Buspirone, a 5-HT1A agonist attenuates anger, aggression and suicidal tendencies in rats.

Urmila Aswar (aswarurmila@gmail.com)
Bharati Vidyapeeth Deemed University Poona College of Pharmacy https://orcid.org/0000-0002-4566-7453
Hrudaya Shende
Sinhgad Technical Education Society's Sinhgad Institute of Pharmacy
Manoj Aswar
SCOP: Sinhgad Technical Education Society's Sinhgad Institute of Pharmacy

Research Article

Keywords: Aggression, Anger, Buspirone, Social isolation, Suicidal tendencies

Posted Date: May 10th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-470695/v1

Abstract

The purpose of the work was to evaluate the effect of buspirone (BUS) on social isolation induced anger, aggression and suicidal tendencies in rats. The male Wistar rats were randomized in 6 groups (n = 6) and caged individually for 14 days to elicit anger and aggression. They were then divided into the following groups vehicle control (no isolation), Stress control (SC), fluoxetine (Flx; 30 mg/kg, p.o), BUS (10 mg/kg, p.o), BUS (20 mg/kg, p.o), BUS (40 mg/kg, p.o). All treatments were administered from day 14 through day 28. On the last day of treatment, assessment of anger, aggression and suicide-related traits were performed. Serum cortisol, blood pressure were measured and magnetic resonance imaging (MRI) of the rat's brain and of BDNF expression were performed. SC group showed significant increase in anger (wheel rolling activity), aggression (increased number of attack bites, wrestling, chasing behavior), irritability score, learned helplessness (number of attempts of escape success and failure, escape latency), increased level of serum cortisol as compared to normal confirming induction of anger, aggression and suicidal ideation. BUS significantly reduced all behavioral traits associated with anger, aggression and suicidal ideation, reduced cortisol levels and significantly increased BDNF compared to stress control. Blood pressure increased substantially in stress control was significantly reduced by BUS, but not by Flx. Neuroimaging studies in stress control brains showed a reduction in amygdala size compared to normal, while animals under BUS treatment mitigated this reduction.

Buspirone has been found to be effective in preventing anger, aggression and suicidal tendencies.

Introduction

Social isolation, a potent stressor in both humans and animals, is one of the important paradigms resulting in anger and aggression(Hartmann *et al.*, 2019)and ultimately leads to suicidal ideation (Malkesman et al., 2009, Locci and Pinna, 2019). Isolation of rats for one week or more can lead to alteration in neurochemical, physiological, anatomical and neuroendocrine system (Hatch *et al.*, 1965). The isolation results in abnormal social behavior, hyper reactivity to handling, anxiety, depression (Filipović et al., 2017), associated altered mood and aversions (Rivera-Irizarry et al., 2020). Similar symptoms are observed in human, social isolation in human is reported to result in reduced concentration during activities, continuous sadness, irritability and insomnia (Santini *et al.*, 2020). Social isolation promulgates into disruption of serotonergic system (Tan et al., 2020), dysregulation of hypothalamus, pituitary and adrenal cortex (HPA axis) and release of high levels of cortisol (Ehlert et al., 2001, Tan et al., 2020), similar effects were observed in socially isolated rats (Mumtaz et al., 2018).

Antidepressants like sertraline, fluvoxamine and fluoxetine has shown to reverse the isolation-induced aggressive behavior (Sánchez and Meier, 1997). Fluoxetine (Flx) is a selective serotonin reuptake inhibitor (SSRI) that acts by blocking the reuptake transporter protein located in the pre-synaptic terminal which blocks the uptake of serotonin into pre-synaptic serotonin neurons. Recent studies suggest that Flx is responsible for an increase in hippocampal neurogenesis and synaptic plasticity (Micheli et al., 2018). Chronic treatment with antidepressants initially inhibits aggression, but later results in neuro adaptive changes. These changes result in severe effects like anger, aggression and suicidal ideation that may be attributed to HPA axis dysregulation or the surge of serotonin at the synapse(Creaney et al., 1991, Teicher et al., 1993). In year 2004, US-FDA has issued a black box warning for use of SSRI as monotherapy in young adults (Kubiszyn and Mire, 2014).

The role of 5-HT1A auto receptor is implicated in aggression via regulation of serotonin level, thus considerable interest has been developed to study its agonists in modulating aggressive behavior(Centenaro *et al.*, 2008). The evidences suggest that chronic treatment with antidepressants increases levels of serotonin locally in mid raphe nuclei hence promoting antidepressant action, but on the other side the rise in serotonin also activates 5-HT1A receptor which inhibit the rate of firing of serotonergic neurons resulting in low levels of serotonin in the raphe nuclei. Hence, to obtain optimum firing of serotonergic neurons, the 5-HT1A receptor must be modulated (Mezzomo *et al.*, 2020). Considering, the role of 5-HT1A in

aggression and potent role of buspirone (BUS), as a partial agonist at 5-HT1A receptor with significant intensity potential, we hypothesized that BUS may prove to be an effective treatment for anger, as an anti-aggressive agent and can be beneficial in the reduction of suicide ideation.

The present study is an attempt to evaluate the effect of 5-HT1A agonist, buspirone in suicidal ideation in socially isolated animals by analyzing negative emotions such as anger, aggression, impulsivity and despair. Anger and fear may encourage avoidance or defensive behavior while sadness and displeasure results into reduced exploratory activities(Guo et al., 2015, Perić et al., 2017). These traits are studied using wheel rolling activity (anger), resident intruder model (aggression and impulsivity), irritability, open field test (OFT) (exploratory behavior) and active avoidance paradigm (behavioral despair).

Materials And Methods

Drugs and Chemicals

Buspirone hydrochloride was purchased from Sigma-Aldrich, USA. Fluoxetine was gifted by Intas Pharmaceuticals Pvt. Ltd, India, Urethane was purchased from Hi-media Laboratories Pvt. Ltd, Mumbai, India, Cortisol kit was purchased from Ray Biotech, GA, USA. cDNA Reverse Transcription Kit was procured form Applied Biosystems, USA. All other chemicals were purchased from local distributors.

Approval of experimental protocol

The experimental protocol was approved by the Institutional Animal Ethical Committee (IAEC) of Sinhgad Institute of Pharmacy, Narhe, Pune, constituted as per the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). The CPCSEA reg. No of the institute is 1139/PO/a/07/CPCSEA and protocol approval number is SIOP/IAEC/2016/05.Adult male Wistar rats (250-300 g) were procured from the National Toxicology Centre, Pune and were housed in diurnal lighting condition (12h/12h) with a temperature of 25±1°C, relative humidity of 45-55%. Animals had free access to food (Standard chow pellet, Nutrivet Life Sciences, Pune) and water ad libitum.

Induction of social isolation induced anger, aggression and suicidal ideation and treatment schedule

The rats were divided into six groups containing 6 rats each. Group 1- Rats (Normal rats) were not isolated and were kept in their home cage with minimal handling. Group II- Stress control; isolated and administered with distilled water. Group III- Standard group was treated with FLX (30 mg/kg, once a day). Group IV to VI were socially isolated and treated with BUS (10, 20 and 40 mg/kg), animals in Group II to VI were individually isolated in polypropylene cages for 14 days (two weeks) followed by treatment with the respective drugs for next 14 days (2 weeks) i.e., from day 14 to day 28(Wei *et al.*, 2010). At the end of treatments, the following behavior parameters were carried out, followed by non-invasive blood pressure (NIBP) measurement and MRI of the brain. The rats were then sacrificed; hippocampus was isolated and used for estimation of BDNF using PCR.

