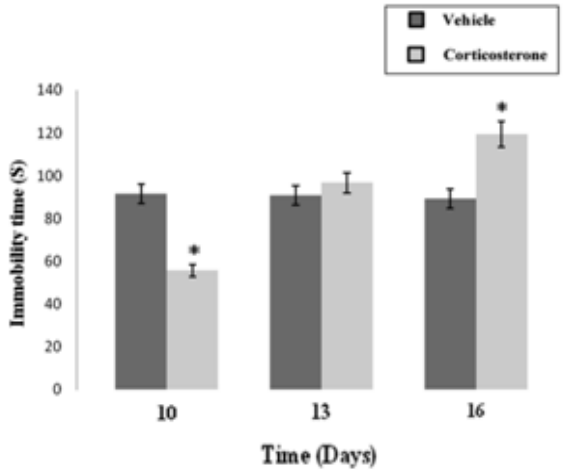


Fig 3: Effects of repeated CORT injections induce depression-like behavior in male mice in the tail suspension test (n=6).
The mean (±S.D.) of time spent immobile during the last 5 min of the tail suspension test is shown. Data was analyzed by using a one-way analysis of variance (ANOVA) with treatment and time as factors. *p<0.05, for the corticosterone vs. vehicle control, Duncan's multiple range test (DMRT).

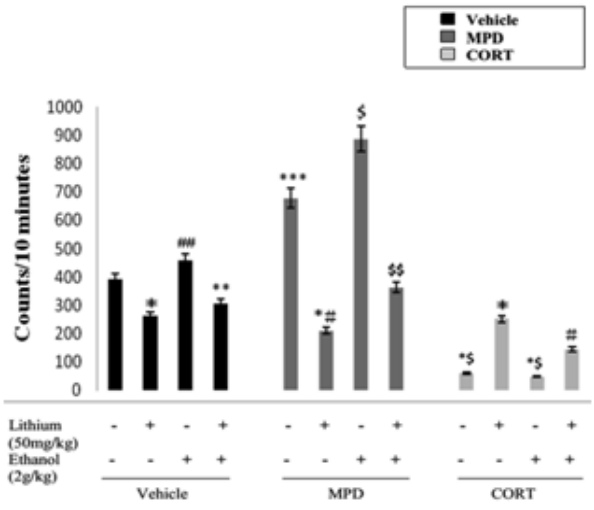


Fig 4: Effect of ethanol, on lithium treated manic and depressive-like behavior mice in terms of counts in 10 min time interval in the actophotometer test.
The plus sign indicates the agent treatment group. The minus sign indicates the vehicle treatment group. Values are expressed as mean (±S.D.) with n=6 in each group; one-way ANOVA followed by Duncan's multiple range test (DMRT). No significant difference was found between CORT and CORT+EtOH treated groups. Significant differences**:** $^{*, \#\#, ***, *\$}$p<0.05 compared with the vehicle-treated control groups. **p<0.05 compared with the vehicle + LiCl treated group. $^{*\#, \$}$p<0.05 compared with the MPD alone treated groups. $^{\$\$}$p<0.05 compared with the MPD + LiCl treated groups. *p<0.05 compared with the CORT alone treated groups. $^{\#}$p<0.05 compared with the CORT + LiCl treated groups.

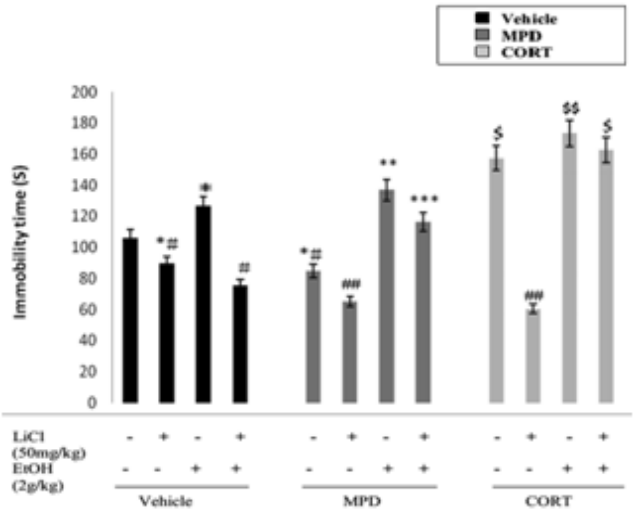
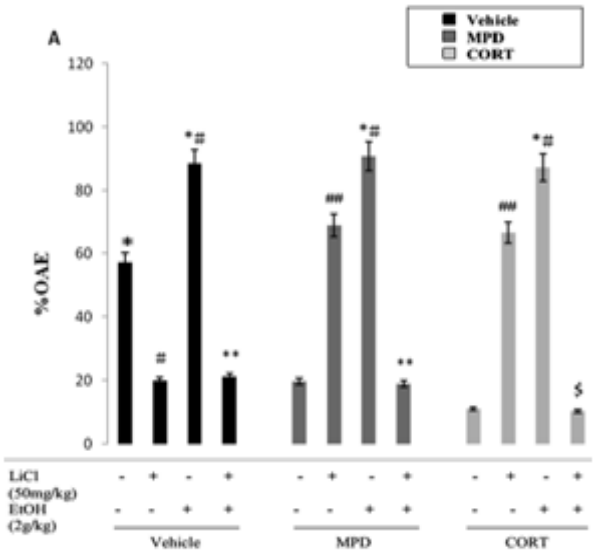


