Influence of Imipramine and Sertraline on Neurodegeneration, Behavioural and Immune Cell Parameters in Rats Under Chronic Unpredictable Stress

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ABSTRACT

Stress is one of the major causes of anxiety & depression. Chronic conditions like anxiety & depression when left untreated may result in neuronal & psychological disorders. The present study is aimed at identifying if chronic unpredictable stress (CUS) can cause neurodegeneration in rats and if imipramine & sertraline (drugs being used for the treatment of depression and anxiety) can protect or recover the brain from neurodegeneration. This study involves 4 groups, namely, Control group (G-I), Chronic unpredictable stress induced group (G-II), CUS + Imipramine (10mg/Kg) treated group (G-III), and CUS + Sertraline (10mg/Kg) treated group (G-IV) with n=6 in each group. The study is conducted for 30 days where stress is induced in G-II, while stress is accompanied with drug treatment in G-III & G-IV. At the end of the 30 day protocol, animals were screened by elevated plus maze and forced swim test . The EPM did not show clear signs of anxiety but the FST has shown signs of depression in G-II & recovery from depression in G-III and G-IV. Serum cortisol levels were assessed and the elevated cortisol levels in G-II clearly indicates that stress has been induced. At the end of the study brains were isolated for histopathological examination and the results indicate that chronic unpredictable stress has induced neurodegeneration in G-II and the drugs Imipramine & Sertraline have shown signs of protection/recovery from neurodegeneration. All the results were analyzed using Graph pad prism (version 6) software.

Keywords: stress, neurodegeneration, anxiety, depression, sertraline, imipramine.

INTRODUCTION

Research has shown that stress is one of the major factors that affects physical and mental well being. This cause and effect relationship between stress and ill health is well explained by the established functionality of the sympathetic-adrenal-medullary (SAM) system and the hypothalamic-pituitary-adrenal (HPA) axis. Research has also shown that stress when suffered for a prolonged period result in neuronal changes. The present study aims at establishing a relationship between chronic unpredictable stress and neurodegeneration. The major disadvantage of both physical stress models and psychological stress models is the development of adaptation /resistance on chronic exposure. The changes in physiological and behavioral responses to chronic stress can be related to the adaptation of the HPA axis. When the same stressor is repeated, the HPA response undergoes desensitization or become stable as it has been reported that rodents repeatedly exposed to restraint stress exhibited a habituated corticosterone response, when they were subsequently challenged with an acute exposure to restraint. On the other hand, the exposure to a multiple stress paradigm produced continued elevation in corticosterone levels, when the animals were subsequently subjected to acute restraint stress. It has also been suggested that the adaptations of HPA axis depend on type, duration and severity of the stress regime. To prevent the development of resistance, Chronic Unpredictable Stress (CUS) models have been developed which involve the use of various physical and psychological stressors in a predetermined manner so that the animal is not able to adapt to the stressor. Adaptation to one type of stressor has been effectively prevented by employing various stressors. Chronic variable stress models have been proven to be more useful as they are devoid of the problem of resistance in the animal species towards the commonly used stressors and also have the advantage of the development of effective and long-term stress response[14]. Since chronic unpredictable stress can cause anxiety or depression [14,15]which may subsequently lead to neurodegeneration, the present research is aimed at studying the effect of chronic unpredictable stress on neurodegeneration and to check if imipramine and sertraline[17,18] can protect or recover the brain from neurodegeneration.

MATERIALS AND METHOD

Selection of animals

24 Healthy young albino male rats weighing 150 - 180 g were obtained from TEENA LABS, Hyderabad. All the pharmacological experimental protocols were approved by the Institutional Animal Ethics Committee (Reg no: MRCP/CPCSEA/IAEC/2011-12/MPCOL/1) and they are grouped accordingly.

Grouping

Number of animals used in each group is 6. Animals are grouped randomly into each group. There are 4 groups in total so total number of animals is 24.

Housing of animals

- After final grouping animals were housed in polypropylene cages (38 cm $\times$ 23 cm $\times$ 10 cm) with six animals in each cage.

- They are maintained under standard laboratory conditions (temperature 25 ± 2 °C, with 12/12 h dark/light cycle)

They were allowed free access to standard dry pellet diet and water *ad libitum*. The rats were acclimatized to laboratory conditions for 10 days before commencement of the experiment.

Experimental Design

Twenty four Wistar Albino male rats of weight 150g-200g were selected for this study. Animals were divided into four groups of six animals each.

Group 1: Control group (saline at 1ml/Kg body weight)

Group 2: chronic unpredictable stress (CUS) induced group

Group 3: CUS + imipramine treated group

Group 4: CUS + sertraline treated group

Dosage

Imipramine: 10 mg/Kg body weight

Sertraline: 10 mg/Kg body weight

Experimental Protocol

The study period of this experiment is 30 days. During this period stress is induced for CUS, imipramine and sertraline groups (2nd, 3rd, and 4th) by different types of stressors. These stressors include isolation, crowding/shaking, warm water swim, mild foot shock, cold water swim, immobilization, sub cutaneous 0.9% saline injection, mild tail pinch, and wetting saw dust all day. After 1hour of inducing stress imipramine 10mg/kg., i.p, is administered for 3rd group, and sertraline 10mg/kg., i.p, is administered for 4th group.

Serum sample preparation

At the end of experimental period, 30 days, approximately 2 to 3 ml of blood was collected from each rat by retro orbital puncture. The blood samples were allowed to clot at room temperature. Serum was separated by centrifugation using cool centrifuge (REMI) at 4000 rpm for 15 min and stored at -20 °C which was later used for estimation of cortisol levels.

Tissue sample preparation

One animal from each group was sacrificed by cervical dislocation and the brain of each rat was removed and washed well with ice cold saline to remove blood and it is placed in formalin solution in a closed container and sent for histopathology study.

