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PREGNENOLONE SULPHATE REVERSED KETAMINE INDUCED BEHAVIORAL CHANGES IN RATS

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ABSTRACT
Pregnenolone and its derivatives have been implicated in neuroprotection and enhance NMDA receptor neurotransmission pointing out their therapeutic potential in schizophrenia. This study was aimed to evaluate the effects of pregnelonone sulphate (Preg S) on behavioural changes and markers of oxidative stress induced by ketamine (Ket) in rats. All rats received intraperitoneal (i.p) injection daily for 14 days with different drug treatments viz. Preg S (11.86 mg/kg), risperidone (2 mg/kg/day) alone and in combination with ketamine (30 mg/kg). Different behavioural tests which included elevated plus maze, spontaneous alternation behaviour, locomotor activity tests were performed after 24 hrs of last dosing followed by the estimation of markers of oxidative stress and acetylcholine esterase (AchE) activity in rat's brain. Ket produced significant changes in behaviour resembling to that of negative symptoms and memory impairment seen in schizophrenic patients. Preg S treated rats spent more time in open arm in elevated plus maze test, decreased percentage alternation and improved locomotor activity as compared to Ket treated rats. The levels of antioxidants such as superoxide dismutase (SOD) and reduced glutathione (GSH) were reduced while thiobarbituric acid reactive substances (TBARS) were elevated. Preg S administration normalized the antioxidant parameters. Preg S improved learning and memory in elevated plus maze, spontaneous alternation behaviour, locomotor activity, decreased AChE activity and reduced oxidative stress. Thus, we may conclude that Preg S offered neuroprotective and antioxidant effect in Ket induced behavioural symptoms.

KEYWORDS: Pregnenolone sulphate, ketamine, neuroprotective, antioxidant, schizophrenia.

1. INTRODUCTION
Schizophrenia (SCZ) is a major mental illness affecting approximately 1% population of world, responsible for causing the changes in perception, thoughts and behaviour.[1] SCZ is characterized by a wide range of symptoms which includes positive symptoms, negative symptoms, cognitive and neuropsychological dysfunction and mood symptoms.[2,3] In addition to the deficits in the cognitive function like attention and memory, it is the leading cause of suicide along with anxiety and depression in about 10% cases.[4]

Despite the availability of better anti-psychotic drugs, complete cure for SCZ has been elusive, and research is continuing for novel anti-psychotics possessing unique pharmacological profile to take care of positive, negative symptoms as well as cognitive deficits.[5-7]

A number of steroid hormones are synthesized in the brain which exists in higher concentrations in the nervous system than in the plasma. It is now known that these neurosteroids are metabolized in the brain from precursor compounds originating from endocrine sources. These neurosteroids are synthesized de novo in the brain from cholesterol.[8,9] Neuroactive steroids are essential for the proper development and functioning of the adult brain and play a major role in the stress response.[10] Based on plasma and cerebral spinal fluid (CSF) level studies in humans and preclinical evaluation of drugs on brain and plasma levels in laboratory animals, it was demonstrated that these steroids may contribute to the pathology and symptoms of some psychiatric illnesses and their levels may be affected by drugs used to treat these disorders.[11-14]

Neurosteroids, such as dihydroepiandrosterone (DHEA), pregnenolone and their derivatives have been implicated in neuroprotection and enhancement of NMDA receptor neurotransmission suggesting therapeutic potential in SCZ, possibly by actions at sigma1 receptors.[15-17] Pregnenolone sulphate (Preg S) is a neurosteroid with excitatory effects in the brain, acting as a potent negative

allosteric modulator of the $GABA_A$ receptor and a weak positive allosteric modulator of the NMDA receptor and agonist of the sigma receptor.[17-19]

With respect to SCZ, Pregnenolone actions on learning and memory in rodent models have been studied as cognitive symptoms were improved and it may be hypothesized that their long-term treatment may improve the outcome and quality of life in patients with SCZ.[20,21] The neurosteroid pregnenolone may represent a promising and mechanistically novel agent for cognitive and negative symptoms in SCZ. Pregnenolone and its sulphated derivative, Preg S enhance learning and memory in animal models at concentrations that are physiologically relevant and known to be present in human brain.[22-24]

In lab animals, the symptoms of SCZ are produced by various techniques including use of chemicals etc. Becker et al reported that sub-chronic treatment of Ketamine (Ket) (30 mg/kg, i.p) induced changes in rat behaviour resembling to that of the symptoms of SCZ[25] and this model was employed in the present research study to observe the schizophrenic behavioural symptoms of animals. Further, the role of oxidative stress has been implicated in the pathophysiology of SCZ[26-28] thus, the present study was carried out to determine the effect of Preg S on behavioural changes and markers of oxidative stress in Ket induced behavioural changes in rats resembling to schizophrenic symptoms in humans.

