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Withania somnifera extract improves cognitive, behavioral and mood disorders in animal model of bipolar disorder

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ABSTRACT: In this paper we examined the *Withania somnifera* extract (WSE) improve cognitive, behavioral and mood disorder in animal model of bipolar disorder. The present study was undertaken to investigate cognitive, behavioral and mood disorder effects of WSE on bipolar disorder of the male/female Wistar albino rats' brain. We have observed after 24 hours of last dosing the behavioral, memory and test were carried out followed by estimation of markers of oxidative stress under six groups using continuous sub-anesthetic dose of Amphetamine (Amph) (1.5 mg/kg, i.p) with WSE (300 mg/kg, i.p) and LiCl (1.5 mEq/kg, i.p.) administered daily for 21 days. We have found the percentage alteration was decreased in rat treated with WSE as compared to control indicate that it also improved spatial memory and learning. We have examined various oxidative parameters such as levels of SOD, GSH and TBARS level as compared to control groups. The reduced level of oxidative stress parameters upon administration of WSE and LiCl indicates ameliorating effects of WSE in oxidative stress. We had concluded that WSE showed improvement in learning and memory as animal treated with WSE spent more time in open arm as compared to Amph with WSE and LiCl treated rats. Hence, we can say that WSE improve cognitive, behavioral and mood disorder in animal model of bipolar disorder.

KEYWORD: *Withania somnifera* extract; cognitive; behavioral; mood disorder; animal model; bipolar disorder

1. INTRODUCTION

Bipolar disorder has cognitive deficits that persist even in euthymic phases [1-3]. Executive functions, working memory, processing speed, episodic memory, fluency, problem-solving and perceptual skills have been reported to consistently have moderate and large measured factors, i.e., a comprehensive cognitive deficit, albeit less severe than some of the deficits reported in schizophrenics [4]. Cognitive deficits were frequently related to poor functional results in schizophrenia [5]. Similarly, even after adjusting for demographic, disease, and mood characteristics, cognitive deficits are associated to lower functioning in bipolar patients in 6 of 8 studies [6]. Furthermore, changes in particular cognitive test scores predicted vocational recovery 3 months following symptomatic recovery in previously employed adults with bipolar disease who have suffered a manic episode [7]. As both a result, it stands to reason, enhancing cognitive capability in bipolar illness should be prioritized in research. Controlled data upon these therapy initiatives, on the other hand, are shockingly scarce. Burdick and colleagues used pramipexole, a dopamine D2/D3 agonist, in an 8-week placebo-controlled experiment to enhance cognition in bipolar disorder patients. However, whenever the sample were confined to something like a strictly euthymic bipolar illness group, two tasks' digits backward and Stroop color showed clinically meaningful therapeutic effects using pramipexole [8]. These factors of cognitive impairment in bipolar disorder are yet unknown. Pharmacologic methods for improving cognition in bipolar disorder [9] have included, among many other things, increasing dopaminergic function [10,11], increasing cholinergic function [12, 13] and lowering hypercortisolemia using a glucocorticoid receptor antagonist [14]. Open research has indeed evaluated cognitive rehabilitation [15].

They investigated the efficacy of a standardized extract of both the medicinal plant *Withania somnifera extract (WSE)* (Sensoril) as either a precognitive agent in bipolar illness. *Withania somnifera (WS)* has now been utilized as a *Rasayana* or an *adaptogen* in Ayurvedic therapy in India for millennia (increasing bodily

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resistance to stress and disease). *WSE* has numerous key bioactive compounds, according to modern chemistry data, including steroidal lactones known as glycowithanolides and sitoindosides, and also Withaferin A [16]. Glycowithanolides have already been demonstrated to have antioxidant, neuroprotective, and memory-enhancing action in the brain [17–20]. Withanolide A from *WSE* with mice, it corrected memory impairments, stimulated dendritic spine and axon regeneration. [21]. Furthermore, in a stress-induced rat model, *WSE* reduced hippocampus cell loss in the CA2 and CA3 regions by 80% [22]. Using stress models, mice given *WSE* showed a considerable reduction in hypercortisolemia and other stress indices [23–25]. *WSE* were demonstrated to exhibit procholinergic even if not glutamatergic or GABAergic actions there in rat brain [26]. Moreover, *WSE* induce cholinesterase inhibitory activity [27–29]. Given the several pharmacologic activities, they hypothesized that additive *WSE* therapy will enhance cognitive skills in people with bipolar illness in general, such as executive functioning, working memory, attention, processing speed and overall memory.

2. RESULTS

2.1. Behavioral parameters

2.1.1. Spontaneous alternation behavior

There was slightly change in the percentage alternation of animals in *WSE* treated groups as compared to control group. Furthermore, as compared with the control group, that percentage change in the Amphetamine (Amph) treated group was significantly lower ($p<0.05$). A significant possible alternation was observed in Amphetamine (Amph) *per se* and *WSE* combination treated groups in comparison to control (Table 1, Figure 1).

Table 1. Effect of i.p. *WSE* & Lithium chloride on spontaneous alteration behavior in rats by using elevate plus maze apparatus

Groups (n=6)	Drug treatment	Dosage (mg/kg)	Percentage alteration
I	Control	Normal saline (0.9%)	45.51±3.89
II	Amphetamine (Amph)	1.5 mg/kg daily for 21 days	67.55± 1.78**
III	*WSE per se*	300 mg/kg body weight, i.p. for 21 days	31.75± 0.82*###
IV	Lithium Chloride (LiCl)	1.5 mEq/kg, pH adjust to 6.5 administered for 21 days	43.11±3.94***###
V	*WSE* + Amph	300 mg/kg body weight, i.p, for 21 days + 1.5 mg/kg daily i.p, for 21 days	61.64±1.12#
VI	LiCl + Amph	1.5 mEq/kg, + 1.5 mg/kg i.p administered daily for 21 days	54.07±3.64##

All values have been expressed as mean ± SEM. 'Analysis of variance (ANOVA) is being used in the statistical analysis, accompanied mostly by Tukey-Kramer multiple comparison test. P <0.05 is regarded very important. * $p<0.05$, ** $p<0.01$, *** $p<0.001$ in compared with the control group, # $p<0.05$, ## $p<0.01$, ### $p<0.001$ in comparison to Amphetamine (Amph).