Behavior Parameters

Assessment of BUS on anger using roller rotation chamber

The in-house roller rotation chamber was developed in the laboratory (Figure 1), the model has been reported previously to measure anger by Awathale et al.,(Awathale *et al.*, 2020) and was used for estimation of anger. Rats were fasted for 24 h and were placed in the Roller rotation chamber. The food was placed in front of rolling wheel in such a way that rat can see/smell food but had no access to it. To obtain food it rolls the wheel in anger and number of wheel rotation signifies anger here. The wheel rotations were recorded using video tracking system (ASTMT2467-SCH80, VJ Instruments, India) for the measurement of parameters like, duration of wheel rolling and number of time's wheel rolled by the animal.

Evaluation of BUS on aggression

Aggression related parameters were measured using the resident intruder paradigm (Wei *et al*., 2010). The aggressive behavior of the test experimental rats on exposure of naïve rat was video graphed for 10 m for following parameters- Attack bites: Biting to the intruder animal, Wrestling: taking upright posture in order to fight, usually showing by both the animals, Chasing behavior: Pursuit of intruder by test animal, with or without physical contact, Attack latency: Latency time to the first attack (in seconds) from the introduction of intruder animal and Tail rattling: Rapid lateral quivering of the tail just before or after attacking. Total aggression score included addition of attack bites, wrestling, chasing behavior and tail rattling. The attack latency (latency time of the first attack in seconds) from the introduction of the intruder mouse was noted and taken as a measure of impulsivity (Malkesman *et al*., 2009).

Evaluation of BUS on irritability

The rats were exposed to an uncomfortable stimulus (a puff of air was blown sharply through a straw onto the back of the animal) and observed for its response. Rats which exhibited enhanced reactivity to stimuli were considered to display irritable behavior and their irritability was rated using 6 category scale as follows(Ho et al., 2001, Malkesman et al., 2009).

Score-0: No irritability

1: Startle response (a complicated involuntary reaction) to air puffed on the rat's back

and to gentle touching of dorsal lumbar region with rod.

2: Biting reaction to gloved hand placed in a cage 1-3 cm in front of the rat snout (Projecting

the nose, jaws, or anterior facial part of animal's head)

3: Biting reaction to gloved hand pushing the rat backward against the cage wall

4: Resistance to capture by gloved hand

5: Resistance to holding

6: Vocalization during test

Total irritability scores of an individual rat were calculated by adding the individual irritability score of that rat.

Evaluation of BUS on learned helplessness

Learned helplessness was performed immediately after irritability measurement using active avoidance paradigms. The rats were placed in avoidance chamber and underwent 15 attempts of avoidance paradigms of 33/ sec each. In each of 33 second attempt, the first 3 second no shock was given (i.e., the rat could not have escaped to the platform before the onset of shock). If rat did not escape the first 3 seconds, the shock for next 30 seconds was given (0.8 mA intensity). If rat jumped at any time during the attempt, is termed as escape and escape latency was measured. If rats failed to jump from the platform, that was called as "escape failure". The number of failed attempts of each rat was also measured Evaluation of standardized extract of Centella asciatica leaves on suicidal behavior related traits in laboratory rats.

Evaluation of BUS on OFT parameters

Open field apparatus consisted 25 (5×5) identical squares (20×20 cm). The squares were virtually subdivided into a peripheral and central sector, (9 central squares (3×3)] and the peripheral sector contain the squares close to the surrounded wall (20 cm high). The animals were placed in the central sector and their activity was video-recorded for the 5

m. Locomotor activity was scored when the animal crossed a sector border with both hind-limbs. The peripheral activity, central activity, total activity, rearing and grooming activities were scored.

Evaluation of BUS on locomotor activity

Each animal was observed for a period of 5 m in a square closed field arena (30x30x30 cm) equipped with 6 photocells in the outer wall. Interruption of beam (locomotor exploratory action) was recorded by means of digital counter Actophotometer (INCO, Ambala, India).

Measurement of hemodynamic parameter

Noninvasive blood pressure (BP) parameter in rat was carried out on the 29th day of the experiment. The rat was kept in the restrainer in such a way that the tail of rat remains outside (At most care was taken to avoid stress and struggle in animals). Pressure cuff was applied on the tail and which was further connected to 8 channel data acquisition system PowerLab (ML 870/P, AD Instruments Pvt. Ltd., Australia) (Aswar et al., 2019).

Measurement of size of amygdala using MRI (Magnetic resonance imaging)

The rats were scanned with a small receiver surface antenna and a clinical MRI scanner [1.5 Tesla (T) Siemens].

Assessment of expression of brain derived neurotrophic factor (BDNF) and concentration of serum cortisol level

Serum Cortisol

On the 30th day (to avoid the stress generated if any, during BP measurement) the blood was withdrawn through retro orbital puncture under anesthesia. Blood samples were collected in tubes and centrifuged at 2500 rpm, and serum was separated. Serum was used for cortisol estimation using the 96-well ELISA kit as per manufacturer's instructions(Bobade et al., 2015).

Quantitative real-time PCR for mRNA measurements

The expression of BDNF mRNA was analyzed using quantitative real-time PCR (qRT-PCR) as previously described with minor modifications (Sagarkar et al., 2017). Briefly, the tissues were homogenized in the Trizol reagent (Ambion, USA), clean-up was carried out using chloroform. The residual DNA was eliminated using a DNA-free™ DNA Removal Kit (Life Technologies, USA). The total RNA reconstituted in nuclease-free water (Invitrogen, USA) was quantified using Biospec Nano Micro-volume UV-Vis Spectrophotometer (Shimadzu, Kyoto, Japan). The quality of the RNA was evaluated by measuring the 260/280 ratio. The 150 ng of total RNA was reverse transcribed using random hexamers and Multiscribe^{TM} reverse transcriptase (Invitrogen, USA) according to the manufacturer's instructions. The PCR conditions used for the reverse transcription were: 25 °C-10 mins; 37 °C-120 mins; and 85 °C-5 min. The cDNA was further subjected to quantitative real-time PCR (qRT-PCR) in StepOne^{TM} RT-PCR System (Applied Biosystems, USA) by using the SYBR green qPCR master mix (Thermo Fisher Scientific, USA) and specific primers for BDNF IX exon and β-actin. The primer sequences used in this study are as follows: BDNF (F-5'- ACCAGGTGAGAAGAGTGATGACCA -3'; R-5' TGGACGTTTGCTTCTTTCATGGGC-3') and β-actin (F-5'- ACTATCGGCAATGAGCGGTTCC-3'; R-5'- CTGTGTTGGCATAGAGGTCTTTACG-3'). Thermal profile used for amplification consisted of three stages: 95 °C-3 mins (1 cycle); 95 °C, 57 °C and 72 °C-30 sec each (40 cycles). All the reactions were performed in triplicates. β-actin was used as a housekeeping gene for the normalization of the data. Fold changes in the BDNF IX mRNA levels were analyzed using $2^{-\Delta\Delta CT}$ method after normalizing to the b-actin mRNA levels as a housekeeping gene (Schmittgen and Livak, 2008). The results are represented as the fold changes in the mRNA levels (± SEMs).