Estimation of Behavioural parameters

The behavioural pattern of animals was determined by elevated plus maze [EPM], forced swim test [FST] and open field test on the last day of the experiment. These tests detect anxiety or depression like behaviour and exploratory behaviour of animals

Estimation immune cell parameters

Immune cell parameters are estimated from the sample of blood which is collected by retro orbital puncture. Differential count is performed in order to determine the effect of stress on different types of blood cells. Specially concentrated on W.B.C and in particular on lymphocytes, monocytes and neutrophils. Blood samples were given to aditya diagnostic centre for differential count.

Statistical analysis

 Statistical analysis was carried out using Graph Pad Prism software (Version 6). The experimental results were expressed as Mean ± SEM, n=6. The intergroup variation between various groups was measured by DUNNETS multiple comparison test.   Results were considerd statistically significant when $P < 0.1$, $P < 0.01$, $P< 0.001$, $P<0.5$.

RESULTS AND DISCUSSION

Table 1: Effect of imipramine and sertraline on elevated plus maze for anxiety in CUS induced rats.

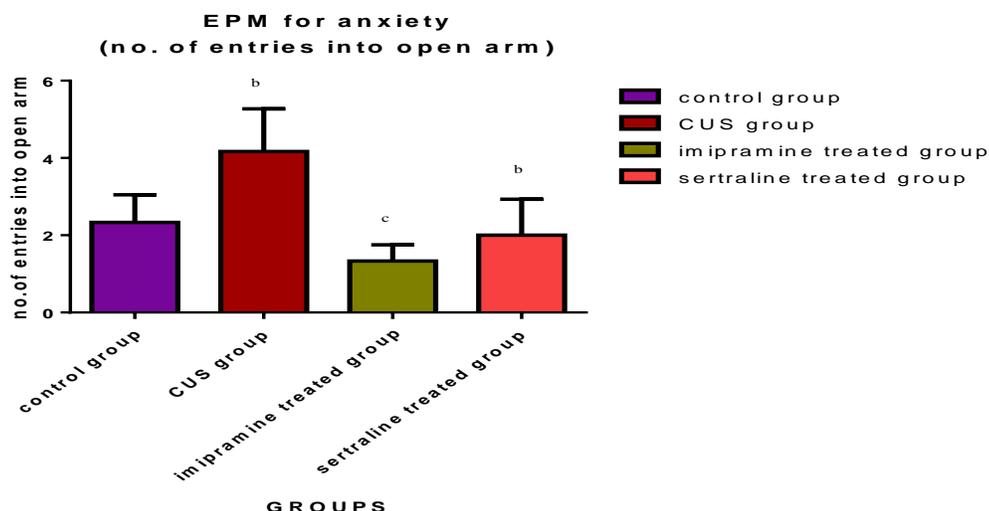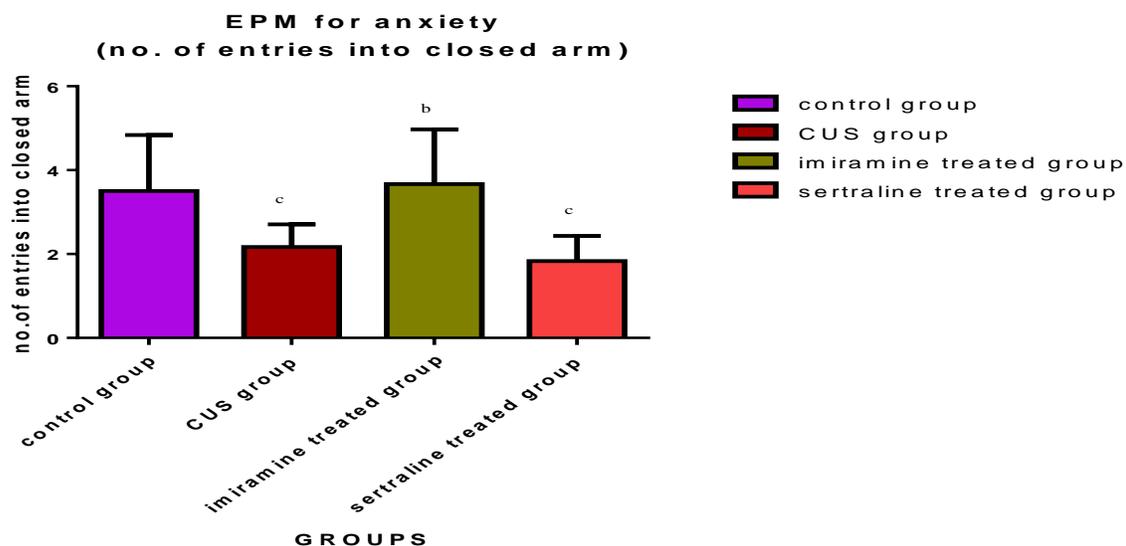| Groups	No.of entries into open arm	No.of entries into closed arm	Total time spent in open arm	Total time spent in closed arm	Rearings	Dips
CONTROL	2.333±0.7149	3.500±1.335	2.000±0.6325	0.000±O.6325	7.167±2.330	26.67±3.801
CUS	4.167±1.108[a]	2.167±0.5426[c]	3.333±0.6280[b]	1.500±0.6583[b]	4.667±1.498[c]	25.83±5.102[b]
IMIPRAMINE	1.333±0.4216[c]	3.667±1.308[b]	2.417±0.7683[a]	2.750±0.8241[b]	6.667±1.706[c]	37.50±5.566[b]
SERTRALINE	2.000±0.9309[a]	1.833±0.6009[c]	1.000±0.4082[c]	3.833±0.4014[b]	6.000±1.342[a]	25.50±1.784

Figure 1: Effect of imipramine and sertraline on elevated plus maze (no. of entries into open arm) for anxiety in CUS induced rats.

Values are expressed as mean ± SEM, n=6.The intergroup variation between various groups was measured by DUNNETS multiple comparison test. [a]P< 0.01 as compared with control, [b]P<0.1 as compared with control, [c]P< 0.5 as compared with control, [d]P<0.001 as compared with control.