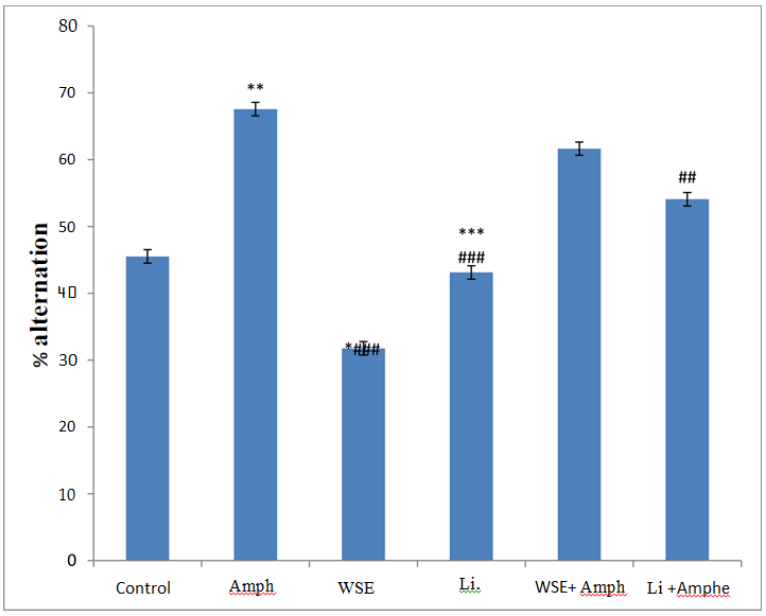

Figure 1. Effects of *WSE* & Lithium chloride on % alteration in elevated plus maze test in rats

All values have been expressed as mean ± SEM. 'Analysis of variance (ANOVA) is being used in the statistical analysis, accompanied mostly by Tukey-Kramer multiple comparison test. P <0.05 is regarded very important. * p<0.05, **p<0.01, *** p<0.001 in compared with the control group, # p+0.05, ## p<0.01, ### p<0.001 in comparison to Amphetamine (Amph).

2.1.2. Elevated plus maze test

Administration of *WSE* (300 mg/kg body weight, i.p. for 21 days) when compared to Amphetamine (Amph) treated (1.5 mg/kg daily for 21 days) groups, the time spent in open arms rose while the time spent in closed arms decreased (Figure 2). Closed arm entrance were statistically significant (P<0.001) in comparison with the control group. In compared to control groups, the total number of open arm entries fell in Amphetamine (Amph) treated groups, and *WSE per se* groups were revealed extremely significant findings (P <0.001) (Table 2, Figure 3-6).

Table 2. Effects of *WSE* & Lithium chloride on Amphetamine (Amph) induced locomotor activity in rats by using Open Field Activity Monitoring System

Groups (n=6)	Drug treatment	Dosage (mg/kg)	% time spent			Total no. of arm entries	
			Open arm		Close arm	Open arm	Close arm
I	Control	Normal saline (1ml of 0.9%)	65± 3.26	36.5±2.56	14.89 ± 1.00	10.33± 0.88	65 ± 3.26
II	Amphetamine (Amph)	1.5 mg/kg daily for 21 days	25.5±2.65**	79.55 ±1.73***	7.61±0.40***	16± 1.15**	25.5±2.65**
III	*WSE per se*	300 mg/kg body weight, i.p. for 21 days	66.75±1.7##	30.44±0.96##	12.36±0.49*##	14.66±0.88*#	66.75±1.7##
IV	Lithium Chloride (LiCl) *Per Se*	1.5 mEq/kg, pH adjust to 6.5 administered for 21 days	61.6±2.7###	35.00±2.24##	15.99±0.31###	11.33±0.79##	61.6±2.7###
V	*WSE*+ Amph	300 mg/kg body weight, i.p, for 21 days + 1.5 mg/kg daily i.p, for 21 days	71.66± 1.39 66.69 ±1.88	32.81±1.31 40.30± 1.17	9.97 ± 0.41 13.67 ± 0.67	9.00± 0.97 8.66± 0.66	71.66± 1.39 66.69 ±1.88
VI	LiCl + Amph	1.5 mEq/kg, pH adjust to 6.5 administered for 21 days + 1.5 mg/kg i.p administered daily for 21 days	65 ± 3.26	36.5±2.56	14.89 ± 1.00	10.33± 0.88	65 ± 3.26

All results were reported as mean + SEM. Analysis of variance (ANOVA) was used for the statistical analysis, accompanied either by Tukey-Kramer multiple comparison test. P<0.05 was seen as substantial. * p<0.05, **p<0.01, *** p<0.001 in comparison with the control group, # p<0.05, ## p<0.01, #4# p<0.001 when compared with Amphetamine (Amph).

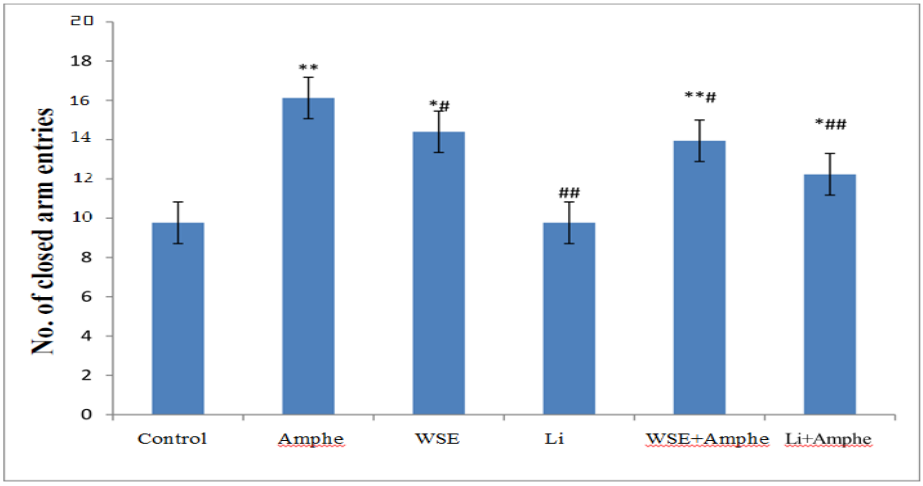

Figure 3. Effect of *WSE* & Lithium chloride on No. of closed arm entries in elevated plus maze test.

All results were reported as mean + SEM. Analysis of variance (ANOVA) was used for the statistical analysis, accompanied either by Tukey-Kramer multiple comparison test. P<0.05 was seen as substantial. * p<0.05, **p<0.01, *** p<0.001 in comparison with the control group, # p<0.05, ## p<0.01, #4# p<0.001 when compared with Amphetamine (Amph).