Results

Effect of BUS (10, 20 and 40) on behavioral parameters

Effect on anger using roller rotation chamber

Table 1 depicts the wheel rolling activity in social isolated rats treated with BUS (10, 20 and 40). It was evident that socially isolated, stressed rats showed a significant (###P<0.001) increase in the duration of wheel rolling, number of time wheel was rotated and wheel biting score as compared to vehicle treated group, the test treatment groups BUS (10, 20 and 40) treatment but not Flx (30) showed reduced wheel rolling activity as compared to stress control. It was evident that BUS but not Flx significantly (***P<0.001, **P<0.01) reduced anger in all the 3 parameters viz. duration of wheel rolling, number of time wheel was rotated and wheel biting score.

Effect of BUS (10, 20 and 40 mg/kg) on aggression

The resident intruder test showed **a s**ignificant rise (P<0.001) in aggressive behavior as observed in increased attack bites, tail rattling, wrestling and chasing behavior in stress control socially isolated rats on day 14. Treatment with BUS dose dependently reduced tail rattling, wrestling, chasing behavior scores, attack bites and increased attack latency. Whereas Flx had no effect in wrestling. BUS (10, 20 and 40mg/kg) as well as Flx significantly (P<0.001) reduced the total aggression score as compared to stress control group (Figure 2a, b, c, d, e, f).

Effect of treatments on irritability

Irritability was measured by counting the total score towards reactivity to the uncomfortable stimuli. There was a significant increase in total irritability score on day 14 in stress control group (P<0.001) as compared to normal rats. Significant reduction in irritation as observed on day 14 in all the groups treated with BUS (10, 20 and 40 mg/kg p.o.) (P<0.001), though no significant reduction was observed in Flx (30 mg/kg) as compared to stress control (Figure 2g).

Effect of treatments on learned helplessness

The data demonstrated significant (P<0.001) increase in escape failure with concomitant reduced escape success of stress control group. Treatment with Flx (30) as well as BUS (P<0.001, P<0.01) significantly improved escape success and reduced escape failure as compared to stress control group (Figure 2h, i).

Effect of treatments on OFT parameters

There was significant reduction in the number of squares crossed, grooming and rearing in stress control group as compared to vehicle treated group. Treatment with Flx (30 mg/kg) as well as BUS (10, 20 and 40 mg/kg) improved the OFT parameters significantly as compared to stress control rats (Table 1).

Effect of treatments on locomotor activity

Significant reduction in the locomotor activity was observed in stress control group as compared to the vehicle control group. Treatment with Flx (30 mg/kg) as well as test groups, BUS (10, 20 and 40 mg/kg) significantly improved locomotor activity (P<0.05, P<0.01, P<0.001) (Figure 2j).

Effect of treatments on noninvasive B.P

Stress controlled animal showed significant rise in blood pressure (P<0.01). Nonsignificant rise in BP was also observed in Flx group. Treatment with BUS (20 and 40) normalized the blood pressure (P<0.05, P<0.001) (Table 2).

Effect of treatments on size of amygdala

It was observed that, stress attenuated the size of the amygdala as compared to vehicle control group (P<0.05), BUS (40) normalized the size of the amygdala (P<0.05) (Figure 3)

Effect of treatments on serum cortisol and BDNF

Serum cortisol was significantly increased in stress control group (P<0.001) as compared to vehicle treated rats. Treatment with Flx as well as BUS reduced it significantly (P<0.001) (Figure 4).

BDNF mRNA expression in the hippocampus of rat brain demonstrated its reduced expression in stress control rats while its expression was increased in Flx (30 mg/kg) and BUS (10, 20 and 40 mg/kg) dose dependently (Table 2).

Discussion

Depression is a neuropsychiatric disorder associated with specific mood alteration (regressive or aggressive), persistent irritability, melancholy, sadness, negative self-concept, and self-inflicted desires (Aswar et al., 2020). The most commonly prescribed anti-depressant drug is an SSRI, but it is accompanied by a black box or a call for caution and is associated with suicidal ideation (Gibbons et al., 2012). Meta-analyses of randomized clinical trials conducted by Brent et al., 2009 suggested the use of SSRI in depressed young adults is found to be associated with suicidal ideation (Brent *et al.*, 2009). Postmortem studies have shown that suicide in depressed patients is more common than in those who do not take antidepressants (Isacsson et al., 2010). Elevated levels of serotonin in the synapse can cause suicidal traits (Graeff, 2004). Although, there are continuing developments observed in the treatment guideline provided by USFDA and other agencies, which suggests closure monitoring of the treatment in suicidal patients, still there is an unmet need to develop the strategy to overcome suicidal ideation. Suicidal traits linked to humans can be modelled in rodents, including aggression, irritability, hopelessness, impulsivity, etc. (Malkesman *et al.*, 2009).

Numerous meta-analytical studies have shown a high correlation between suicide risk and anxiety, impulsiveness, risk of violence (aggression) and anger (Gvion and Levi-Belz, 2018). As well, other behaviors such as irritability and desperation are strongly associated with suicidal ideation (Kashden et al., 1993, Orri et al., 2018). Stress induced by social isolation, which leads to aggressiveness, is an established model for the induction of depression, used in this study (Shimizu et al., 2016). After isolation, we evaluated behavioral parameters such as wheel rolling activity, resident intruder paradigm to measure anger, aggression, irritability and learned helplessness test demonstrating hopelessness (Sáenz et al., 2006, Malkesman et al., 2009, Aswar et al., 2013). Impulsivity is also characterized by elevated blood pressure and elevated cortisol, therefore the present study also measured non-invasive blood pressure and serum cortisol in rats (Smith and Vale, 2006).

The wheel rolling activity was carried out as per the instrument designed by Awathale et al.,(2020). The instrument was manufactured from a wooden box, covered on three sides and having a window at the front with a wheel. When the fasted rat was kept in the box it could see and smell food without having access to it. As already reported that fasting increases anger, and hence increase in wheel rolling activity and biting of the wheel in stress control group as compared to the vehicle control group which are in line with the previous studies (Awathale *et al.*, 2020).

Aggressive behavior is a natural behavior in all animal species (Koolhaas *et al.*, 2013). Flx and BUS, reduces anger as seen in the reduction of rolling activity of the wheels due to their anti-depressant and calming effect (Podhorna and Krsiak, 2000). Among several reported methods, RIT is an appropriate model for studying aggression in rats. The paradigm involves the introduction of an unfamiliar rat into the home cage of the resident rat, which result in aggressive behavioral pattern like, attack bites, wrestling, chasing behavior, tail tattling, and attack. In this study, 14 days of social isolation caused significant aggression in rats. The BUS treatment (10, 20 and 40 mg/kg) showed a reduction in all RIT parameters relative to stress control indicating a reduction in aggression (Fig. 3). The serotonin system in the brain (particularly the modulation of the firing and the release of 5-HT of the serotonin neuron, via the 5-HT1A pre-synaptic automatic receptors)

have striking influences on aggression under certain conditions. Certain 5-HT1A and 1B receptor agonists such as fluprazine, DU28412, DU27725, eltoprazine, batoprazine showed a prominent anti-aggressive and serene effect (Cremers et al., 2000, de Boer and Newman-Tancredi, 2016). Since BUS is a 5-HT1A agonist, its anti-aggressive effect could be attributed to the optimum release of serotonin in the synapse. Although a better understanding of 5HT receptor subtypes is required to use models of anger and aggression.