Figure 2: Effect of imipramine and sertraline on elevated plus maze (no. of entries into closed arm) for anxiety in CUS induced rats.

Values are expressed as mean ± SEM, n=6.The intergroup variation between various groups was measured by DUNNETS multiple comparison test. [a]P< 0.01 as compared with control, [b]P<0.1 as compared with control, [c]P< 0.5 as compared with control, [d]P<0.001 as compared with control.

E P M f o r a n x i e t y
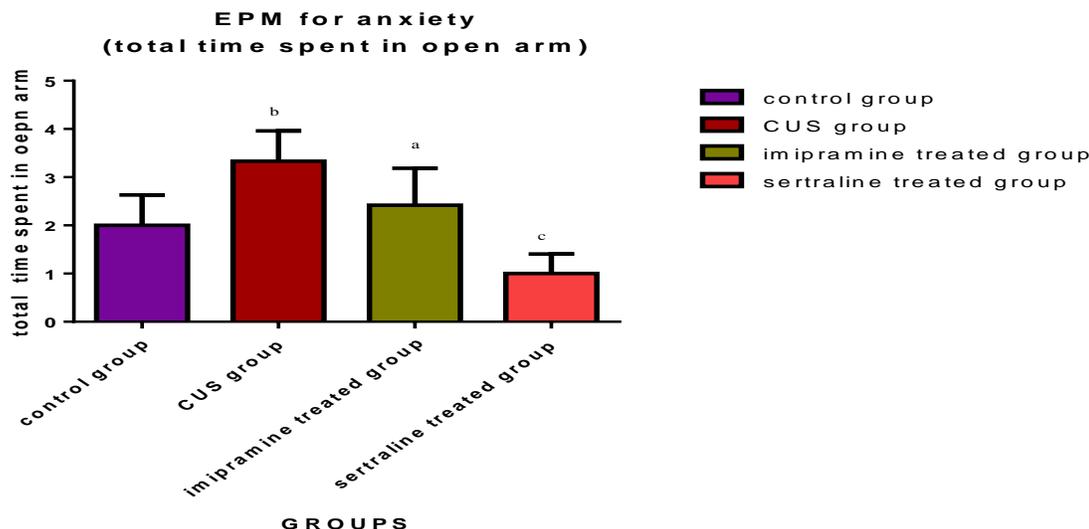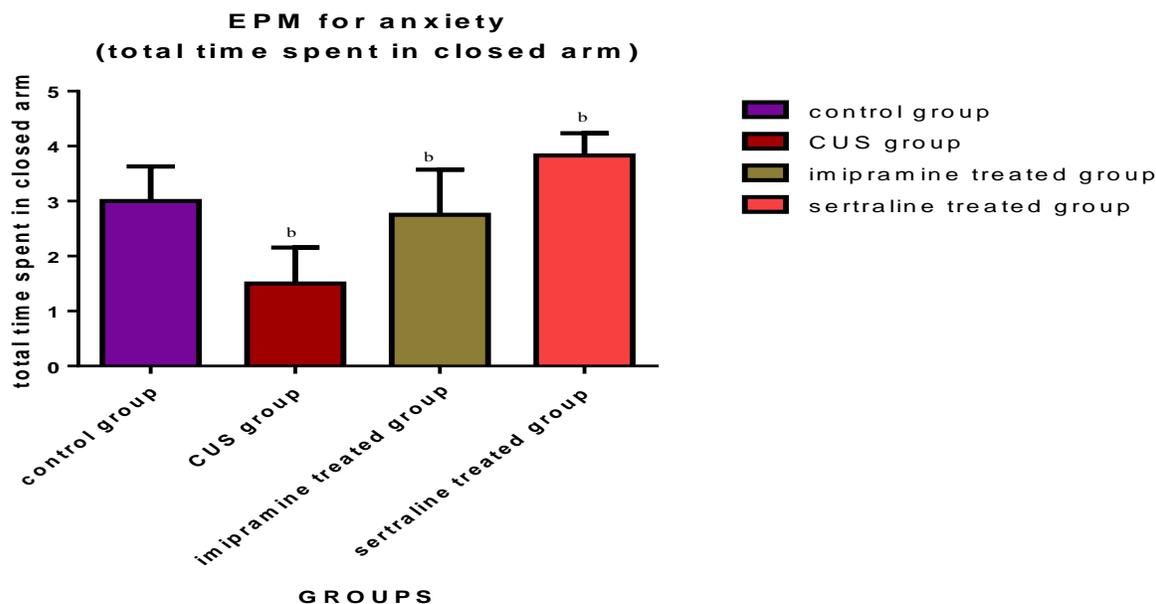**(t o t a l t i m e s p e n t i n o p e n a r m)**

Figure 3: Effect of imipramine and sertraline on elevated plus maze (total time spent in open arm) for anxiety in CUS induced rats.

Values are expressed as mean ± SEM, n=6.The intergroup variation between various groups was measured by DUNNETS multiple comparison test. [a]$P< 0.01$ as compared with control, [b]$P<0.1$ as compared with control, [c]$P< 0.5$ as compared with control, [d]$P<0.001$ as compared with control.

E P M f o r a n x i e t y
(t o t a l t i m e s p e n t i n c l o s e d a r m)

Figure 4: Effect of imipramine and sertraline on elevated plus maze (total time spent in closed arm) for anxiety in CUS induced rats.