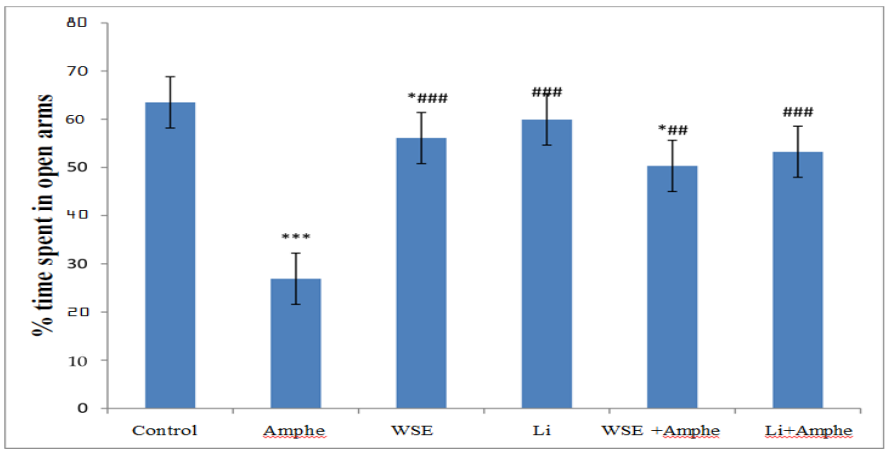

Figure 4. Effects of *WSE* & Lithium chloride on % time spent in open arms on elevated plus maze test in rats

All results were reported as mean + SEM. Analysis of variance (ANOVA) was used for the statistical analysis, accompanied either by Tukey-Kramer multiple comparison test. P<0.05 was seen as substantial. * p<0.05, **p<0.01, *** p<0.001 in comparison with the control group, # p<0.05, ## p<0.01, #4# p<0.001 when compared with Amphetamine (Amph).

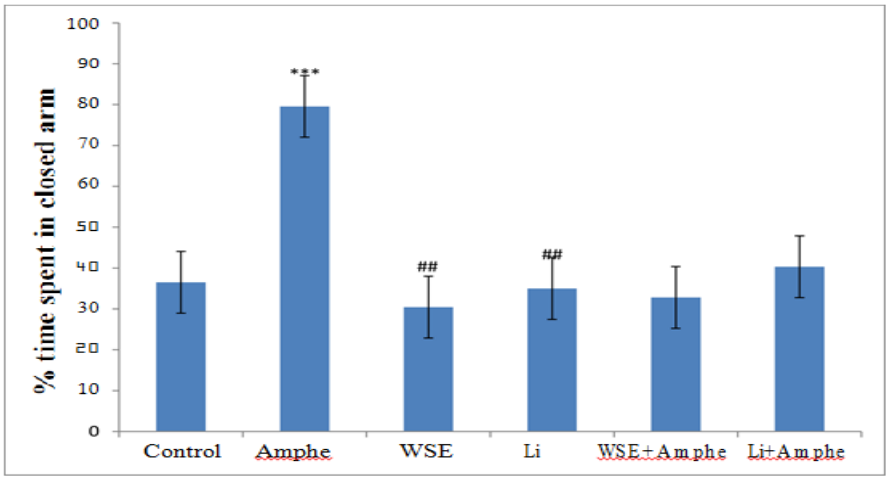

Figure 5. Effect of *WSE* & Lithium chloride on %time spent closed arm in elevated plus maze test in rats

All results were reported as mean + SEM. Analysis of variance (ANOVA) was used for the statistical analysis, accompanied either by Tukey-Kramer multiple comparison test. P<0.05 was seen as substantial. * p<0.05, **p<0.01, *** p<0.001 in comparison with the control group, # p<0.05, ## p<0.01, #4# p<0.001 when compared with Amphetamine (Amph).

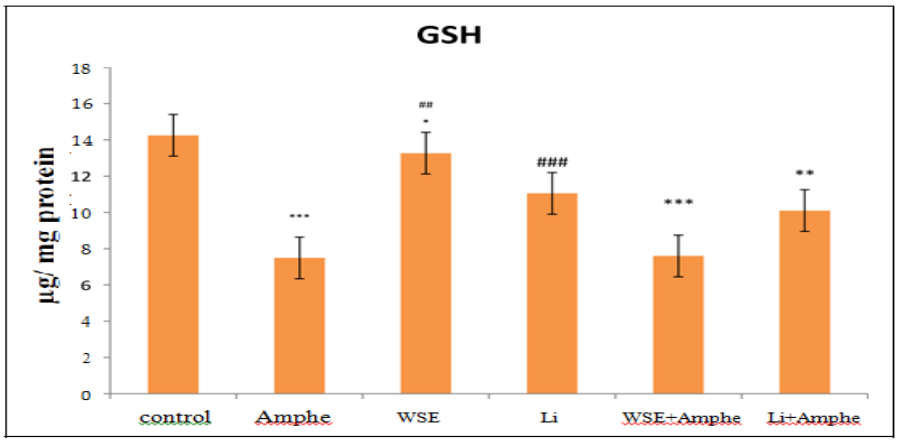

Figure 6. Effects of *WSE* and Lithium chloride on GSH level in rat brain sample

All results were reported as mean + SEM. P<0.05 was seen as substantial. * p<0.05, **p<0.01, *** p<0.001 in comparison with the control group, # p<0.05, ## p<0.01, #4# p<0.001 when compared with Amphetamine (Amph).

2.1.3. Locomotor activity monitoring

Total movement time was increased in Amphetamine (Amph) treated groups as compared with control groups, where *WSE per se* groups showed highly significant as compared with Amphetamine (Amph) group (P<0.001). Rest time was increased in *WSE* treated groups as compared to Amphetamine (Amph) treated groups showed hyperlocomotion activity. Horizontal locomotor activity was increased in Amphetamine (Amph) treated group as compared to control groups (Table 3).

Table 3. Effects of *WSE* & Lithium chloride on Amphetamine (Amph) induced locomotor activity in rats by using Open Field Activity Monitoring System

Groups (n=6)	Treatment	Horizontal activity(cm)	Move time (s)	Rest time (s)	Average dist/move (cm)	Mean velocity (cm/s)	Total movement ()
I	Control (1ml/kg,i.p) saline (0.9%)	2068.37 ±98.65	243± 15.68	682.57±14.98	2.58 ±0.17	2.73±0.11	563.38 ±23.78$^{\#}$
II	Amphetamine (Amph) (1.5 mg/kg,i.p)	6082.62± 252.70**	738±16.87**	246 ±15.94**	5.16± 0.35**	8.19 ±0.07**	1689±78.26**
III	*WSE per se* (300 mg/kg,i.p)	1974.71 ±92.51$^{**\#\#}$	165±8.79$^{**\#\#}$	671±8.57$^{*\#}$	1.89±0.06$^{*\#\#}$	2.81±0.06$^{\#}$	452.93±26.31$^{*\#\#}$
IV	LiCl *per se (1.5 mEq/kg, i.p.)*	1052 ±35.69$^{**\#\#\#}$	232±25.68$^{\#\#}$	693.67±10.31$^{*\#\#\#}$	2.36±0.04$^{*\#\#}$	2.63±0.02$^{\#\#\#}$	611.52±11.04$^{**\#\#\#}$
V	(*WSE* + Amph 300 mg/kg, i.p. +1.5 mg/kg, i.p.)	3032 ±203.54	308±15.73	612.65±15.54	3.15± 0.13	3.18±0.12	862.73±27.54
VI	LiCl + Amph (1.5 mEq/kg,i.p. +1.5 mg/kg , i.p)	4031± 263.27	302±24.78	602.70±25.73	3.24 ± 0.24	3.82 ±0.21	903.21 ±22.13