2.0. MATERIALS AND METHODS

2.1. Animals

Wistar albino rats, weighing 200-230 gm, were procured from the Central Animal House Facility, Hamdard University, New Delhi, India. The animals were kept in polypropylene cages under standard laboratory conditions (12 hours' light/dark cycles) and had free access to a commercial pellet diet and water *ad libitum*. The animal house temperature was maintained at 25 ±2 ºC. This study was approved by the Institutional Animal Ethics Committee (IAEC) [Reg. No1115] Jamia Hamdard, New Delhi on dated 25 Feb 2016.

2.2. Preparation and drug Administration

All the drugs solutions were administered intraperitoneally (i.p) for 14 days to rats. Preg S was procured from Sigma Aldrich Bangalore and was administered at a dose of 11.86 mg/kg/day, i.p. It was dissolved in 0.9% normal saline and sonicated for 10 min in ultrasonicated bath.[15] Ketamine was purchased from Troikaa Pharmaceutical Ltd and was administered (30 mg/kg/day, i.p).[25] Risperidone (2 mg/kg/day, i.p) was dissolved in 0.4 $molL^{-1}$ tartaric acid.[29]

Behavioural symptoms were induced by intraperitoneal injection of Ket (30 mg/kg/day) for two weeks. Sub-anaesthetic dose of ketamine produced behavioural symptoms like locomotor activity changes seen in elevated plus maze.[25]

2.3. Treatment schedule

Wistar albino rats of either sex were used in the present study. The duration of study was 14 days. The Animals were divided randomly into 6 groups each containing of 6 animals. Group-I Normal Control, Group-II Ketamine, Group-III, Preg S *per se*, Group-IV Risperidone *per se*, Group-V Preg S + Ketamine, Group-VI Risperidone + Ketamine. In group I, rats were administered normal saline (1 ml/kg body weight, i.p). Group II, Ketamine (30 mg/kg/d, i.p), Group III, received Preg S *per se* (11.86 mg/kg/d, i.p. dissolved in 0.9% NaCl), Group IV, received Risperidone *Per se* (2 mg/kg/day, i.p), Group V, received Preg S (11.86 mg/kg, i.p) + Ketamine (30 mg/kg i.p) and Group-VI- Risperidone (2 mg/kg/day, i.p) + Ketamine (30 mg/kg i.p) respectively.

After 24 hrs of last dosing, the behavioural tests were carried out followed by the estimation of markers of oxidative stress and acetylcholine esterase activity in rat's brain.

2.4. Behavioural tests

2.4.1. Spontaneous Alternation Behaviour (SAB)

This method was used to study the effect of drugs on learning and memory. Spontaneous alternation behaviour (SAB) was performed in a plus maze to assess effect of drugs on short term memory with respect to spatial orientation and perception as per earlier reported method.[30] The animals were placed in plus maze. The maze (85 cm height) was constructed of wood painted grey and contained a central platform (25 cm diameter), from which radiated four symmetrical arms (55cm long× 10 cm wide), with 12 cm wall. After being placed in the central platform, rats were allowed to traverse the maze freely for 12 min. The number and sequence of entries were recorded. An alternation was defined as entry into four different arms on an overlapping quintuple set. Five consecutive arm choices within the total set of arms choices constitute a quintuple set. A quintuple consisting of arm choices A, B, A, C, D was considered as an alternation, while the set with A, B, A, C, B did not. Using this procedure, percentage alternation is equal to the ratio of actual alternation to possible alternation × 100. Possible alternation sequences are equal to the no of arms entries minus 4.