Irritability, a sensation characterized by the reduction of temperament control, generally leads to irreversible verbal or behavioral attacks. Many studies suggest that irritability is strongly associated with suicidal thoughts and suicide attempts (Conner et al., 2004). The measure of irritability in rodents is defined as an extreme reaction to relatively minor tactile or auditory stimuli when the animal becomes wild or agitated. Different behavioral paradigms attempt to assess and monitor irritability in rats. We used total irritability score after applying a discomfit stimulus in rats and scored them on 6 points (Ho *et al*., 2001). In the present study, social isolation stress was found to increase irritability (mean of total irritability score) in stress control group, while treatment with BUS showed the reduction in irritability scores. However, the fluoxetine-treated rat did not prevent irritability due to stress. The lack of impact of fluoxetine on irritability scores is consistent with previous reports (Jain et al., 1992, Koukopoulos et al., 2005). No significant changes were observed in rearing and grooming parameter of OFT by Flx (30 mg/kg) and BUS (10, 20 mg/kg) suggesting anti anger, anti-aggressive effects of BUS without concomitant sedative and motor impairment effect.

We carried out the active avoidance paradigm to study learned helplessness behavior in socially isolated rats. The principle underlying the test is based on the hypothesis that animals will normally attempt to escape an aversive stimulus (i.e., foot shock). When the stimulus is inevitable, they eventually stop trying to escape, displaying “desperation” or hopelessness (Banerjee et al., 2012). The current study shows significantly increased escape latencies, the number of failed attempts and successes, attempts in stress control group as compared with normal rats. The results demonstrated induction of learned helplessness in stress control groups while treatment with BUS (10, 20 and 40 mg/kg) treated group showed significant prevention of learned helplessness. The model means the role of stress adaptive behavior in normal animals due to the activation of postsynaptic 5-HT1A receptors in the dorsal hippocampus that are reported to mediate this behavior. High level of blood cortisol as a consequence of chronic social stress is known to impair stress adaptation mechanism, predisposing animals to learned helplessness (Joca et al., 2003).

The high cortisol level in stress control group evident in the present study indicated HPA axis activation. The significant decrease of cortisol level in BUS (all the doses) treated animals as well as reduction in learned helplessness behavior by BUS confirmed amelioration of the HPA axis. Prior to cortisol estimation NIBP measurement showed a significant rise in BP in stress control animals as assumed, there was a nonsignificant rise in BP in Flx treatment group too, the result corroborates with the previous findings (Griebel et al., 1999). Marked reduction in BP by BUS at higher dose indicated in stress control group is in accordance with previous and might involve 5-HT1A receptor in regulation of BP (Taylor et al., 1989).

Important parts of the CNS, such as the amygdala, hypothalamus, orbitofrontal cortex and peri-aqueductal grey, have played a major role in the aggressiveness and related disorders. Various neuro-imaging studies, such as MRI, EEG, have shown that patients with aggressive behavior have either a small size of hippocampus, amygdala, hypothalamus (Siever, 2008, Rosell and Siever, 2015). Smaller amygdala was observed in the aggressive population (n=25) as compared to non-aggressive population (n=29) (Bobes *et al*., 2013). Bilateral amygdala ablation in dogs and primates showed a decrease in aggressiveness (Adolphs et al., 1995). The current studies are consistent with previous data; we found a reduction in amygdala size in the stress control group relative to normal animals. BUS (40) treated group improved the size of amygdala indicating amelioration of aggressive behavior (Gerritsen *et al*., 2017). Flx had no marked effect on its advocating its limited role in prevention of aggression.

Various biomarkers can be used to assess anger, aggression and vulnerability to suicide, Brain-derived neurotrophic factor (BDNF) is one such factor (Molteni *et al.*, 2010). BDNF is responsible for the function, sprouting, neuronal survival, differentiation and synaptic plasticity (Maynard *et al.*, 2016). BDNF disruption in rodents and humans is associated with depression (Maynard *et al.*, 2016) as well as stress (Notaras and van den Buuse, 2020) and treatment with antidepressants up-regulates it (Björkholm and Monteggia, 2016). Our findings are consistent with the above evidences, the stress control group had reduced levels of BDNF and significant increase was demonstrated in BUS and Flx group.

Behavioral, biochemical, genetic expression of BDNF and neuro-imaging findings in the present study strongly support the effectiveness and safe use of the BUS as compared to Flx for the reduction of anger and aggression and suicidal ideation.

A potential mechanism of preferential use of BUS versus Flx can be attributed to its partial agonist activity established on the serotonin receptor, while Flx is a selective serotonin reuptake inhibitor. Flx works by decreasing serotonin reuptake and ultimately increasing the accumulation of serotonin in the synaptic cleft. Excessive accumulation of serotonin in the synapses is associated with suicidal ideation (Stahl, 1998, Albert et al., 2011). Serotonin is released from the vesicle in the synaptic cleft, where it interacts with the post and presynaptic receptors. The feedback loop governs the concentration of 5HT in the synaptic cleft. However, chronic administration of the SSRI desensitizes this loop and the regulatory neurotransmission of serotonin is blocked (Sangkuhl et al., 2009).

Pre synaptically, the 5-HT1A receptor acts as a “brake” to inhibit the activity of the entire 5-HT system and is thought to delay the antidepressant response. Unlike SSRI, BUS does not block the reuptake of serotonin, it is a partial agonist, increases serotonin concentration at certain levels and periodically block 5-HT1A receptors located both at pre-synaptic and post-synaptic membrane, which result in a decrease in the accumulation of 5-HT. Because BUS is not causing serotonin accumulation in the synapse, hence BUS would be preferable over Flx for the prevention of anger, aggression associated with depression and the further ideation of suicide in human.

Conclusion

The current study has first time demonstrated the effect of BUS as well as Flx on anger using preclinical model- using Roller rotation chamber and found both of them to be equipotent. Secondly, Flx which is already reported to induce suicidal ideations, has un-favorable effects on BP and was not found to prevent irritability hence 5-HT1A modulator BUS, reduced behavioral (Resident intruder paradigm, irritability, OFT, locomotor activity), improved serum cortisol level, size of amygdala, BDNF, may be considered as a treatment for anger, aggression and suicidal ideation.

Declarations

Ethics- Protocol approval number - SIOP/IAEC/2016/05

Consent to Participate- Yes

Consent to Publish- Yes

Authors' contributions- UA conceptualized and designed the study and wrote the manuscript, HS performed the study and MA analyzed the study and wrote the manuscript.

Funding Source- NA.

Conflicts of interest/Competing interests- The authors declare that they have no conflict of interest.

Availability of data and material (data transparency)- The authors declare that all data were generated in-house and that no paper mill was used. The raw data is submitted with the manuscript in excel format.