All the values were expressed as mean ±SEM. Statistical analysis was carried out using analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparison test. P <0.05 was considered significant. * p<0.05, **p<0.01, *** p/0.001 when compared with control, # p<0.05, ## p<0.01, ### p<0.001 when compared with Amphetamine (Amph).

2.2. Biochemical parameters

2.2.1. Reduced glutathione GSH

WSE (300 mg/kg body weight, i.p. for 21 days) treatment showed significant increase in GSH levels (p<0.05) and Amphetamine (Amph) administration (1.5 mg/kg daily for 21 days) resulted in decrease in GSH level and their combination led to an increase in GSH level as compared to their respective controls (Table 4, Figure 7).

Table 4. Effects of *WSE* & Lithium chloride on GSH, TBARS and SOD in rats

Groups (n=6)	Drugs treatment	GSH (µg/mg of protein)	TBARS (nmol/mg of protein)	SOD (Unit/mg of protein)
I	Control (1ml/kg,i.p) saline (0.9%)	14.25 ± 0.40	6.28 ± 0.16	171.8 ± 13.86
II	Amphetamine (Amph) (1.5 mg/kg,i.p)	7.49 ± 0.38***	7.52 ± 0.11**	76.74 ± 6.94***
III	*WSE per se* (300 mg/kg,i.p)	11.26 ± 0.28$^{*\#\#}$	6.14 ± 0.25$^{\#\#}$	164.83 ± 16.16$^{*\#\#}$
IV	*WSE* + Amph (300 mg/kg,i.p. +1.5 mg/kg, i.p.)	9.6 ± 0.63$^{\#\#}$	6.95 ± 0.14	127.61 ± 5.26$^{**\#\#}$
V	LiCl *per se* (2 mEq/kg.ip)	13.85 ± 0.27$^{\#\#\#}$	4.05 ± 0.21$^{***\#\#\#}$	175.44 ± 12.83$^{\#\#\#}$
VI	LiCl + Amph (1.5 mEq/kg,i.p. +1.5 mg/kg, i.p)	10.1 ± 0.87	6.28 ± 0.23$^{\#\#}$	140.32 ± 8.17$^{***\#\#\#}$

All data was presented as average + SEM and analyzed using ANOVA as well as the Tukey-Kramer multiple comparison test. P values of less than 0.05 were considered significant, while P values of less than 0.001 were considered very impressive. The total number of animals within every group is N=6* p<0.05, **p<0.01, *** p<0.001 in comparison to control, # p<0.05, ## p<0.01, ### p<0.001 in comparative to Amphetamine (Amph).

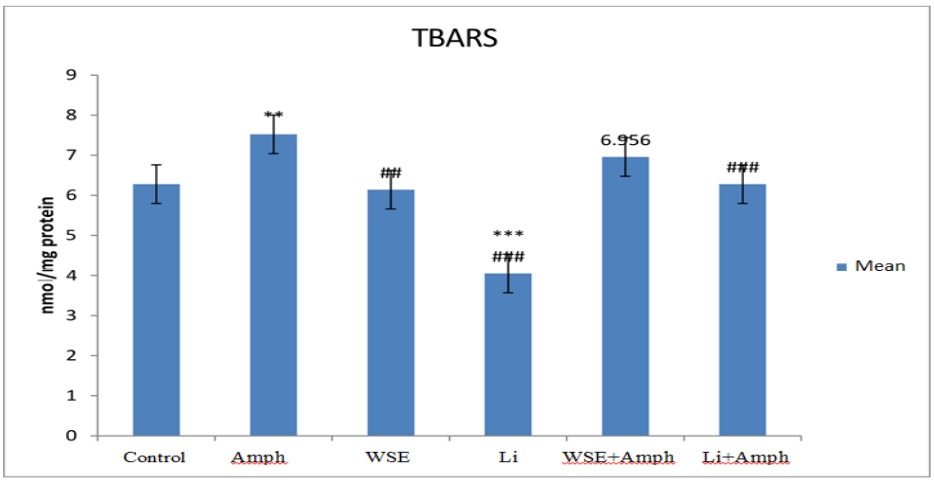

Figure 7. Effects of *WSE* & Lithium chloride on TBARS level in rat brain sample

P value greater than 0.05 is considered as significant, there is significant difference between control group and Amphetamine treated group (P<0.01). *WSE* show significance (p<0.01) as compared with toxic group.

2.2.2. TBARS

WSE (300 mg/kg body weight, i.p. for 21 days) When compared with the control group, there was a decrease in TBARS levels (p<0.05). When compared to a control group, Amphetamine (Amph) generated a higher TBARS score (p<0.01). The combination of *WSE* & Amphetamine (Amph) treatment resulted in a somewhat higher decrease of TBARS than just the control groups (Table 4, Figure 8).

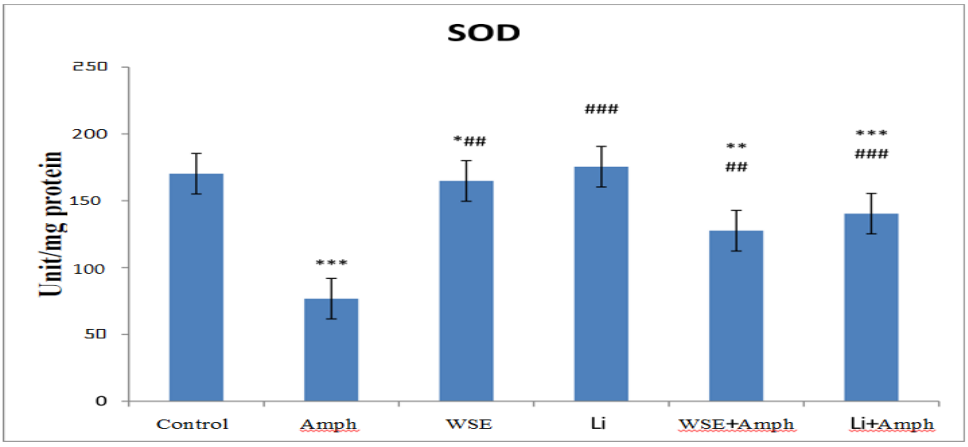

Figure 8. Effects of *WSE* & Lithium chloride on SOD in rat brain sample

Marker show significant difference as *** (P< 0.001)) vs control, ### (P< 0.001) vs toxic, there is less significant difference between *WSE* and Amphetamine treated group as compared to Lithium chloride treated group. P value less than 0.05 is considered significant.