Elevated Plus Maze Test (EPMT)

Behavioural symptoms were measured in the elevated plus-maze 2 weeks after the final injection. The maze was made of black polyvinyl chloride and had two open and two closed arms (50×10×40 cm) mounted 50 cm above the floor. The floor of the arms was smooth. Light levels were 30 or 400 lx. Bright illumination is considered more stressful to animals. A rat was placed in the central platform of the apparatus facing a closed arm. This is expressed as the animal spending more time in the enclosed arms. This model is based on rodent's

aversion of open spaces which involves avoidance of open areas by confining movements to enclose space.[31]

2.4.2. Locomotor Activity monitoring

It was observed in open field arenas consisting of an acrylic box (40.6×40.6×40.6 cm^3) accommodated with two photo beam frames (16 beams/dimension; 2.5 cm between beams; Coulbourn Instruments, Allentown, PA). The horizontal locomotor activity was recorded by the lower frame (2.5 cm above the arena floor) while the upper frame (15 cm above the floor) records rearing. The open field chamber was joined to a computer running software (True Scan 2.0 version, Coulbourn Instrument, Allentown, PA) that records beam break (100 ms sampling rate). Rats were kept for half an hour in home cage for habituation. Then they were place in an open field chamber for half an hour prior to observe the locomotor activity.[4] Locomotor activity was recorded for 20 minutes during which different parameters of horizontal locomotion activity were recorded for each rat.

2.4.4. Biochemical estimations

Brain was harvested by decapitation, immediately after behavioural studies, weighed and kept at -70°C till the time it was used for assay of oxidative stress marker i.e. thiobarbituric acid reactive substances (TBARS), reduced glutathione (GSH), superoxide dismutase (SOD), were measured to establish antioxidant properties of Preg S and acetylcholine esterase activity (AChE) in rats brain.

2.4.5. TBARS estimation[32]

One ml of the suspension medium was taken from the supernatant of the 10% tissue homogenate and centrifuged at 10,000 rpm. 0.5 ml of 30% TCA followed by 0.5 ml of 0.8% TBA was added to it. The tubes were covered with aluminum foils and were kept in a shaking water bath for 30 min at 80^{o}C. After 30 min, the tubes were taken out and were kept in ice-cold water for 10 min. They were then centrifuged at 3000 rpm for 15 min. The absorbance of the supernatant was read at 540 nm at room temperature against an appropriate blank. Blank consisted of 1.0 ml distilled water, 0.5ml 30% TCA, 0.5ml 0.8% TBA.

2.4.6. GSH level[33]

A known weight of tissue ranging from (300-600 mg) was homogenized in 5-8 ml of 0.02 M EDTA and then 4.0 ml of cold distilled water was added to it. After mixing it well, 1 ml of 50% trichloroacetic acid (TCA) was added and shaken intermittently for 10 min using a vortex mixer. After 10 min the contents were transferred to centrifuge tube (rinsed in EDTA) and centrifuged at 6000 rpm for 15 min. Following centrifugation, 2ml of the supernatant was mixed with 4.0 ml of 0.4 M tris buffer (pH 8.9). The whole solution was mixed well and 0.1 ml of 0.01 M DTNB was added to it. Absorbance was read within 5 min of the addition of DTNB at 412 nm against a reagent blank with no homogenate.

2.4.7. SOD activity[34]

The supernatant was assayed for SOD activity by following the inhibition of pyrogallol autoxidation. 100 µl of cytosolic supernatant was added to Tris HCL buffer (pH 8.5). The final volume of 3 ml was adjusted with the same buffer. At least 25µl of pyrogallol was added and change in absorbance at 420 nm was recorded at 1 min interval for 3 min. The increase in absorbance at 420 nm after the addition of pyrogallol was inhibited by the presence of SOD.