Code availability (software application or custom code)- NA

References

1. Adolphs R, Tranel D, Damasio H, Damasio AR (1995) Fear and the human amygdala. J Neurosci 15: 5879-5891. https://doi.org/10.1523/JNEUROSCI.15-09-05879.1995
2. Albert PR, Le François B, Millar AM (2011) Transcriptional dysregulation of 5-HT1A autoreceptors in mental illness. Mol Brain 4: 1-14. https://doi.org/10.1186/1756-6606-4-21
3. Aswar M, Bhalekar M, Trimukhe A, Aswar U (2020) Self-microemulsifying drug delivery system (SMEDDS) of curcumin attenuates depression in olfactory bulbectomized rats. Heliyon 6: e04482. https://doi.org/10.1016/j.heliyon.2020.e04482
4. Aswar U, Kalshetty P, Thakurdesai P, Mohan V (2013) Evaluation of standardized extract of Centella asciatica leaves on suicidal behavior related traits in laboratory rats [PZD-3 received PC Dadiya Award [C]. 46th Annual Conference of Indian Pharmacological Society and International Conference on Translational Medicine. pp. 16-18
5. Aswar U, Mahajan U, Kandhare A, Aswar M (2019) Ferulic acid ameliorates doxorubicin-induced cardiac toxicity in rats. Naunyn Schmiedebergs Arch Pharmacol 392: 659-668. https://doi.org/10.1007/s00210-019-01623-4
6. Awathale SN, Dudhbhate BB, Rahangdale RR, Borkar CD, Subhedar NK, Kokare DM (2020) Denial of food to the hungry rat: A novel paradigm for induction and evaluation of anger-like emotion. J Neurosci Methods 341: 108791. https://doi.org/10.1016/j.jneumeth.2020.108791
7. Banerjee R, Ghosh A, Mondal A (2012) Effects of chronic stress and antidepressant treatment on behavioral, physiological and neurochemical aspects in male and female rats. Al Ameen J Med Sci 5(2): 165-176
8. Björkholm C, Monteggia LM (2016) BDNF–a key transducer of antidepressant effects. Neuropharmacology 102: 72-79. https://doi.org/10.1016/j.neuropharm.2015.10.034
9. Bobade V, Bodhankar SL, Aswar U, Vishwaraman M, Thakurdesai P (2015) Prophylactic effects of asiaticoside-based standardized extract of Centella asiatica (L.) Urban leaves on experimental migraine: Involvement of 5HT1A/1B receptors. Chin J Nat Med 13: 274-282. https://doi.org/10.1016/S1875-5364(15)30014-5
10. Bobes MA, Ostrosky F, Diaz K, Romero C, Borja K, Santos Y, Valdés-Sosa M (2013) Linkage of functional and structural anomalies in the left amygdala of reactive-aggressive men. Soc Cogn Affect Neurosci 8: 928-936. https://doi.org/10.1093/scan/nss101
11. Brent DA, Emslie GJ, Clarke GN, Asarnow J, Spirito A, Ritz L, Vitiello B, Iyengar S, Birmaher B, Ryan ND (2009) Predictors of spontaneous and systematically assessed suicidal adverse events in the treatment of SSRI-resistant depression in adolescents (TORDIA) study. Am J Psychiatry 166: 418-426. https://doi.org/10.1176/appi.ajp.2008.08070976
12. Centenaro LA, Vieira K, Zimmermann N, Miczek KA, Lucion AB, de Almeida RMM (2008) Social instigation and aggressive behavior in mice: role of 5-HT 1A and 5-HT 1B receptors in the prefrontal cortex. Psychopharmacology 201: 237-248. https://doi.org/10.1007/s00213-008-1269-6
13. Conner KR, Meldrum S, Wieczorek WF, Duberstein PR, Welte JW (2004) The association of irritability and impulsivity with suicidal ideation among 15-to 20-year-old males. Suicide Life Threat Behav 34: 363-373. https://doi.org/10.1521/suli.34.4.363.53745
14. Creaney W, Murray I, Healy D (1991) Antidepressant induced suicidal ideation. Hum Psychopharmacol: Clinical and Experimental 6: 329-332. https://doi.org/10.1002/hup.470060411
15. Cremers TI, de Boer P, Liao Y, Bosker FJ, den Boer JA, Westerink BH, Wikström HV (2000) Augmentation with a 5-HT1A, but not a 5-HT1B receptor antagonist critically depends on the dose of citalopram. Eur J Pharmacol 397: 63-74. https://doi.org/10.1038/sj.npp.1301287

16. de Boer SF, Newman-Tancredi A (2016) Anti-aggressive effects of the selective high-efficacy 'biased'5-HT 1A receptor agonists F15599 and F13714 in male WTG rats. Psychopharmacology 233: 937-947. https://doi.org/10.1007/s00213-015-4173-x
17. Ehlert U, Gaab J, Heinrichs M (2001) Psychoneuroendocrinological contributions to the etiology of depression, posttraumatic stress disorder, and stress-related bodily disorders: the role of the hypothalamus–pituitary–adrenal axis. Biol Psychol 57: 141-152. https://doi.org/10.1016/S0301-0511(01)00092-8
18. Filipović D, Todorović N, Bernardi RE, Gass P (2017) Oxidative and nitrosative stress pathways in the brain of socially isolated adult male rats demonstrating depressive-and anxiety-like symptoms. Brain Struct Funct 222: 1-20. https://doi.org/10.1007/s00429-016-1218-9
19. Gerritsen L, Milaneschi Y, Vinkers CH, Van Hemert AM, Van Velzen L, Schmaal L, Penninx BW (2017) HPA axis genes, and their interaction with childhood maltreatment, are related to cortisol levels and stress-related phenotypes. Neuropsychopharmacology 42: 2446-2455. https://doi.org/10.1038/npp.2017.118
20. Gibbons RD, Brown CH, Hur K, Davis JM, Mann JJ (2012) Suicidal thoughts and behavior with antidepressant treatment: reanalysis of the randomized placebo-controlled studies of fluoxetine and venlafaxine. Arch Gen Psychiatry 69: 580-587. https://doi.org/1001/archgenpsychiatry.2011.2048
21. Graeff FG (2004) Serotonin, the periaqueductal gray and panic. Neurosci Biobehav Rev 28: 239-259. https://doi.org/10.1016/j.neubiorev.2003.12.004
22. Griebel G, Cohen C, Perrault G, Sanger DJ (1999) Behavioral effects of acute and chronic fluoxetine in Wistar–Kyoto rats. Physiol Behav 67: 315-320. https://doi.org/10.1016/S0031-9384(98)00298-4
23. Guo Y, Zhang H, Gao J, Wei S, Song C, Sun P, Qiao M (2015) Study of genes associated with the 'anger-in'and 'anger-out'emotions of humans using a rat model. Exp Ther Med 9: 1448-1454. https://doi.org/10.3892/etm.2015.2246
24. Gvion Y, Levi-Belz Y (2018) Serious suicide attempts: systematic review of psychological risk factors. Front Psychiatry 9: 56. https://doi.org/10.3389/fpsyt.2018.00056
25. Hartmann A, Lisboa SF, Sonego AB, Coutinho D, Gomes FV, Guimarães FS (2019) Cannabidiol attenuates aggressive behavior induced by social isolation in mice: Involvement of 5-HT1A and CB1 receptors. Prog Neuro-Psychopharmacol Biol Psychiatry 94: 109637. https://doi.org/10.1016/j.pnpbp.2019.109637
26. Hatch A, Wiberg G, Zawidzka Z, Cann M, Airth J, Grice H (1965) Isolation syndrome in the rat. Toxicol Appl Pharmacol 7: 737-745. https://doi.org/10.3758/BF03336555
27. Ho Y-J, Liu T-M, Tai M-Y, Wen Z-H, Chow RS-S, Tsai Y-F, Wong C-S (2001) Effects of olfactory bulbectomy on NMDA receptor density in the rat brain:[3H] MK-801 binding assay. Brain Res 900: 214-218. https://doi.org/10.1016/S0006-8993(01)02297-1
28. Isacsson G, Rich CL, Jureidini J, Raven M (2010) The increased use of antidepressants has contributed to the worldwide reduction in suicide rates. Br J Psychiatry 196: 429-433 . https://doi.org/10.1192/bjp.bp.109.076166
29. Jain U, Birmaher B, Garcia M, Al-Shabbout M, Ryan N (1992) Fluoxetine in children and adolescents with mood disorders: a chart review of efficacy and adverse effects. J Child Adolesc Psychopharmacol 2: 259-265. https://doi.org/10.1089/cap.1992.2.259
30. Joca SRL, Padovan CM, Guimarães FS (2003) Activation of post-synaptic 5-HT1A receptors in the dorsal hippocampus prevents learned helplessness development. Brain Res 978: 177-184. https://doi.org/10.1016/S0006-8993(03)02943-3
31. Kashden J, Fremouw WJ, Callahan TS, Franzen MD (1993) Impulsivity in suicidal and nonsuicidal adolescents. J Abnorm Child psychol 21: 339-353. https://doi.org/10.1007/BF00917538
32. Koolhaas JM, Coppens CM, de Boer SF, Buwalda B, Meerlo P, Timmermans PJ (2013) The resident-intruder paradigm: a standardized test for aggression, violence and social stress. J Vis Exp 77: 4367. https://doi.org/3791/4367