Values are expressed as mean ± SEM, n=6.The intergroup variation between various groups was measured by DUNNETS multiple comparison test. [a]$P< 0.01$ as compared with control, [b]$P<0.1$ as compared with control, [c]$P< 0.5$ as compared with control, [d]$P<0.001$ as compared with control.
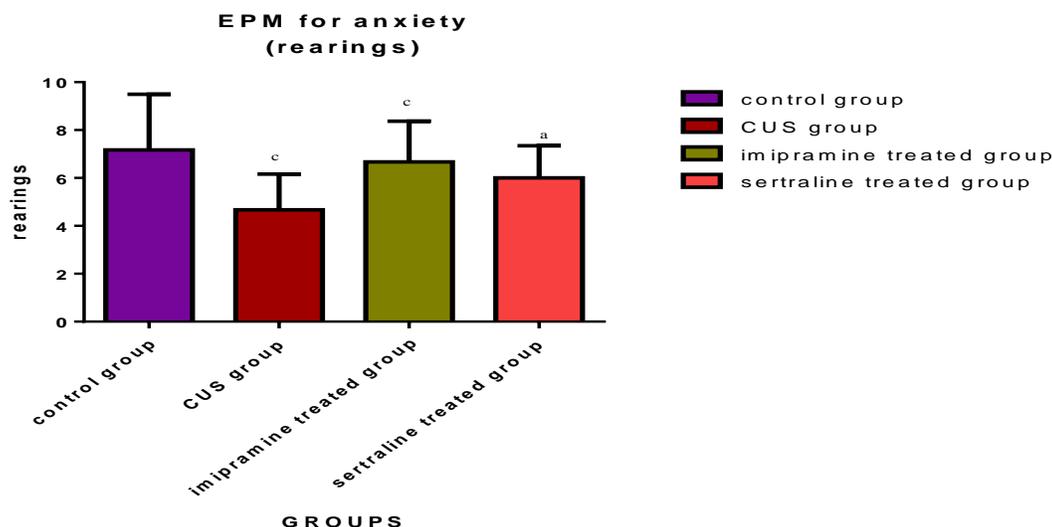
Figure5: Effect of imipramine and sertraline on elevated plus maze (rearings) for anxiety in CUS induced rats.

Values are expressed as mean ± SEM, n=6.The intergroup variation between various groups was measured by DUNNETS multiple comparison test. [a]P< 0.01 as compared with control, [b]P<0.1 as compared with control, [c]P< 0.5 as compared with control, [d]P<0.001 as compared with control.
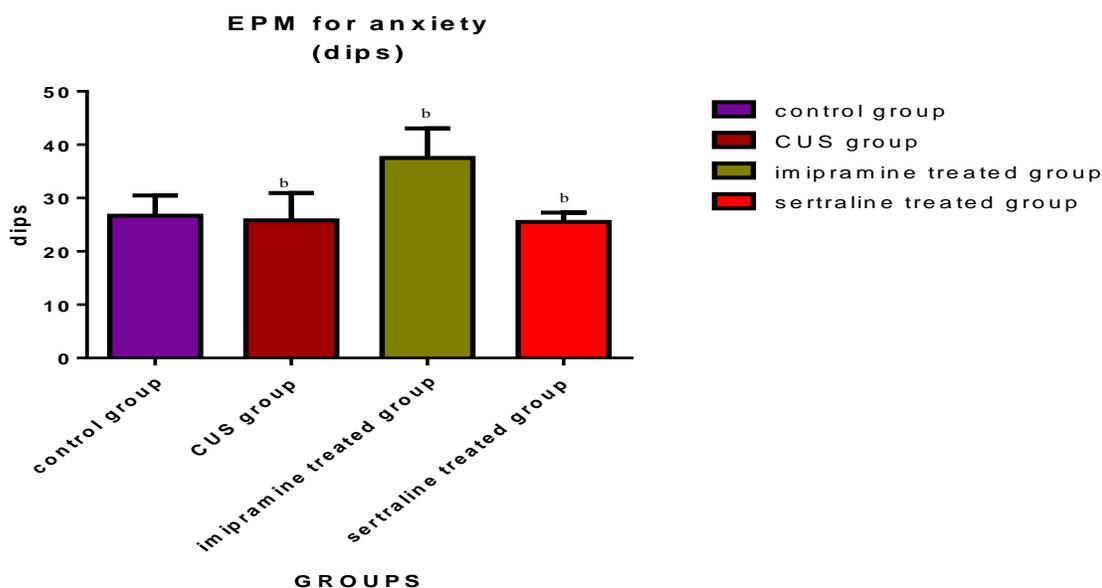

Figure 6: Effect of imipramine and sertraline on elevated plus maze (dips) for anxiety in CUS induced rats.

Values are expressed as mean ± SEM, n=6.The intergroup variation between various groups was measured by DUNNETS multiple comparison test. [a]P< 0.01 as compared with control, [b]P<0.1 as compared with control, [c]P< 0.5 as compared with control, [d]P<0.001 as compared with control.

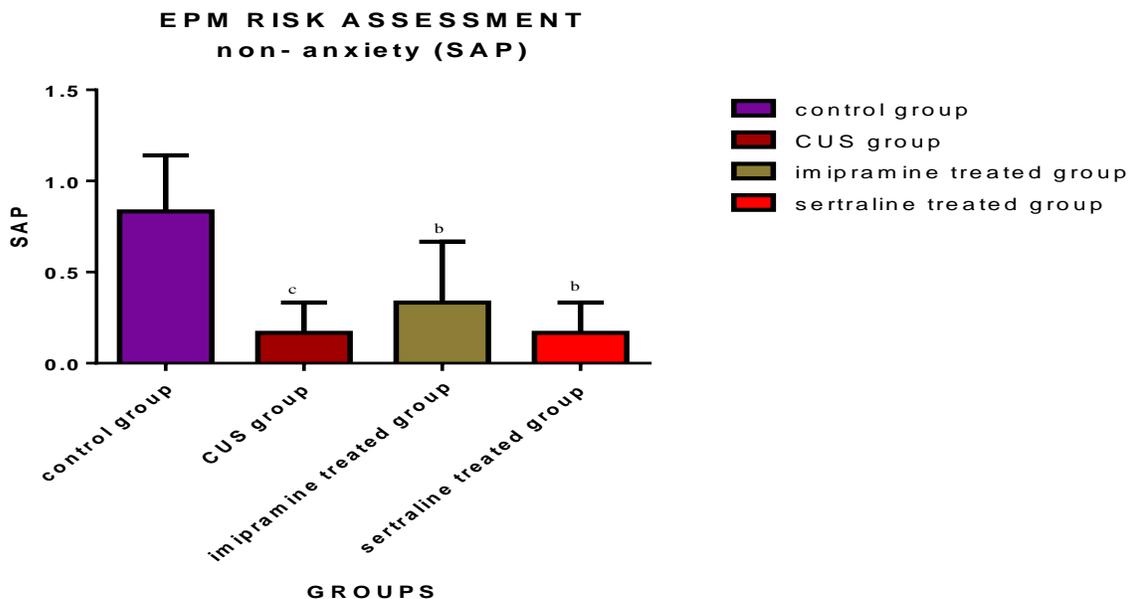**Table 2: Effect of imipramine and sertraline on elevated plus maze for risk assessment (non-anxiety) in CUS induced rats.**