2.2.3. SOD activity

SOD values showed significant increase in *WSE* groups as compared to Amphetamine (Amph) group (P<0.001), while *WSE* showed slightly difference as compared to control group (p<0.05). However, when combination of *WSE* and Amphetamine (Amph) administered showed marked decreases in SOD value as compared to control groups (Table 4, Figure 9).

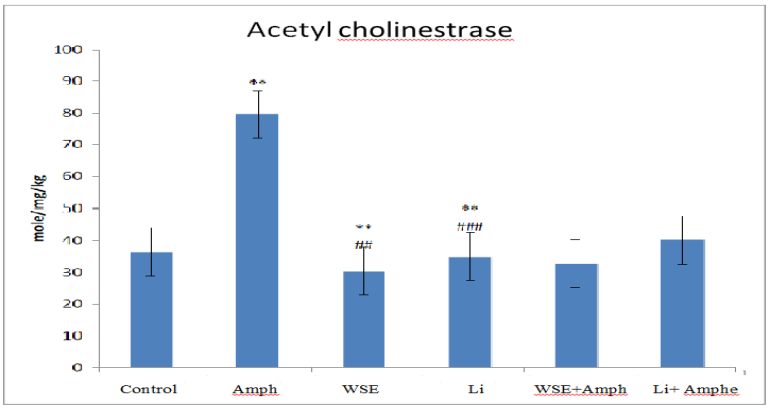

Figure 9. Effects of *WSE* on acetylcholinesterase in elevated plus maze test in rat

2.3. Acetylcholinesterase activity

Amphetamine (Amph) (1.5 mg/kg daily for 21 days) produced significant increase in acetylcholinesterase level as compared to their control ($p<0.05$). Administration of *WSE* lead to decrease in acetyl cholinesterase level as compared to their control (Table 5, Figure 10).

Table 5. Effects of *WSE* & Lithium chloride on acetylcholinesterase in elevated plus maze test in rat

Groups (N=6)	Drug treatment (14 days)	Dosage (mg/kg)	Acetylcholinesterase mole/mg/protein
I	Control (NS)	1 ml	18.48 ± 1.05
II	Amphetamine (Amph)	30	27.37 ± 2.02*
III	*WSE per se*	11.86	6.74 ±1.04**##
IV	Lithium Chloride(LiCl) *per se*	2	3.06 ±0.68**###
V	*WSE* + Amph	11.86 + 30	10.03 ±0.93*###
VI	LiCl+ Amph	2 +30	11.90 ± 1.28

All results have been presented as mean + SEM, and ANOVA and Tukey-Kramer multiple comparison tests are being used to analyze them. P values of less than 0.05 were considered significant, while P values of less than 0.001 were regarded very significant. The total number of animals within every group is N=6* $p<0.05$, **$p<0.01$, *** $p<0.001$ when compared with control, # $p<0.05$, 4# $p<0.01$, ### $p<0.001$ when compared with Amphetamine (Amph).

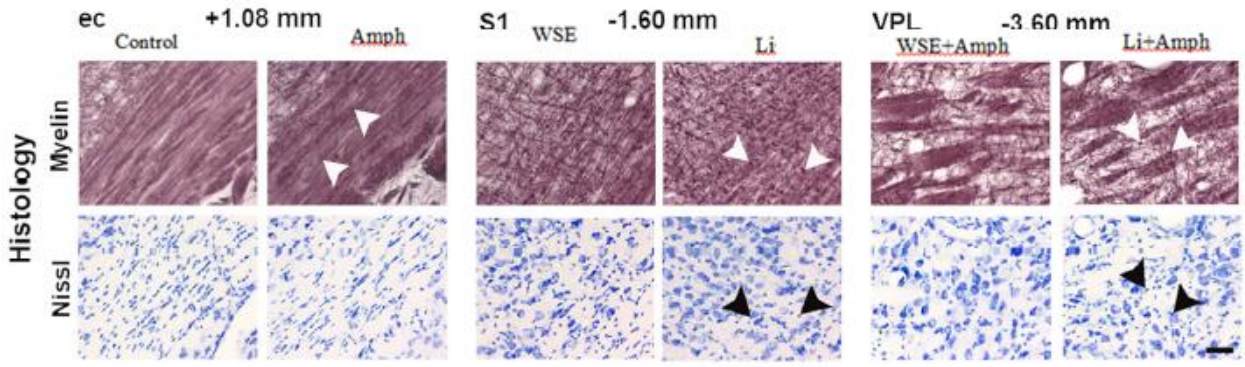

Figure 10. Representative whole-brain myelin and Nissl stained section of bipolar disorder animal model at 1.08-3.60 mm

2.4. Histopathology

In the present study, the decrease in infarct volume in animals supplemented with *WSE* correlates well with the improved locomotor and cognitive functions. This protective mechanism of *WSE* could be

attributed to the free radical scavenging, as well as thrombolytic and membrane stabilizing properties of withanolides present in *WS* root extract as reported earlier [30-32].

The tissue microstructure was subjected to a thorough quantitative histological examination. In vivo diffusion tensor imaging DTI identified increasing microstructural tissue abnormalities there in grey and white matter associated with axonal injury and gliosis from of the acute to subacute phases following varied dosage treatments (Figure 10).

This study found glial abnormalities in the prefrontal white matter with bipolar disorder. Changes in the populations of oligodendrocytes and astrocytes in white matter in severe psychiatric diseases may reflect disturbances in the architectural or metabolic support of axons.

2.5. Radiological study

We have observed the whole-brain voxel-wise group analysis of FA, AD, MD, and RD parameters comparing dose of *WSE* on Amphetamine (Amph) and lithium for animals at day 21. The rats showed significantly reduced FA, AD, MD, and RD parameters.