33. Koukopoulos K, Sani G, Albert MJ, Minnai GP, Koukopoulos AE (2005) Agitated depression: spontaneous and induced. Bipolar disorders. Cambridge University Press, Cambridge, pp. 157-186
34. Kubiszyn T, Mire SS (2014) A review of recent FDA drug safety communications for pediatric psychotropics. J Child Fam Stud 23: 716-727. https://doi.org/10.1007/s10826-012-9706-x
35. Locci A, Pinna G (2019) Social isolation as a promising animal model of PTSD comorbid suicide: neurosteroids and cannabinoids as possible treatment options. Prog Neuro-Psychopharmacol Biol Psychiatry 92: 243-259. https://doi.org/10.1016/j.pnpbp.2018.12.014
36. Malkesman O, Pine DS, Tragon T, Austin DR, Henter ID, Chen G, Manji HK (2009) Animal models of suicide-trait-related behaviors. Trends Pharmacol Sci 30: 165-173. https://doi.org/10.1016/j.tips.2009.01.004
37. Maynard KR, Hill JL, Calcaterra NE, Palko ME, Kardian A, Paredes D, Sukumar M, Adler BD, Jimenez DV, Schloesser RJ (2016) Functional role of BDNF production from unique promoters in aggression and serotonin signaling. Neuropsychopharmacology 41: 1943-1955. https://doi.org/10.1038/npp.2015.349
38. Mezzomo NJ, Müller TE, Franscescon F, Michelotti P, Souza TP, Rosemberg DB, Barcellos LJ (2020) Taurine-mediated aggression is abolished via 5-HT1A antagonism and serotonin depletion in zebrafish. Pharmacol Biochem Behav 199: 173067. https://doi.org/10.1016/j.pbb.2020.173067
39. Micheli L, Ceccarelli M, D'Andrea G, Tirone F (2018) Depression and adult neurogenesis: positive effects of the antidepressant fluoxetine and of physical exercise. Brain Res Bull 143: 181-193. https://doi.org/10.1016/j.brainresbull.2018.09.002
40. Molteni R, Cattaneo A, Calabrese F, Macchi F, Olivier JD, Racagni G, Ellenbroek BA, Gennarelli M, Riva MA (2010) Reduced function of the serotonin transporter is associated with decreased expression of BDNF in rodents as well as in humans. Neurobiol Dis 37: 747-755. https://doi.org/10.1016/j.nbd.2009.12.014
41. Mumtaz F, Khan MI, Zubair M, Dehpour AR (2018) Neurobiology and consequences of social isolation stress in animal model—A comprehensive review. Biomed Pharmacother 105: 1205-1222. https://doi.org/10.1016/j.biopha.2018.05.086
42. Notaras M, van den Buuse M (2020) Neurobiology of BDNF in fear memory, sensitivity to stress, and stress-related disorders. Mol Psychiatry 25: 2251-2274. https://doi.org/10.1038/s41380-019-0639-2
43. Orri M, Galera C, Turecki G, Forte A, Renaud J, Boivin M, Tremblay RE, Côté SM, Geoffroy M-C (2018) Association of childhood irritability and depressive/anxious mood profiles with adolescent suicidal ideation and attempts. JAMA psychiatry 75: 465-473. https://doi.org/1001/jamapsychiatry.2018.0174
44. Perić I, Stanisavljević A, Gass P, Filipović D (2017) Fluoxetine reverses behavior changes in socially isolated rats: role of the hippocampal GSH-dependent defense system and proinflammatory cytokines. Eur Arch Psychiatry Clin Neurosci 267: 737-749. https://doi.org/10.1007/s00406-017-0807-9
45. Podhorna J, Krsiak M (2000) Behavioural effects of a benzodiazepine receptor partial agonist, Ro 19-8022, in the social conflict test in mice. Behav Pharmacol 11: 143-151. https://doi.org/1097/00008877-200004000-00006
46. Rivera-Irizarry JK, Skelly MJ, Pleil KE (2020) Social isolation stress in adolescence, but not adulthood, produces hypersocial behavior in adult male and female C57BL/6J mice. Front Behav Neurosci 14. https://doi.org/10.3389/fnbeh.2020.00129
47. Rosell DR, Siever LJ (2015) The neurobiology of aggression and violence. CNS Spectr 20: 254-279. https://doi.org/10.1017/S109285291500019X
48. Sáenz JCB, Villagra OR, Trías JF (2006) Factor analysis of forced swimming test, sucrose preference test and open field test on enriched, social and isolated reared rats. Behav Brain Res 169: 57-65. https://doi.org/10.1016/j.bbr.2005.12.001Get
49. Sagarkar S, Bhamburkar T, Shelkar G, Choudhary A, Kokare DM, Sakharkar AJ (2017) Minimal traumatic brain injury causes persistent changes in DNA methylation at BDNF gene promoters in rat amygdala: a possible role in anxiety-like

behaviors. Neurobiol Dis 106: 101-109. https://doi.org/10.1016/j.nbd.2017.06.016

50. Sánchez C, Meier E (1997) Behavioral profiles of SSRIs in animal models of depression, anxiety and aggression. Psychopharmacology 129: 197-205. https://doi.org/10.1007/s002130050181
51. Sangkuhl K, Klein T, Altman R (2009) Selective serotonin reuptake inhibitors (SSRI) pathway. Pharmacogenet Genomics 19: 907. https://doi.org/1097/FPC.0b013e32833132cb
52. Santini ZI, Jose PE, Cornwell EY, Koyanagi A, Nielsen L, Hinrichsen C, Meilstrup C, Madsen KR, Koushede V (2020) Social disconnectedness, perceived isolation, and symptoms of depression and anxiety among older Americans (NSHAP): a longitudinal mediation analysis. Lancet Public Health 5: e62-e70. https://doi.org/10.1016/S2468-2667(19)30230-0
53. Schmittgen, T.D., Livak, K.J., 2008. Analyzing real-time PCR data by the comparative C(T) method. Nat. Protoc. 3, 1101-1108. https://doi.org/10.1038/nprot.2008.73
54. Shimizu K, Kurosawa N, Seki K (2016) The role of the AMPA receptor and 5-HT3 receptor on aggressive behavior and depressive-like symptoms in chronic social isolation-reared mice. Physiol Behav 153: 70-83. https://doi.org/10.1016/j.physbeh.2015.10.026
55. Siever LJ (2008) Neurobiology of aggression and violence. Am J Psychiatry 165: 429-442. https://doi.org/10.1176/appi.ajp.2008.07111774
56. Smith SM, Vale WW (2006) The role of the hypothalamic-pituitary-adrenal axis in neuroendocrine responses to stress. Dialogues Clin Neurosci 8: 383. https://doi.org/31887/DCNS.2006.8.4/ssmith
57. Stahl SM (1998) Not so selective serotonin reuptake inhibitors. J Clin Psychiatry 59: 333-343. https://doi.org/10.4088/JCP.V59N0701
58. Tan O, Martin LJ, Bowen MT (2020) Divergent pathways mediate 5-HT1A receptor agonist effects on close social interaction, grooming and aggressive behaviour in mice: Exploring the involvement of the oxytocin and vasopressin systems. J Psychopharmacol 34: 795-805. https://doi.org/10.1177/0269881120913150
59. Taylor J, Harris N, Krieman M, Vogel W (1989) Effects of buspirone on plasma catecholamines, heart rate, and blood pressure in stressed and nonstressed rats. Pharmacol Biochem Behav 34: 349-353. https://doi.org/10.1016/0091-3057(89)90325-0
60. Teicher MH, Glod CA, Cole JO (1993) Antidepressant drugs and the emergence of suicidal tendencies. Drug Saf 8: 186-212. https://doi.org/10.2165/00002018-199308030-00002
61. Wei S, Zhang H, Gao J, Xue L, Sun P, Chao Y, Xue G, Qiao M (2010) Impact of social isolation and resident intruder stress on aggressive behavior in the male rat. Neural Regen Res 5: 1175-1179. https://doi.org/10.18632/oncotarget.22007