Fig 5: Effect of ethanol, on lithium treated manic and depressive-like behavior mice in terms of counts in 5 min time interval in the tail suspension test.
The plus sign indicates the agent treatment group. The minus sign indicates the vehicle treatment group. Values are expressed as mean (±S.D.) with n=6 in each group; one-way ANOVA followed by Duncan's multiple range test (DMRT). No significant difference was found between CORT and CORT+LiCl+EtOH treated groups. Significant differences**:** $^{*\#, *, \#, **, \$}$ p<0.05 compared with the vehicle-treated control group. $^{\#\#}$ p<0.05 compared with the MPD alone treated groups. ***p<0.05 compared with the MPD + LiCl treated groups. $^{\#\#, \$\$}$ compared with the CORT alone treated groups.



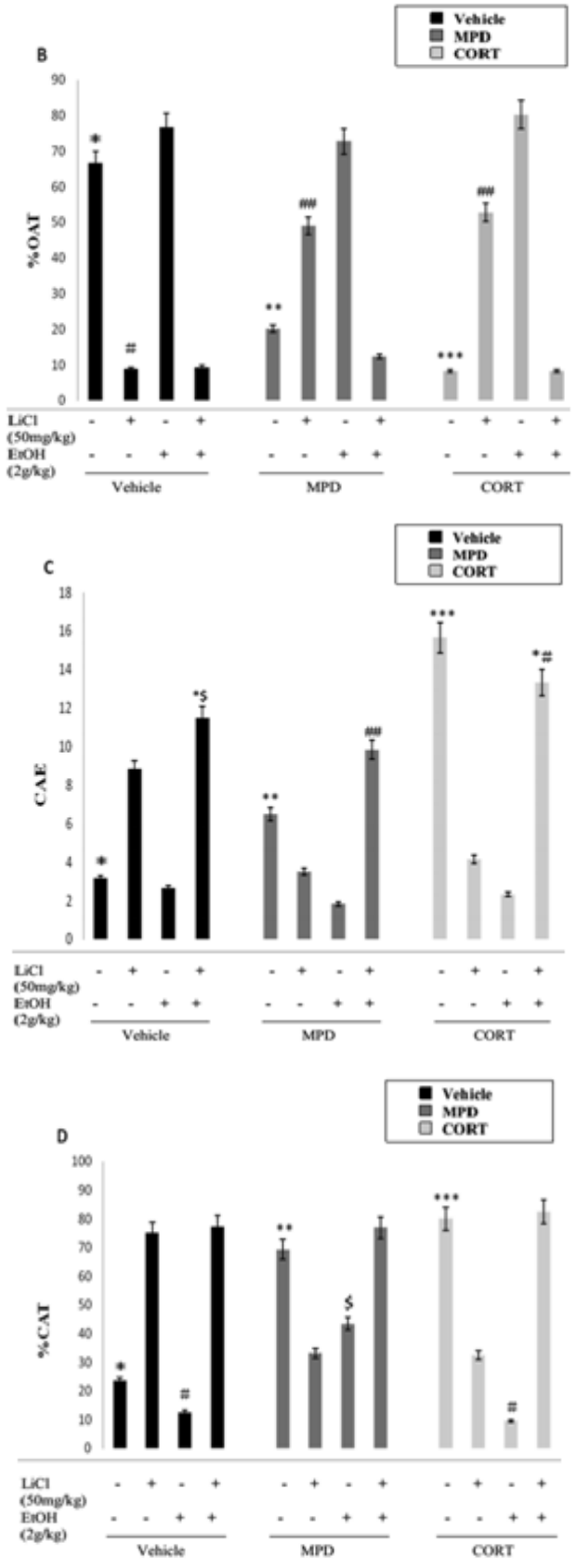


Fig 6: Effect of ethanol, on lithium treated manic and depressive-like behavior mice over a 5 min time period in the elevated plus maze test.