Group sroups	Stretch Attend Posture E
Control	0.8333±O.3073
CUS	0.1667±0.1667[c]
Imipramine	0.3333±0.3333[a]
Sertraline	0.1667±0.1667[a]

EPM RISK ASSESSMENT non- anxiety (SAP)

Figure 7: Effect of imipramine and sertraline on elevated plus maze (SAP) for risk assessment non- anxiety in CUS induced rats.

Values are expressed as mean ± SEM, n=6.The intergroup variation between various groups was measured by DUNNETS multiple comparison test.

[a]$P < 0.01$ as compared with control, [b]$P<0.1$ as compared with control, [c]$P< 0.5$ as compared with control, [d]$P<0.001$ as compared with control.

Table 3: Effect of imipramine and sertraline on force swim test for depression in CUS induced rats.

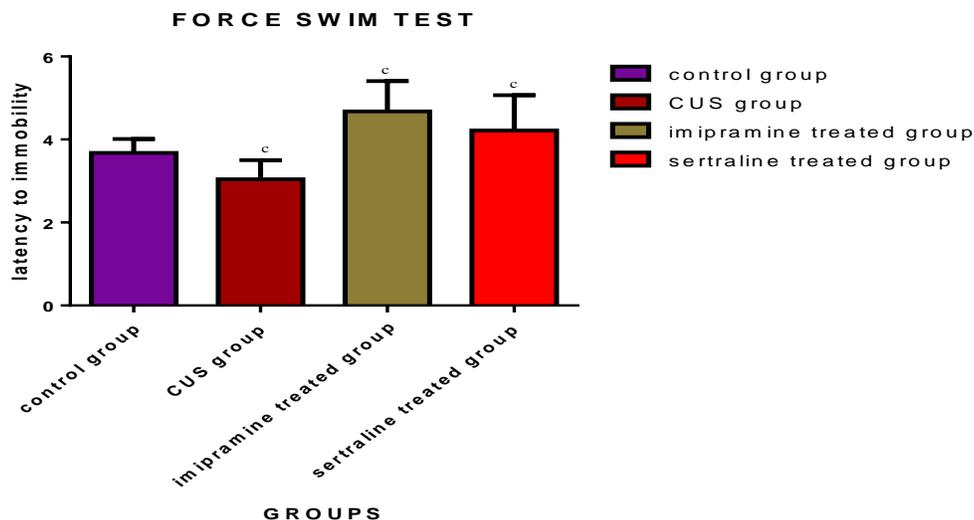| Groups	Latency To Immobility
Control	3.678±0.3329
CUS	3.042±0.4614[c]
Imipramine	4.675±0.7334[c]
Sertraline	4.218±0.8487[c]

FORCE SWIM TEST

Figure 8: Effect of imipramine and sertraline on force swim test for depression in CUS induced rats.

Values are expressed as mean ± SEM, n=6.The intergroup variation between various groups was measured by DUNNETS multiple comparison test. [a]$P< 0.01$ as compared with control , [b]$P<0.1$ as compared with control, [c]$P< 0.5$ as compared with control, [d]$P<0.001$ as compared with control

Table 4: Effect of imipramine and sertraline on open field test for exploratory behavior and anxiety in CUS induced rats

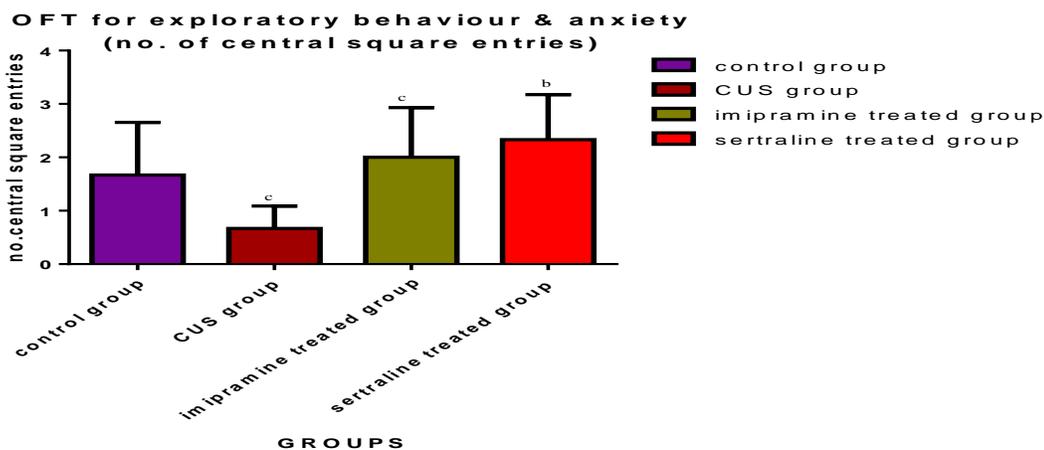| Groups	No.of cenral square entries	No.of periferal square entries
Control	1.667±0.9888	39.50±2.814
CUS	0.6667±0.4216[C]	21.67±4.201[d]
Imipramine	2.000±0.9309[C]	28.67±3.383[d]
Sertraline	2.333±0.8433[b]	38.17±3.219[a]

Figure 9: Effect of imipramine and sertraline on open field test (no. of central square entries) for exploratory behavior and anxiety in CUS induced rats.