Tables

Table 1. Effect of BUS (10, 20 and 40 mg/kg) on (a) parameters for measurement of anger- Wheel rolling activity, (b) parameters for measurement of exploratory behavior- Open field parameters.

Parameters	Vehicle	SC	Flx (30)	BUS (10)	BUS (20)	BUS (40)
Wheel rolling activity using Roller rotation chamber						
Duration of wheel rolling	37.67±5.80	129.7±8.46$^{###}$	32.83±5.134***	38.33±3.018***	31.67±4.62***	41.67±16.17***
No. of times wheel rolled	26.00±1.87	61.50±7.247$^{###}$	26.83±4.772***	46.86±5.534**	36.50±4.43***	38.00±6.683***
Number of wheel biting	10.50±2.26	19.33±2.32$^{###}$	7.000±2.000***	5.833±1.327***	6.833±0.94***	4.667±0.988***
Open field parameters						
Number of squares crossed	78.60±9.64	68.00±10.14$^{###}$	14.20±1.20***	63.40±5.97^{ns}	81.00±10.83	76.00±5.03
Grooming	12.17±1.778	16.33±3.303$^{###}$	7.60±1.21*	9.667±1.77	4.333±0.494***	9.400±2.34
Rearing	5.000±0.816	8.667±0.760$^{###}$	1.80±0.37***	3.000±0.82***	4.500±1.17**	5.167±0.47*

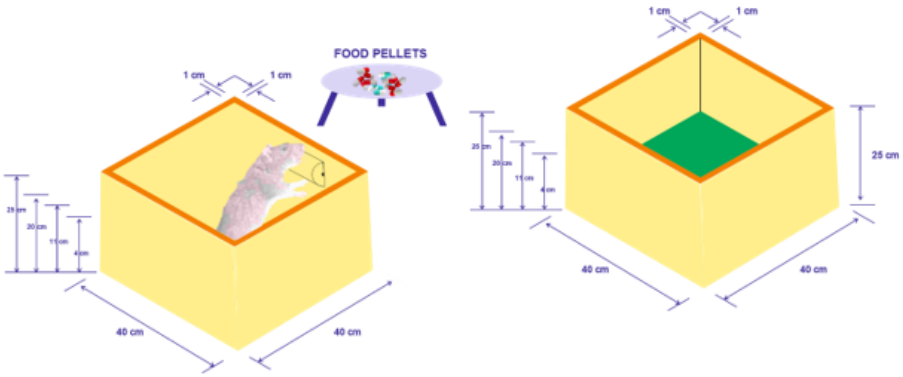
Data was expressed as mean ± SEM (n=6). Statistical significances were determined using one way ANOVA followed by Dunnett's post hoc test. $^{###}$P<0.001 as compared to vehicle, *P<0.05, **P<0.01 ***P<0.001 compared to stress control. SC- Stress control, Flx- Fluoxetine, BUS- Buspirone.

Table 2. Effect of BUS (10, 20 and 40 mg/kg) on Physiological parameters (a) Blood pressure (b) BDNF mRNA level

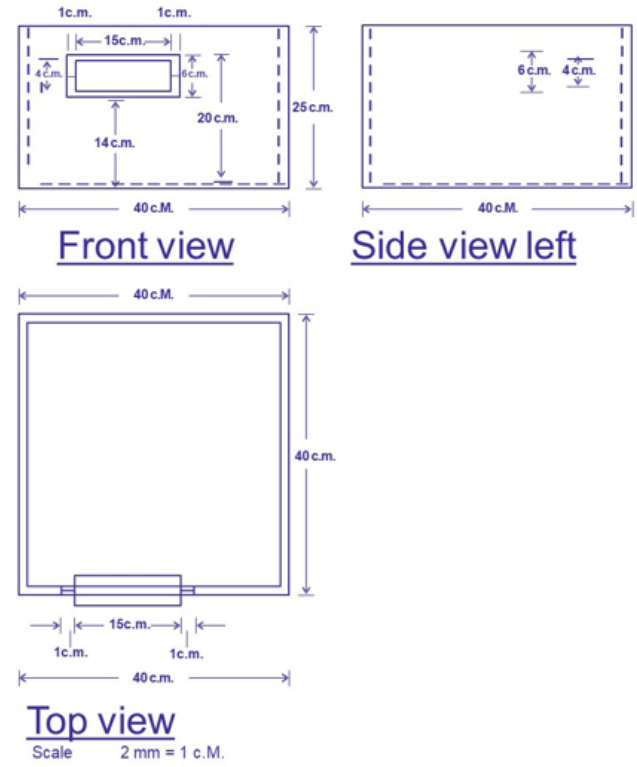
Parameter	Vehicle	SC	Flx (30)	BUS (10)	BUS (20)	BUS (40)
Blood pressure (mmHg)	114.1±4.26	145.0± 3.07$^{#}$	121.6±15.46	132.4±2.88	118.8±5.14*	102.4±8.38***
BDNF mRNA level	1.44±0.05	0.311±0.07$^{##}$	1.46±0.21**	1.06±0.36**	1.32±0.029**	1.41±0.24**

Data is expressed as mean ± SEM (n=6). Statistical significances were determined using one way ANOVA followed by Dunnett's post hoc test. $^{#}$P<0.05, $^{##}$P<0.01 as compared to vehicle, *P<0.05, **P<0.01 ***P<0.001 as compared to stress control. SC- Stress control, Flx- Fluoxetine, BUS- Buspirone.

Figures



a) Isometric view, Dimensions Taken In Isometric Scale (I.E True Dix0.815)



b) Design of Roller rotation chamber

Figure 1

Design of Roller rotation chamber used for wheel rolling activity.