2.4.8. Estimation of brain AChE[35]

Rat brain was harvested by decapitation, immediately after elevated plus maze test, weighed and kept at -70°C until AChE assay. The whole brain AChE activity was measured according to the method of Ellman et al., 1961. A known weight of the brain tissue was homogenized in 0.32 M sucrose solution to get a 10% homogenate that was centrifuged at 3000 rpm for 15 minutes followed by centrifugation at 10,000 rpm for 10 min at a constant temperature 4°C. 1 ml. of supernatant was mixed with 9 ml of sucrose solution to get a 1% post mitochondrial supernatant (PMS). Test samples were prepared by mixing 2.7 ml of phosphate buffer, 0.1ml of DTNB and 0.1 ml of PMS. Reaction mixture was taken in a cuvette and pre-incubated for 5 min and 0.1 ml of acetyl thiocholine iodide was added to the mixture to initiate the reaction and immediately absorbance was recorded at 412 nm for 3 min interval. Protein was determined according to well established method.[36]

2.5. Statistical Analysis

Data were expressed as the mean ± SEM. For a statistical analysis, group means was compared by one-way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparison tests which can be used to identify differences between groups. P value < 0.05 was considered significant. Statistical analysis was carried out using Graph Pad Prism 5.00.288 (Graph pad software San Diego, CA).

3.0. RESULTS

3.1. Spontaneous Alternation Behaviour (SAB)

There was slightly decrease in the percentage alternation of animals in pregnenolone sulphate *per se* (11.86 mg/kg, Preg S) and risperidone (2 mg/kg, Risp) groups as compared to control group. However, a significant increased in the percentage alternation was observed in combination with ketamine (30 mg/kg, Ket) as compared to control group ($p<0.05$ (Fig 1).

3.2. Elevated plus maze test (EPMT)

Administration of Preg S increased the time spent in open arms and reduced the time spent in closed arms as compared to ket treated groups where closed arm entry was highly significant ($P<0.001$) as compared to control group. Total no. of open arm entries decreased in ket treated groups when compared with control groups and Preg S *per se* groups showed highly significant results ($p< 0.001$) (Table1).

3.3. Locomotor activity monitoring

Total movements, move time, move distance, mean velocity were increased and rest time was decreased in Ket treated group as compared with control group. However combination of Preg S and Ris pre-treated groups showed highly significant reduction in total movements, move time, move distance, mean velocity and increased in rest time as compared with Ket group ($P<0.001$) (Table 2).

3.4. Biochemical parameters

3.4.1. GSH

Preg S (11.86 mg/kg) treatment showed significant increase in GSH levels ($p<0.05$) and Ket administration (30 mg/kg) resulted in decrease in GSH level however, co-administration showed an increase in GSH level as compared to their respective controls (Table 3).

3.4.2. TBARS

Ket pre-treatment produced increased in TBARS value as compared to control group ($p<0.01$). Preg S showed a reduction in TBARS level ($p<0.05$) as compared to control group. Combination of Preg S and ketamine administration resulted in reduction of TBARS value slightly more than control groups (Table 3).

3.4.3. SOD activity

SOD activity was significantly reduced in ketamine group ($P< 0.001$), however combination of Preg S and Ket showed marked increased in SOD activity as compared to control groups. (Table 3).

3.4.4. AChE activity

Ket (30 mg/kg) produced significant increased in acetyl cholinesterase activity as compared to their control ($p<0.05$). Combination of Preg S and Ket treated animals showed decreased in AChE activity as compared to control group (Fig. 2).

4.0. DISCUSSION

Intraperitoneal injection of multiple sub-anaesthetic dose of Ket (30 mg/kg) for two weeks as reported by earlier reported study[25] demonstrated behavioural symptoms and memory impairment similar to that of schizophrenic symptoms. The present study evaluated Preg S in behavioural tests which include EPMT and SAB including the locomotor activity (LA) in rats.

During the EPMT, total time spent in closed arm, total time spent in open arms, % preference to closed arm and open arm were recorded, animal spent more time in closed arm as compared to open arm in group treated with ketamine but when it was treated with Preg S, there was an enhancement in the time spent in open arms as compared to closed arm which showed that rat demonstrated aversion toward open arm entries which indicates that anxiety and stress like symptoms were reduced which is a core symptoms of schizophrenia. Others symptoms also recorded in elevated plus maze like freezing to open space for long time. The present study demonstrated relieving and improvement in the stress and anxiety by Preg S which may be useful in ameliorating the symptoms of SCZ.

SAB has been used as a measure of recording short term memory.[37] In the present study it was also observed that upon administration of Ket, the % alteration was increased as compared with saline treated groups in elevated plus maze- test. Combination of Preg S with Ket reduced the % alternation suggesting its role in learning and memory and anti-amnestic effect. These behavioural observations were corroborated with a decrease in AChE activity in rat brain by Preg S thus, confirming its anti-amnesic activity against Ket induced amnesia in elevated plus maze model.