Values are expressed as mean ± SEM, n=6.The intergroup variation between various groups was measured by DUNNETS multiple comparison test. ^{a}P< 0.01 as compared with control, ^{b}P<0.1 as compared with control, ^{c}P< 0.5 as compared with control, ^{d}P<0.001 as compared with control
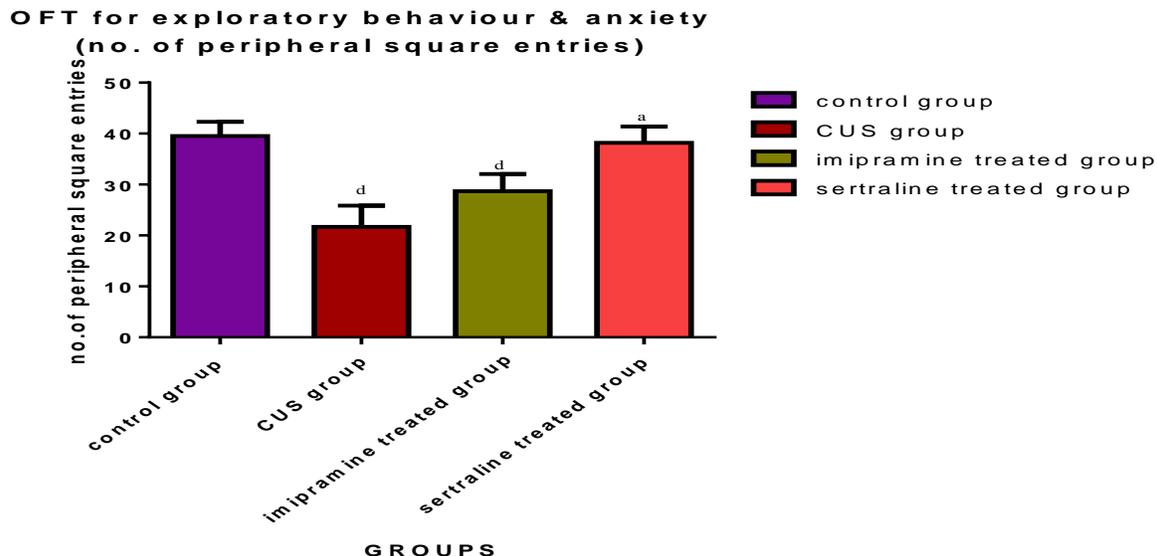

Figure 10: Effect of imipramine and sertraline on open field test (no. of peripheral square entries) for exploratory behavior and anxiety in CUS induced rats.

Values are expressed as mean ± SEM, n=6.The intergroup variation between various groups was measured by DUNNETS multiple comparison test. ^{a}P< 0.01 as compared with control, ^{b}P<0.1 as compared with control, ^{c}P< 0.5 as compared with control, ^{d}P<0.001 as compared with control

Table 5: Effect of imipramine and sertraline on open field test for anxiety in CUS induced rats.

Groups	Stretch attend posture
Control	1.167±0.4014
CUS	0.0±0.0^a
Imipramine	0.3333±2.108^a
Sertraline	1.0000±0.3651^a

Table 6: Effect of imipramine and sertraline on immune cell parameters (lymphocyte count) in CUS induced rats.

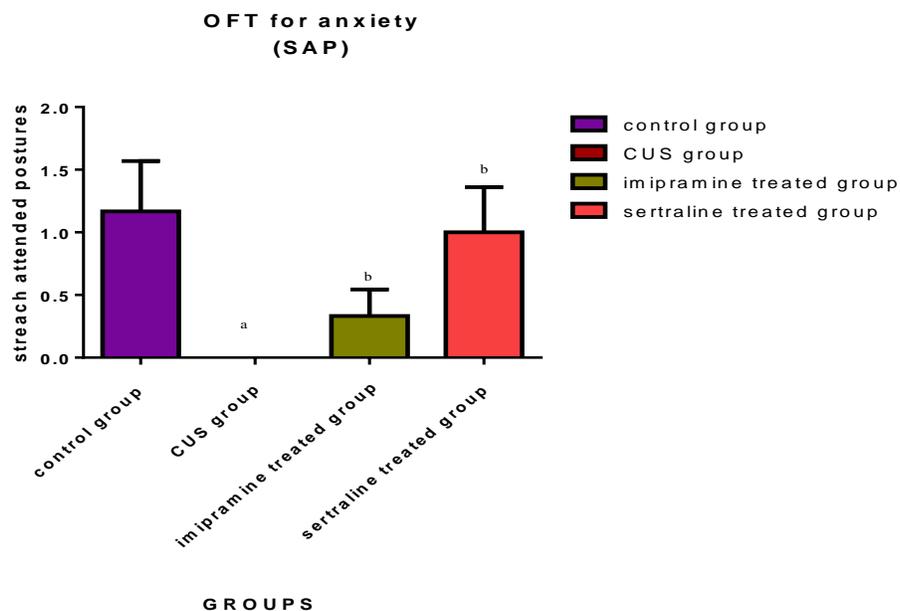| Groups	Lymphocytes
1. Control	77.5±0.5000
2. CUS	79±1.000^a
3. Imipramine	77.00±1.000^a
4. Sertraline	70.00±3.000^a

Figure 11: Effect of imipramine and sertraline on open field test (SAP) for exploratory behavior and anxiety in CUS induced rats.