The plus sign indicates the agent treatment group. The minus sign indicates the vehicle treatment group. Data for the open arms and the closed arms were analyzed independently of one another. **A:** % of open arm entries of the maze. **B:** % of time spent in the open arms of the maze. **C:** number of entries in the closed arms of the maze. **D:** % of time spent in the closed arms of the maze. Values for a specific behavior segment that do not share a common superscript are significantly different at P<0.05. All data are expressed as mean (±S.D.) with n=6 in each group; one-way ANOVA followed by Duncan's multiple range test (DMRT).

Table 1: Schedule of drug treatment*

		Induction phase				Expression phase			
Group (N=6)	Day 0	Day 1	Days 2-7	Days 8-9	Days 10-14	Days 15	Days 16-21
I	H	S	Vehicle	WO	Vehicle	WO	Vehicle
II	H	S	Vehicle + LiCl @13: 00	WO	Vehicle + LiCl @13: 00	WO	LiCl @13:00
III	H	S	Vehicle + EtOH @14: 00	WO	Vehicle + EtOH @14: 00	WO	EtOH @14: 00
IV	H	S	Vehicle + LiCl @13:00 + EtOH @14: 00	WO	Vehicle + LiCl @13: 00 + EtOH @14: 00	WO	LiCl @13:00 + EtOH @14:00
V	H	S	MPD @12: 00	WO	MPD @12: 00	WO	MPD @12: 00
VI	H	S	MPD @12: 00	WO	MPD @12: 00+LiCl @13: 00	WO	MPD @12: 00+LiCl @13: 00
VII	H	S	MPD @12: 00	WO	MPD @12: 00+ EtOH @14: 00	WO	MPD @12: 00+ EtOH @14: 00
VIII	H	S	MPD @12: 00	WO	MPD @12: 00+LiCl @13: 00 + EtOH @14: 00	WO	MPD @12: 00+LiCl @13: 00 + EtOH @14: 00
IX	H	S	CORT @ 12:00	WO	CORT @ 12:00	WO	CORT @ 12:00
X	H	S	CORT @ 12:00	WO	CORT @ 12:00	WO	CORT @ 12:00+LiCl @13: 00
XI	H	S	CORT @ 12:00	WO	CORT @ 12:00	WO	CORT @ 12:00+ EtOH @14: 00
XII	H	S	CORT @ 12:00	WO	CORT @ 12:00	WO	CORT @ 12:00+LiCl @13:00 EtOH @14:00

*H= habituation; S= 0.9% Saline (i.p.); WO= washout period. MPD=2. 0 mg/kg methylphenidate (i.p.); CORT=20.0mg/kg corticosterone (i.p.); LiCl=50mg/kg lithium chloride (p.o.); EtOH=2.0 g/kg ethanol (p.o.). All mice were habituated to the activity chambers for 48-72 h prior to the beginning of the experiment. In the 1st day morning all mice were received intraperitoneal saline injection. On the 2nd day onwards the drug treatments was started for all the 12 groups. Methylphenidate (2.0 mg/kg) and Corticosterone (20.0 mg/kg) were given

intraperitoneally whereas; lithium (50 mg/kg) and ethanol (2.0g/kg) were given by oral gavage. However in MPD and CORT groups (VI, VII, VIII, X, XI, XII) lithium and ethanol were administered after the significant changes in the behavioral activity, which will be monitored by using actophotometer and Tail suspension test.

In the present study, similar sensitization to hyperlocomotion has been observed with repeated administration of ethanol (2g/kg, p.o.) in vehicle+EtOH treated groups.[34,35] However, we also support the previous findings that ethanol enhanced stimulation of locomotor activity in MPD+EtOH (2g/kg, p.o.) treated groups.[36,37] CORT+EtOH treated group showed significant decrease in motor activity.[38] In addition to this ethanol enhanced locomotor activity in manic-like behavior mice and attenuates motor activity in depressive-like behavior mice.

In this paper, we also investigated the combined effect of lithium and ethanol in manic and depressive-like behavior in mice. We found that there was a significant increase in locomotor activity in MPD+LiCl+EtOH treated groups.[39] Another interesting observation of the present study was the significant decrease in locomotor activity in CORT+LiCl+EtOH treated groups.[33] Therefore ethanol modulates the lithium treatment and differentially modifies behavioral responses in the methylphenidate and corticosterone groups.