Total movement time, rest time and horizontal activity were recorded in activity monitoring system to assess the LA. Total movement time was increased in ketamine treated groups as compared with control groups. Preg S combination groups showed a highly significant reduction in LA as compared with ketamine group. N-Methyl-D Aspartate (NMDA) receptor antagonists, phencyclidine and dizocilpine (MK-801), also produce similar behavioural effects in rodents characterized by increased LA similarly as our observations. In particular, NMDA antagonist-induced hyperlocomotion has been used to compare the effects of typical and atypical anti-psychotic drugs in the NMDA-model of schizophrenic symptoms.[38] Thus, Preg S may be a good candidate to have effects produced by NMDA receptor antagonistic activity.[8] Recent studies provide additional evidence that Preg S may function as an endogenous neurotransmitter or neuromodulator, creating renewed interest in the identification of novel neuroactive steroid targets for pharmacological intervention.[24,39]

Evidence for increased oxidative stress in chronic SCZ patients is primarily based on the altered levels of antioxidants enzymes, free radical production or reactive oxygen species (ROS) can cause cellular damage or neuronal death, because oxidation of cellular components like lipid, protein and DNA and alteration of signalling pathway that finally promote the damage of cells.[40]

Multiple Sub-anaesthetic dose of Ket (30 mg/kg, i.p) showed significant ($p<0.01$) increased in TBARS value as compare to control treated groups. Impaired antioxidant defences are suggested to participate in the pathophysiology of schizophrenia and other neurodegenerative conditions. Altered SOD and increased lipid peroxidation, measured by the TBARS, are increased in schizophrenic patients.[41] Preg S showed a highly significant reduction in TBARS level, suggesting its neuroprotective and/or anti-oxidant properties.

GSH represents main cellular non-protein antioxidant and redox regulator in protecting nervous tissue against ROS[42] and in modulating redox sensitive sites,

including NMDA receptors. GSH level was significantly ($p< 0.001$) decreased in ketamine treated group as compared to control group, after Preg S administration GSH level was increased, combination of Preg S and ketamine show slightly increased in GSH level as compared to Ket which also showed neuroprotective and/or anti-oxidant properties of drug.

SOD is an important enzyme in reducing oxidative stress, in the present study there was a decrease in Ket treated rats, but highly increased in Preg S treated group showed that neurosteroids play important role to preventing oxidative damage cause neuronal cell death and apoptosis in schizophrenia by restoring this enzyme.

AChE was increased in Ket treated rats ($p<0.001$) as compared to control, Preg S showed marked decreased in AChE activity. ACh release from rat hippocampus is linked to cognitive function[43]s. Thus, indirectly our study confirms that Preg S increased the Ach levels and improved the cognitive function.

Table 1: Effect of Pregnenolone sulphate on Elevated Plus Maze test in rats.

Groups (n=6)	Drug treatment	Dosage (mg/kg)	% Time spent		Total no. of Arm entries	
			Open arm	Close arm	Open arm	Close arm
I	Normal Control	1 ml	65 ± 3.26	36.5 ± 2.56	14.89 ± 1.00	10.33 ± 0.88
II	Ketamine	30	25.5 ± 2.65**	79.55 ±1.73***	7.61 ± 0.40***	16 ± 1.15**
III	Preg S *per se*	11.86	66.75 ± 1.7##	30.44 ± 0.96##	12.36±0.49*##	14.66 ±0.88*#
IV	Risp *Per se*	2	61.6 ± 2.7###	35.00 ± 2.24##	15.99±0.31###	11.33±0.79##
V	Preg S + ket	11.86 + 30	71.66 ± 1.39	32.81±1.31	9.97 ± 0.41	9.00 ± 0.97
VI	Risp+ ket	2 + 30	66.69 ±1.88	40.30± 1.17	13.67 ± 0.67	8.66 ± 0.66

All the values were expressed as mean ±SEM and each data point was the average of 6 animals in each group (n=6). 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 ketamine. Preg S= Pregrenolone Sulphate; Risp =Risperidone; ket=Ketamine

Table 2: Effects of Pregnenolone sulphate on ketamine induced locomotor activity in rats.