Values are expressed as mean ± SEM, n=6.The intergroup variation between various groups was measured by DUNNETS multiple comparison test. [a]P< 0.01 as compared with control, [b]P<0.1 as compared with control, [c]P< 0.5 as compared with control, [d]P<0.001 as compared with cont.
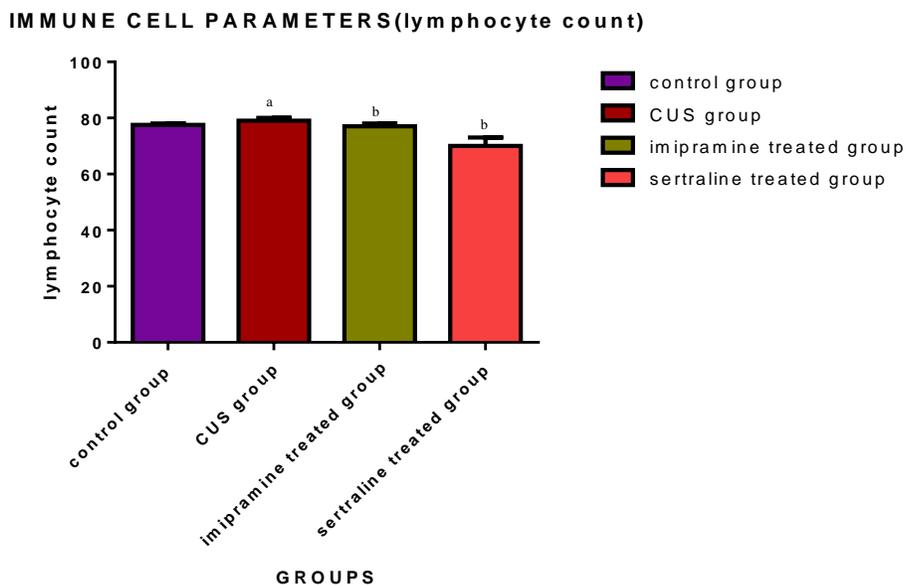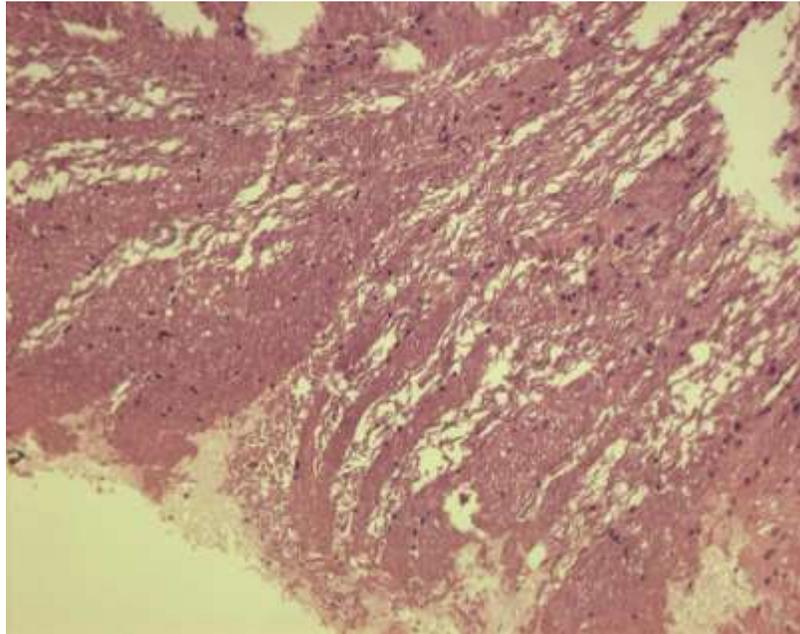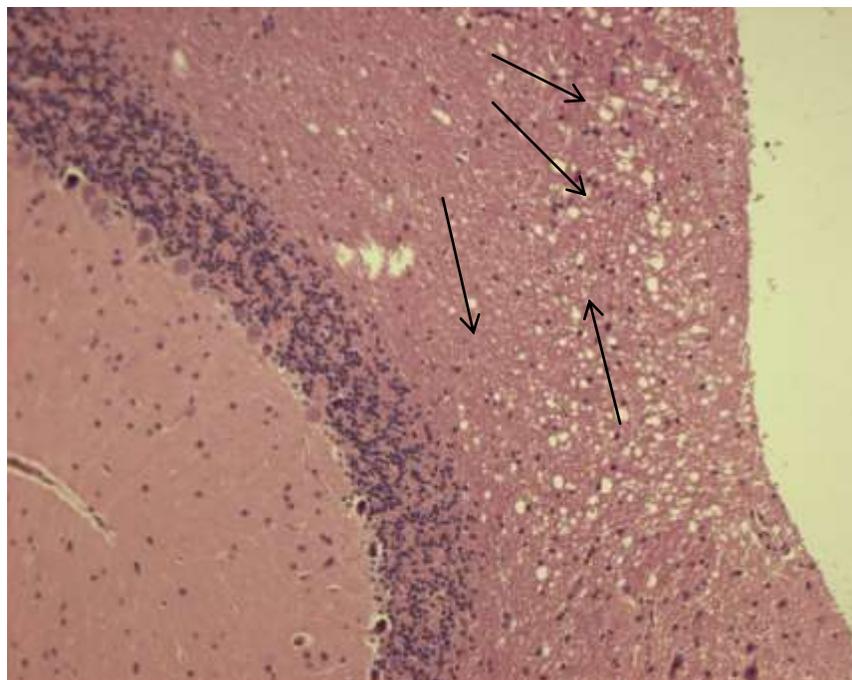