Whole-brain voxel-wise group analysis of FA, AD, MD, and RD parameters comparing dose of *WSE* on Amphetamine (Amph) and lithium for animals at day 21. FA, AD, MD, and RD values were all considerably lower in the rats. 1 – p, whereby p is the permutation-based FWE corrected p value after TFCE enhancement of the test statistic; a corrected $p<0.05$ were judged significant (blue-light blue colour scale). Axial diffusivity usually abbreviated as AD; fractional anisotropy is abbreviated as FA; mean diffusivity is abbreviated as MD; while radial diffusivity is abbreviated as RD (Figure 12).

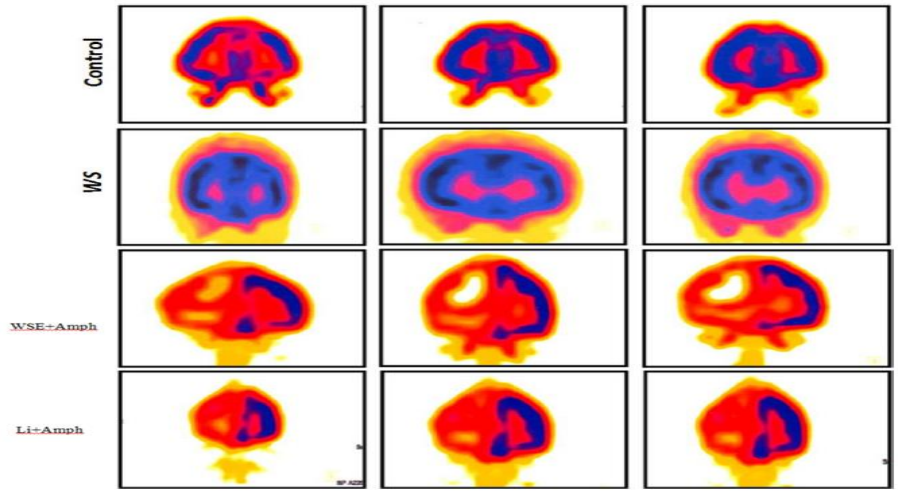

Figure 12. Single photon emission computerized tomography (SPECT) imaging
The use of MRI and histologic investigations together expands our understanding of in vivo DTI's ability to identify microstructural tissue brain changes following different therapies. And use in vivo diffusion tensor imaging DTI, this work may open up a new window to identifying progressive moderate microstructural tissue damage.

We have studied radiological such as MRI and PET of brain were performing to see the various anatomical changes in different part of brain of rat. In contrast to bipolar illness animal models, brain SPECT pictures both control and *WSE* supplemented rats exhibited increased retention of ^{99m}Tc-ECD inside the brain, indicating improved blood flow within brain areas.

A multiple linear regression test was used to analyses the connection between the DTI and histologic parameters from of the ST and CD investigations in white and grey matter. Overall impact of two components, AI & CD, to FA or AD (DTI parameter) was assessed using our regression model; AI &CD. The results of both the multiple linear regression test are summarized in Table 6, including contains the animal model statistics (R2 adjusted and F statistic), the t statistics for AI and CD [t(AI) and t(CD)], as well as the associated q values in Table 6 (Figure 13).

Table 6. Multiple linear regression between quantitative DTI and histologic analysis of bipolar disorder rats

	FA				(ADX10^{-3}mm^2 / s			
	R^2 adj	F stat	t(AI)	t(CD)	R^2 adj	F stat	t(AI)	t(CD)
Control	0.20	4.29	2.91*	-0.78	0	0.87	1.17	-0.79
Amph	0.72**	35.4	3.00	-2.37	0.69**	31.4	4.54***	-0.32
Li	0.08	2.19	-0.35	-2.06	0.03	1.47	-0.36	1.01
WSE+Amph	0.15	3.37	-1.96	-2.03	0	0.59	0.28	-0.98
Li+Amph	0.53**	32.0	5.02***	-4.63***	0.48***	26.5	5.45**	-3.17**

The use of MRI and histologic investigations together expands our understanding of in vivo DTI's ability to identify microstructural tissue brain changes following different therapies. And use in vivo diffusion tensor imaging DTI, this work may open up a new window to identifying progressive moderate microstructural tissue damage.

3. DISCUSSION

The effects of WSE on behavioral symptoms and memory impairment induced by a two-week intraperitoneal injection of numerous subanesthetic doses of amphetamine (Amph) (1.5 mg/kg, i.p.)). Alessandra Tiziana Pean et al. (2014) used operant self-administration paradigms to investigate the influence of WSE on ethanol consumption motivation [33]. Wistar rats were taught to self-administer ethanol (10%) by poking their noses. The effects of WSE (75-300 mg/kg) on provision and maintenance, ethanol breakpoint under a progressive reinforcement schedule, effects of deprivation, and reinstatement of seeking behavior were examined. Behavioral characteristics were recorded in the elevated-plus-maze test and in spontaneous alternating behavior, including locomotor activity in rats.

As with the elevated-plus-maze test, the total time spent in the closed arm, the total time spent on the side arms, and the percent preference for the closed arm and the open arm were recorded. Animals in the amphetamine group (Amph) spent much more time in the closed arm than in the open arm, although the time spent in the open arm increased compared with the closed arm as they were treated with WSE, suggesting that the rats had an aversion to open-arm entries. Other symptoms have already been observed in high difficulty mazes, such as freezing in the open for a prolonged period of time. In the current study, WSE was shown to relieve tension and anxiety, suggesting that it may be effective in treating symptoms of bipolar disorder.

Spontaneous alternation behavior (SAB) as a measure of short-term memory acquisition. In the current study, it was found that when amphetamine (Amph) was administered, the percent change on the Elevation and Maze Test was higher than when saline was administered. The percent change was lower when WSE was combined with amphetamine (Amph), indicating a role in learning and an anti-amnestic effect. WSE reduced AChE activity in rat brain, indicating an anti-amnesic effect against amphetamine (Amph)-induced amnesia in the elevated and maze paradigms [33].

To measure locomotor activity, total movement duration, resting time, and total horizontal activity were recorded with an activity monitoring system. Compared with the control groups, the amphetamine (Amph)-treated groups showed longer total movement duration. Compared with the amphetamine (amph) group, the WSE combination group showed a highly significant reduction in locomotor activity. The N-methyl-D-aspartate (NMDA) receptor antagonists phencyclidine and dizocilpine (MK-801) have comparable effects in rats associated with increased locomotor activity, as we have seen. In particular, hyperlocomotion triggered by NMDA antagonists has been used to compare the effects of typical and atypical APDs in the NMDA model of schizophrenic symptoms [34]. Thus, WSE may be a good candidate for effects induced by NMDA receptor antagonism [35]. Recent studies provide further evidence that WSE may function as an endogenous neurotransmitter or neuromodulator, generating renewed interest in identifying new neuroactive steroid targets for pharmacological intervention. Ashwagandha leaf-derived water extract (ASH-WEX) was evaluated for its neuroprotective properties. In specialized cells exposed to glutamate, with or without ASH-WEX, cell viability and expression of markers of glial and neuronal cell differentiation were examined. When exposed to glutamate, RA-differentiated C6 and IMR-32 cells lose their neuronal networks and die, leading to an increase in the stress protein HSP70. Pretreatment with ASH-WEX prevented glutamate-induced cell death and partially reversed the glutamate-induced changes in HSP70. In addition, studies of the neural cell adhesion molecule (NCAM) neuronal plasticity marker and its polysialylated version, PSA-NCAM, have shown that ASH-WEX holds therapeutic promise in preventing glutamate-induced excitotoxicity-induced neurodegeneration [36].