Groups (n=6)	treatment	Horizontal activity(cm)	Move time (s)	Rest time (s)	Average dist/move (cm)	Mean velocity (cm/s)	Total movement (#)
I	Normal Control (1ml/kg, i.p)	2068.37 ±98.65	243± 15.68	682.57 ±14.98	2.58 ±0.17	2.73± 0.11	563.38 ±23.78
II	Ketamine (30 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	Preg S *per se* (11.86 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	Risp *per se* (2 mg/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	Preg S (11.86 mg/kg, i.p) + ket (30 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	Risp (2 mg/kg, i.p) + ket (30 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 and each data point was the average of 6 animals in each groups (n=6). 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, when compared with control, # p<0.05, ## p<0.01, ### p<0.001 when compared with ketamine. Preg S =Pregrenolone Sulphate; Risp =Risperidone; ket=Ketamine

Table 3. Effects of Pregnenolone sulphate 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	Normal Control (1ml/kg, i.p)	14.25 ± 0.40	6.28 ± 0.16	171.8 ± 13.86
II	Ketamine (30 mg/kg,i.p)	7.49 ± 0.38***	7.52 ± 0.11**	76.74 ± 6.94***
III	Preg S *per se* (11.86 mg/kg, i.p)	11.26 ± 0.28*##	6.14 ± 0.25##	164.83 ± 16.16*##
IV	Risp *per se* (2 mg/kg.i.p)	13.85 ± 0.27###	4.05 ± 0.21***###	175.44 ± 12.83###
V	Preg S (11.86 mg/kg,i.p) + ket (30 mg/kg, i.p)	9.6 ± 0.63##	6.95 ± 0.14	127.61 ± 5.26**##
VI	Risp (2 mg/kg, i.p) + ket (30 mg/kg, i.p)	10.1 ± 0.87	6.28 ± 0.23##	140.32 ± 8.17***###

All values were expressed as mean ± SEM, analyzed by ANOVA followed by Tukey- Kramer multiple comparison test. P values <0.05 was considered significant and P value <0.001 was considered extremely significant. N= 6 number of animals in each group***** p<0.05, **p<0.01, *** p<0.001 when compared with control, ## p<0.01, ### p<0.001 when compared with ketamine. Preg S =Pregrenolone Sulphate; Risp =Risperidone; ket=Ketamine

CONCLUSION

Ket (30 mg/kg i.p) administered for 14 days produced significant changes in behaviour resembling to that of negative symptoms as well as cognitive memory impairment seen in schizophrenia. Preg S treated rats spent more time in open arm in EPMT, decreased percentage alteration and improved LA as compared to Ket treated rats. These observations indicated the improvement in learning and memory with Preg S. Oxidative stress was observed in Ket treated rats and various antioxidant parameters were estimated. The levels of SOD and GSH were reduced and TBARs level was elevated as compared to control groups. Upon Preg S administration, ameliorating effects was observed in anti-oxidant parameters. Our results showed that neurosteroid, Preg S improved learning and memory in EPM, SAB, LA, enhance cognitive effect by decreasing AChE and reduced oxidative stress. Thus, it may be concluded that Preg S may have neuroprotective and anti-oxidant effect. Further research may be warranted by executing multiple dose dependent studies in different models of SCZ with the estimation of neurotransmitters to confirm its role in SCZs.

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Conflict of Interest

All the authors declare that they have No conflict of interest.

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Ethical Statement

All the experiments have been conducted as per ethical standards.

Figure Legends

Figure 1: Effect of Pregnenolone Sulphate on Spontaneous Alteration Behaviour in rats.

All the values were expressed as mean ± SEM and each data point was the average of 6 animals in each group (n=6). 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 ketamine. Preg S = Pregrenolone Sulphate; Risp =Risperidone; Ket=Ketamine.

Figure 2: Effects of Pregnenolone Sulphate on acetyl cholinesterase esterase activity in elevated plus maze test in rat.

All the values were expressed as mean ± SEM and each data point was the average of 6 animals in each group (n=6). 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 ketamine. Preg S =Pregrenolone Sulphate; Risp =Risperidone; Ket = Ketamine

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