Figure 12: Effect of imipramine and sertraline on immune cell parameters (lymphocyte count) in CUS induced rats.
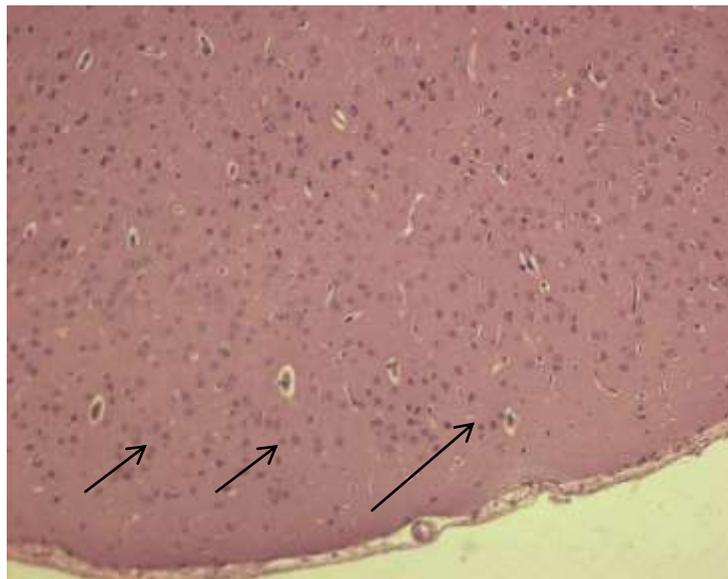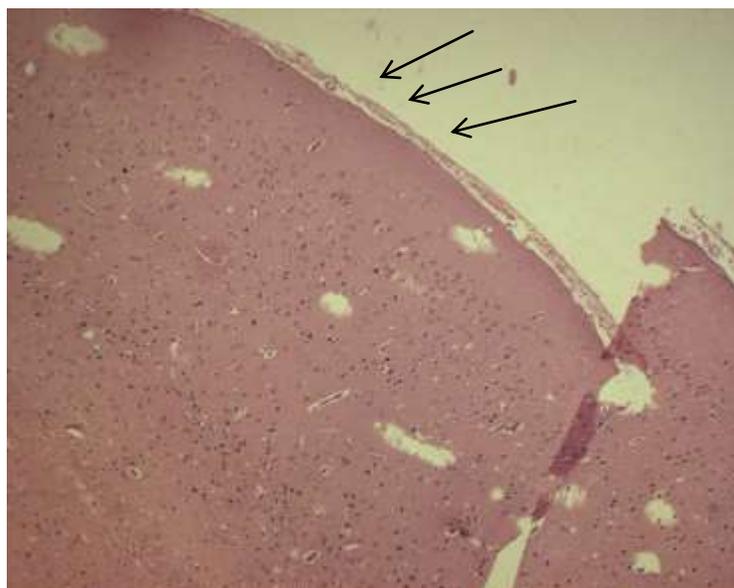
Histopathology

GROUP 1 (CONTROL): No demyelination/ degeneration is noticed in the cerebral hemispheres

GROUP 2 (CUS): Moderate demyelination and degeneration noticed in the cerebral hemispheres. Cerebellum appeared normal.

GROUP 3 (IMIPRAMINE): Cerebral hemispheres appeared normal. Mild meningeal thickness noticed surrounding the cerebral hemispheres.

GROUP 4 (SERTRALINE): Cerebral hemisphere appeared normal. But mild meningeal thickening surrounding the cerebral hemispheres.

Cortisol assay

Serum samples were collected from 1st and 2nd group animals as mentioned in methodology, and were sent to *SANZYME LIMITED* located near Shamshabad, Hyderabad for estimation of cortisol levels.

The reports include cortisol levels as mentioned below:

Control group (1st group) - 19.54 μg/dL (mean value, n=6)

CUS group (2nd group)- 25.855 μg/dL (mean value, n=6)

Discussion on different studies and assays

- In elevated plus maze 1st 2nd 3rd 4th 5th and 6th parameters indicate anxiety behavior of animals and 7th parameter indicates risk assessment. If the animal is showing more open arm entries then it means that it is less anxious. If it is showing more closed arm entries then it is more anxious. Based on this consideration some animals are anxious and some are less anxious.

- In force swim test Latency to immobility is nothing but time taken for animals to immobilize. Latency to become immobile was defined as the time at which the rat first initiated a stationary posture that did not reflect attempts to escape from the water. Decreased latency to immobility indicates depressive behavior while increased latency to immobility indicates anti-depressant action.

- In open field test increased central square entries indicate that the animal is less anxious and more exploratory in nature. Increased peripheral square entries indicate that the animal is more anxious and less exploratory. Increased SAP indicates that the animal is in anxiety.

- By observing results of cortisol assay,(control and CUS group animals cortisol levels) it is clear that during stress conditions cortisol levels in blood increases, compared to that of control levels.

- In immune cell parameter analysis, though the W.B.C count is non uniform, there is an increase in lymphocyte count.

- Based on the histopathology reports and images, 2nd group shows moderate neurodegeneration` while 3rd and 4th group animals show that anti-depressant drugs which were used simultaneously with chronic unpredictable stress, prevent neurodegeneration.

CONCLUSION

- Based on the results of EPM, there are no clear signs of anxiety.

- Based on the above discussions about force swim test we can conclude that 2nd group (CUS) animals are having less latency to immobility indicating that they are depressed. But 3rd and 4th group animals show more latency to immobility which indicate they are less depressed. Chronic unpredictable stress causes depression to 2nd 3rd and 4th groups but imipramine and sertraline decreases depression which is why 3rd and 4th group animals are less depressed than 2nd group animals.

- Based on the above discussions about open field test, 2nd group animals show increased peripheral square entries which indicate they are anxious and remaining group animals show

increased central square entries which indicate these animals are more exploratory and less anxious.

- Based on the cortisol assay results it is clear that stress has been induced to CUS group animals as their cortisol levels are high compared to that of control group animals.

- W.B.C and monocyte count is non uniform. But lymphocyte count shows an increase in number in CUS group and decrease in number in antidepressant drug treated groups. This supports previous studies that chronic stress causes *lymphocytosis* (i.e, increased lymphocyte count).

- Inducing chronic unpredictable stress causes neurodegeneration in rats, but treating the chronic unpredictable stress induced groups simultaneously with imipramine and sertraline prevents neurodegeneration.

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