Although oxidation of cellular components such as lipids, proteins, and DNA, as well as changes in signaling pathways, could indeed cause cellular damage or neuronal death, changes in antioxidant enzyme levels and free radical formation are the main indicators of increased oxidative stress in patients with chronic bipolar disorder or reactive oxygen species (ROS) [37,38]. Participants were selected according to specific criteria. *W. somnifera* significantly reduced neurological impairments in the brains of mice caused by oxidative stress induced by various chemical and physical stressors. The altered oxidative as well as other stress indicators in different areas of the mouse brain were also drastically improved by WS.

Multiple subanesthetic doses of amphetamine (Amph) (1.5 mg/kg, i.p.) and LiCl (1.5 mEq/kg, i.p.) showed significant ($p<0.01$) (Philippe Cappeliez and Elizabeth Moore 1990; Ahmed et al., 2013). Compared to the control groups, TBARS levels increased. Impaired antioxidant defenses may play a role in the pathogenesis of bipolar disorder, schizophrenia, and other neurodegenerative diseases. In patients with schizophrenia and dipolar disorder, superoxide dismutase (SOD) and lipid peroxidation as measured by thiobarbituric acid reactive substances (TBARS/MDA) are increased [39]. WSE showed a significant reduction in TBARS levels, confirming its neuroprotective and/or antioxidant properties. In a type of randomized, double-blind, placebo-controlled pilot clinical trial, WS showed an encouraging effect in improving immediate or general memory and cognitive performance in adults with moderate cognitive impairment [40]. A randomized, double-blind, placebo-controlled companion study determined whether WSE helped improve cognitive impairment in individuals with bipolar disorder [41]. In a clinical trial, WS was found to improve cognitive and motor performance in healthy individuals. [42]. A likely mechanism behind the neuroprotective effects of WS could be mediated by many mechanisms. Many of these studies suggest that withanoside IV [43], withanone [44], withaferin A [45], withanolide A and some other bioactive compounds, as well as the synergistic effect of several compounds present in WSE, may contribute to the beneficial effect of WS in pathogenesis [46, 47].

GSH is the most important nonprotein antioxidant and redox regulator in the neurological system, shielding it from reactive oxygen species and modifying redox sensitive locations, such as NMDA receptors [48]. GSH level has been significantly ($p<0.001$) lower in the Amphetamine (Amph) treated group comparison to the control group, but it was expanded within a week of *WSE* administration. Combination of *WSE* and Amphetamine (Amph) showed higher GSH levels than Amphetamine (Amph), suggesting that the drug has neuroprotective and/or antioxidant properties [49].

SOD appears to be an important enzyme in reducing oxidative stress; in this study, it was found to be lower in amphetamine (Amph)-treated rats but significantly higher in WSE-treated rats, suggesting that neurosteroids play an important role in preventing oxidative damage, neuronal cell death, and apoptosis in bipolar disorder while restoring the above enzyme [50]. Moreover, oxidative stress impairs lipid-rich white matter [51]. Consequently, oxidative stress might be responsible for myelin-related deficits in SZ and bipolar disorder [52]. The pathophysiology of the disease could well be influenced by oxidative stress, which could also disrupt cellular signaling cascades [53].

Acetylcholinesterase level was increased in amphetamine (Amph) treated rats compared to control ($p<0.001$), while acetylcholinesterase level was significantly lower in WSE. The release of acetylcholine (ACh) in the rat hippocampus was associated with cognitive function. Thus, this study indirectly suggests that WSE increases Ach levels and improves cognitive performance. In addition, long-term treatment with fenproporex decreased acetylcholinesterase activity in the prefrontal cortex and striatum of rats, whereas no differences were found in the hippocampus and hypothalamus. Thus, the results suggest that continuous fenproporex treatment decreases acetylcholinesterase activity in the rat brain. Longer-term administration of fenproporex did not alter the short- or long-term memory of rats (IA or CMIA) [54].

It has been widely reported that MCA occlusion can induce selective behavioral and histopathological changes that closely resemble the histological, biochemical, and clinical features of ischemic stroke in humans [55]. The animal model showed a significant increase in infarct volume in both cortical and striatal brain regions, indicating a significant loss of active dehydrogenases in these brain regions. Cerebral infarcts result from mechanisms involving secondary excitotoxicity and free radical formation, which in turn are associated with the occurrence of brain edema and resultant tissue damage.

FA, AD, and MD was reduced further caudally at -5.60 mm within external capsule, corpus callosum, or auditory cortex. The hippocampus & midbrain additionally showed signs of Alzheimer's disease. RD were exclusively reduced in the auditory cortex; it was at the preceding level. Its exterior capsule, corpus callosum, internal capsule, & cingulum all had lower FA, AD, and MD levels. All the characteristics within somatosensory cortex, auditory cortex, and hippocampus was reduced in grey matter regions. Associated with reductions in FA, AD, & MD were detected in MRI along the external capsule up towards the

cingulum. Following treatment using *WSE*, Amphetamine (Amph) and Lithium Chloride, changes in AD and MD could be seen in the white matter, including the corpus callosum including fimbria, and that in the grey matter in the somatosensory cortex, although variations in RD have only been detected with in somatosensory cortex.

These results are in corroboration with an earlier report, wherein, *WSE* pre-supplementation to animals was shown to significantly reduce the infarct volume in *in-vivo* diffusion-weighted imaging studies in experimental model of ischemic stroke [56]. Moreover, it has been suggested that *WSE* has the propensity to enhance cyclic AMP levels in ischemic rats, which have shown to reverse stroke induced vasospasm in central vessels [57]. Additionally, WS supplementation has been shown to cause modulation of adenosine, which further causes inhibition of an excitatory amino acid (glutamate), thereby ameliorating ischemia reperfusion injury induced excitotoxic response [58].