In the TST, animals are placed in an inescapable situation and the antidepressant-like activity is expressed by the decrease of immobility time [40]. The immobility in the TST represents a failure of persistence in escape-directed behavior and effective antidepressant treatments in the TST [27,41]. In the present study vehicle+LiCl treated group produces a significant reduction in the immobility time as compared to vehicle-treated control. Hence this finding supports the previous report that long-term lithium administration in mice exerts robust antidepressant-like effects in the TST.[42] On the other hand, vehicle+EtOH treated groups significantly increase the immobility time.[43] However, lithium may produce a synergistic antidepressant-like effect in Vehicle+LiCl+EtOH treated groups by reducing the immobility time in the TST.

We have also observed that MPD treated groups significantly reduce the immobility time when compared to vehicle-treated control. However, MPD enhancing motor activity may give a "false" positive effect in the TST, decreasing the immobility time by stimulating locomotor activity.[44,45,46] We demonstrate that MPD+LiCl treated group produced a significant decrease in immobility time when assessed in the TST. Therefore, lithium may serve as an anti-immobility effect in manic-like behavior in mice. However, MPD+EtOH treated group caused a significant elevation of immobility time in the mice TST compared with the CORT groups ($P<0.05$). Our observations revealed that MPD+LiCL+EtOH treated group significantly increase the immobility duration as compared to MPD+LiCL treated group ($p<0.05$). An interesting finding of the present study is that the ethanol attenuates the lithium treatment and increase the immobility duration in manic-like behavior in mice.

Tail suspension tests are the accepted stress models of depression. Repeated CORT treatment caused a significant elevation of immobility time in the mice TST compared with the vehicle-treated control ($P<0.05$) [47,48]. We support the previous findings that corticosterone (20 mg/kg, i.p) injections significantly induce depressive-like behavior in mice.[18] The reduction of immobility was comparable to observed effects after administration of lithium in depressive-like behavior in mice. Therefore lithium reverses its effect on animal model of depression and may serve as an anti-depressant like effect [45]. Similarly in our study we observed that ethanol enhance immobility period and also modulates the lithium treatment in a significant manner ($P<0.05$).

The elevated plus-maze is one of the most widely used models for the study of anxiety-related processes in animals. It is noticeable that chronic administration of MPD (2mg/kg, i.p.) and CORT (20 mg/kg) for 21 days significantly increase in the number of entries into the closed arm and decrease in the percentage of time spent in the open arm provides evidence that animals behaved differently to vehicle-treated controls in open-arm measures of anxiety even after repeated testing indicates, anxiety-like behaviors. Previous studies showing that repeated long-term methylphenidate treatment causes an increase in anxiety [49,50].
Studies have shown that MPD+LiCl and CORT+LiCl treated groups significantly increase in % OAT and % OAE, induced anti-anxiety like effect and improved exploratory activity. The results of the present study confirm the anxiolytic properties in vehicle+EtOH, MPD+EtOH and CORT+EtOH treated group mice as evidenced by

an increased proportion of open arm entries after the administration of ethanol at doses of (2 g/kg, p.o.) [51,52]. Likewise, ethanol combined with lithium treated groups significantly increased in closed arm entries and decreased in open arm entries indicates anxiogenic effect. Therefore the results revealed that ethanol attenuates the lithium treatment and increase the anxiety-like behaviors in manic and depressive-like behavior in mice.

Overall, the behavioral evidence supports our hypothesis that lithium treatment (50 mg/kg p.o.) exhibit antidepressant and anti-anxiety like effects in manic and depressive-like behavior in mice. However, we evaluated the effect of ethanol while administered lithium during manic and depressive-like developmental periods demonstrates anxiogenic activity and reduction in exploratory behavior. Based on the behavioral assessment we also found that ethanol impair the lithium treatment and enhanced the locomotor sensitization activity in manic-like behavior in mice and decreased anti-depressant activity in depressive-like behavior in mice. We also confirm that ethanol worsen the condition of the bipolar disorder patient even in the regular intake of mood stabilizer.

This study gives a clear indication about the effect of inducer and its impact on the physiology and biochemistry of the experimental models hence this study forms the base for further investigation of neurophysiological changes in the brain with reference to ethanol stimulation of manic and depressive patients.

5. CONCLUSION

Further researches have to be done yet for these entire conclusion, and more importantly, it is necessary to clarify the mechanism by which ethanol elicits behavioral effects in manic and depressive-like behavior in mice by altering the transduction of cellular signal generated by different neurotransmitter systems. The present findings may therefore be relevant to the underlying pathophysiology of bipolar disorder.

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