They employed 2D histologic sections in just this investigation, which limited the analysis to 2D and left out the full 3D histopathologic interpretation. ST analysis of 3D histologic images acquired by advanced 3D imaging technologies like as confocal, multi-photon, or electron microscopy is the focus of future research [59].

4. CONCLUSION

They concluded that continuous subanesthetic doses of amphetamine (Amph) (1.5 mg/kg, i.p.) with WSE (300 mg/kg, i.p.) and LiCl (1.5 mEq/kg, i.p.) administered daily for 21 days showed significant changes in behavior in bipolar disorder. It can induce negative symptoms as well as cognitive memory impairment. In EPM, WSE showed improvement in learning and memory performance, as WSE-treated animals spent more time in the open arm than amphetamine (amph)-treated rats. The percent change was lower in WSE-treated rats compared with controls, suggesting that it also improved spatial memory and learning. Various oxidative parameters were estimated, oxidative stress was observed, the levels of SOD and GSH were reduced, and TBARS levels increased compared with the control groups, but the reduced ions in oxidative stress after WSE administration indicated an improvement in the effects of WSE on oxidative stress. Our results showed that WSE and LiCl improved learning and memory performance in the Raised-Plus maze, spontaneously altered behavior, locomotor activity, effects on the brain by lowering AchE, and reduced oxidative stress. This suggests that WSE may have neuroprotective and antioxidant effects. Our findings need to be further confirmed by multiple dose-dependent studies in different animal models with neurotransmitter assay to confirm the role of WSE in bipolar disorder.

5. MATERIALS AND METHODS

All chemicals and reagents were used A Grade (99.9%) purity such as 5,5-Dithiobis (2 –nitro benzoic acid) (DTNB) (SRL); Di ethyl ether (SD Fine Chem ltd) ; Ethylene diamine tritactic acid disodium salt (EDTA, Sod. salt) LiCl (SD Fine Chem ltd.); Folin's Ciocalteu Reagent (SD Fine Chem ltd.); Methanol (SD Fine Chem ltd.); Pot. Dihydrogen ortho phosphate (SRL); Potassium chloride (SRL); Pyrogallol (SRL); Sodium chloride (SRL); Sodium phosphate dibasic (SRL); Sodium Potassium Tartrate (SDFL); Standard TEP Reagent (SIGMA Aldrich); Thiobarbituric Acid (TBA) (SD Fine chem ltd.); Trichloroacetic Acid, Amphetamine (SD Fine Chem Ltd.); Tris Buffer (SRL); Tris HCL (SRL), Amphetamine injection purchased from Apollo pharmacy, New Delhi, and fresh root of *Withania somnifera* from Khaadi Bawli Market, Old Delhi and authenticated by senior botanist Dr. Sarfaraz Hussain and a pouch of sample submitted to the University for reference.

5.1. Methods of extraction of *WSE*

The root of *WS* were air-dried and ground into a coarse powder. After that, the coarse powder (500 g) was extracted separately with distilled water and 95 percent w/v alcohol using a cold maceration procedure for 24 and 72 hours, respectively. Under lowered pressure and vacuum, both extracts were filtered through muslin cloth and evaporated. The aqueous extract yielded 43 percent w/w, while the alcoholic extract yielded 29 percent w/w. Alcoholic extract was suspended in water and fractionated with petroleum ether, chloroform, ethyl acetate, and n-butanol, yielding 5.8%, 3.9, 2.1, and 1.2 percent w/w fractional yields from each solvent, respectively.

5.2. Animals

In this study, Wistar albino rats of both sexes were used as a prospective animal model of bipolar disorder. This study lasted 21 days. The animals were divided into six groups of six animals each. Wistar

albino rats weighing 200-230 grams were obtained from the Institute for Industrial Research & Toxicology-Ghaziabad, India. Approved by the Institutional Animal Ethics Committee (IAEC) [Project proposal no-IIRT/IAEC/2021/144] on September 06, 2021. Animals were housed in polypropylene cages under typical laboratory conditions (12 h light/dark cycle) with unlimited access to commercial pelleted food and water. The temperature inside the animal housing was maintained at 25±2 ^{o}C.

5.3. Tissue processing and histology

Following euthanasia by cervical dislocation, the brains were taken from the skull and postfixed in 4% PFA for 4 hours. After that, the brains were put in a cryoprotective solution (20% glycerol in 0.02 M potassium PBS, pH 7.4) for 36 hours. We quickly froze the brains in dry ice after cryoprotection and kept them at 70°C till cutting. We sectioned a subset of brains in the coronal plane using a sliding microtome (30mm, 1-in-5 series). The first series of sections were preserved at 20°C in 10% formalin, and the subsequent series in cryoprotectant tissue-collecting solution (30% ethylene glycol, 25% glycerol in 0.05 M sodium phosphate buffer). We used Nissl staining (thionine) to evaluate the cytoarchitectonics in the first series of sections, gliosis [increased cell density (CD)].

5.4. Apparatus

5.4.1. Elevated plus maze

The Elevated Plus Maze (EPM) test is used to detect anxiety-related behavior in rodent model of CNS disorder. A "+"-shaped maze elevated above the floor with two oppositely positioned closed arms, two oppositely positioned open arms, and a center region makes up the EPM device. Subjects' behavior is recorded using a video camera set above the maze and evaluated using a video tracking system as they freely explore the maze. To quantify anxiety-like behavior, the preference for open arms over closed arms (represented as a percentage of entries and/or a percentage of time spent in open arms) is measured.

5.4.2. Locomotor activity in an open field environment

The open field chambers are Plexiglas square chambers (27.3 x 27.3 x 20.3 cm; Med Associates Inc., St Albans, VT) surrounded by infrared photobeam sources (16 x 16 x 16)

5.5. Treatment schedule

Animals were divided into six groups each groups contain 6 wistar rats, normal saline was given to the control group, amphetamine 1.5 mg/kg, i.p, daily administered for 21 days to induce bipolar like symptoms (Amphetamine Model) [60]. 300mg/kg of *WSE* was given i.p for 21 days to the third group [61]. Combination of *WSE* and amphetamine was given i.p for 21 days to the fourth group, Lithium chloride 1.5 mEq/kg alone was administered i.p for 21 days as a gold standard comparative study. 1.5 mEq/kg Lithium chloride in combination with amphetamine 1.5mg/kg, i.p was given to the sixth group.

5.6. Statistical analysis

All the values were expressed as mean ±SEM. Statistical analysis was carried out using analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparison test. P <0.05 was considered significant. * p<0.05, **p<0.01, *** p/0.001 when compared with control, # p<0.05, ## p<0.01, ### p<0.001 when compared with Amphetamine (Amph).

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