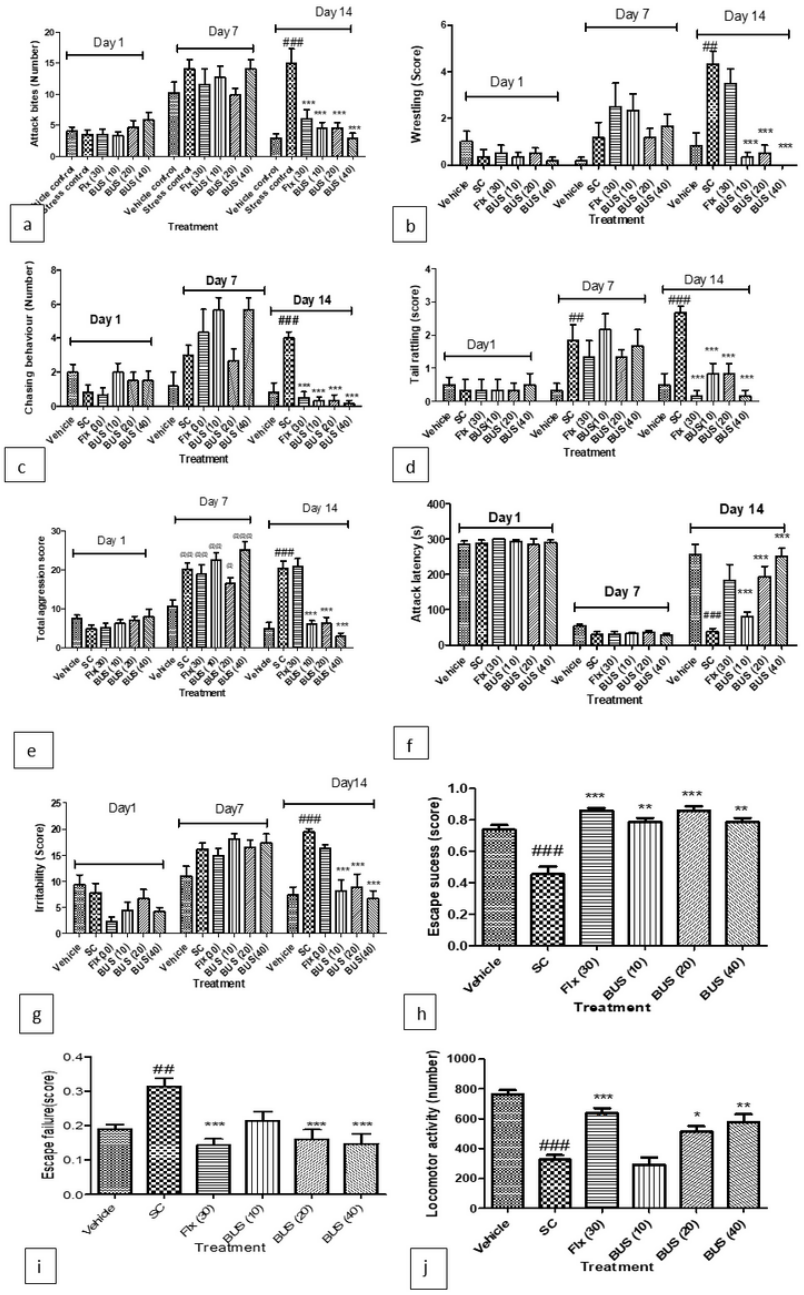
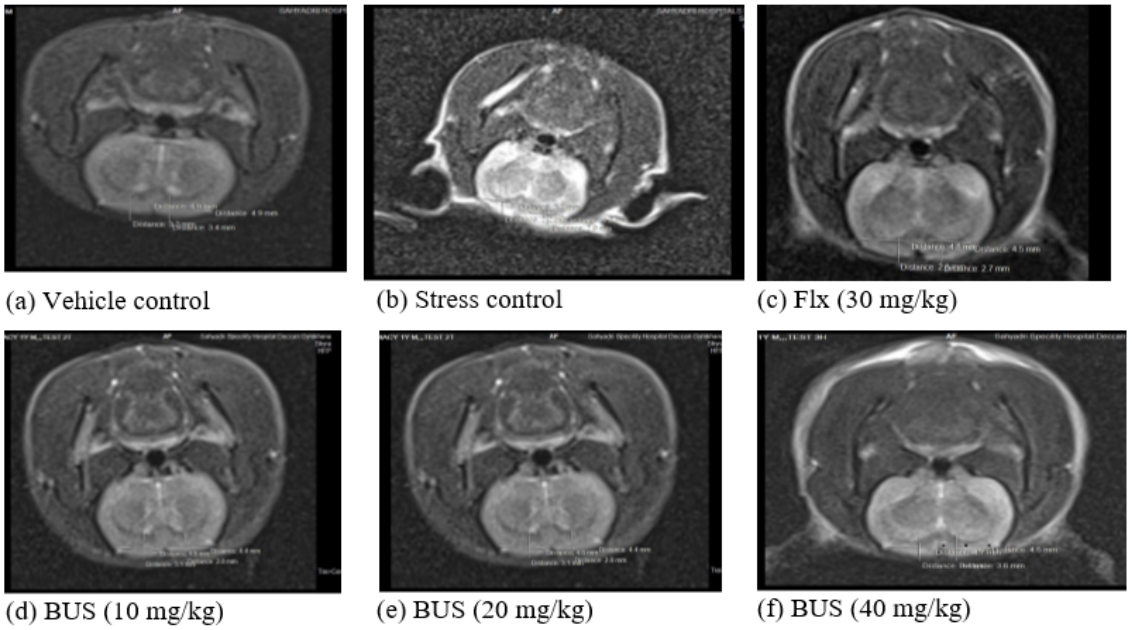


Figure 2

Effect of BUS (10, 20 and 40 mg/kg) on behavioral parameters including (a) Attack bites (b) Wrestling (c) Chasing behavior (d) Tail rattling (e) Total aggression score (f) Attack latency (g) Irritability (h) Escape success (i) Escape failure (j) Locomotor activity. Data was expressed as mean ± SEM (n=6). Statistical significances were determined using two way ANOVA followed by Bonferroni's post hoc test (a-g) while one way ANOVA followed by Dunnett's post hoc test was used for h-j. ##P<0.01, ###P<0.001 compared to vehicle treated group, *P<0.05, **P<0.01, ***P<0.001 as compared to SC group. SC- Stress control, Flx- Fluoxetine, BUS- Buspirone



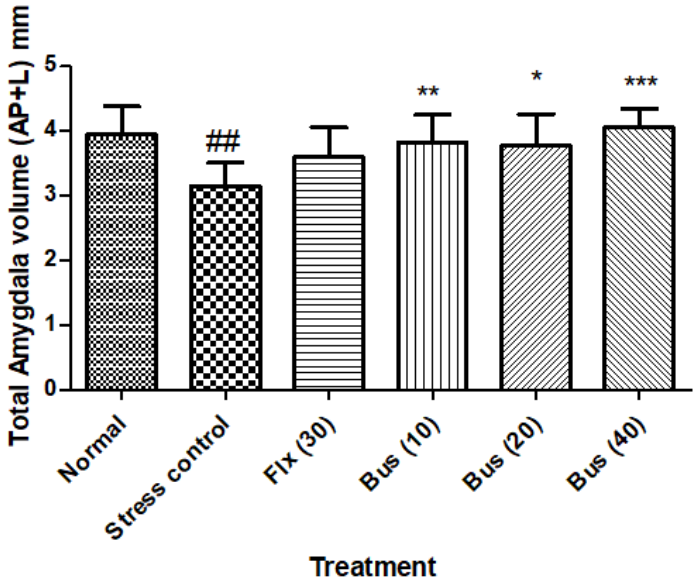


Figure 3

Effect of BUS (10, 20 and 40 mg/kg) on the size of amygdale using MRI Data was expressed as mean ± SEM (n=6). Statistical significances were determined using one way ANOVA followed by Dunnett's post hoc test. #P<0.05 compared to vehicle treated group, *P<0.05 as compared to SC group. SC- Stress control, Flx- Fluoxetine, BUS- Buspirone.

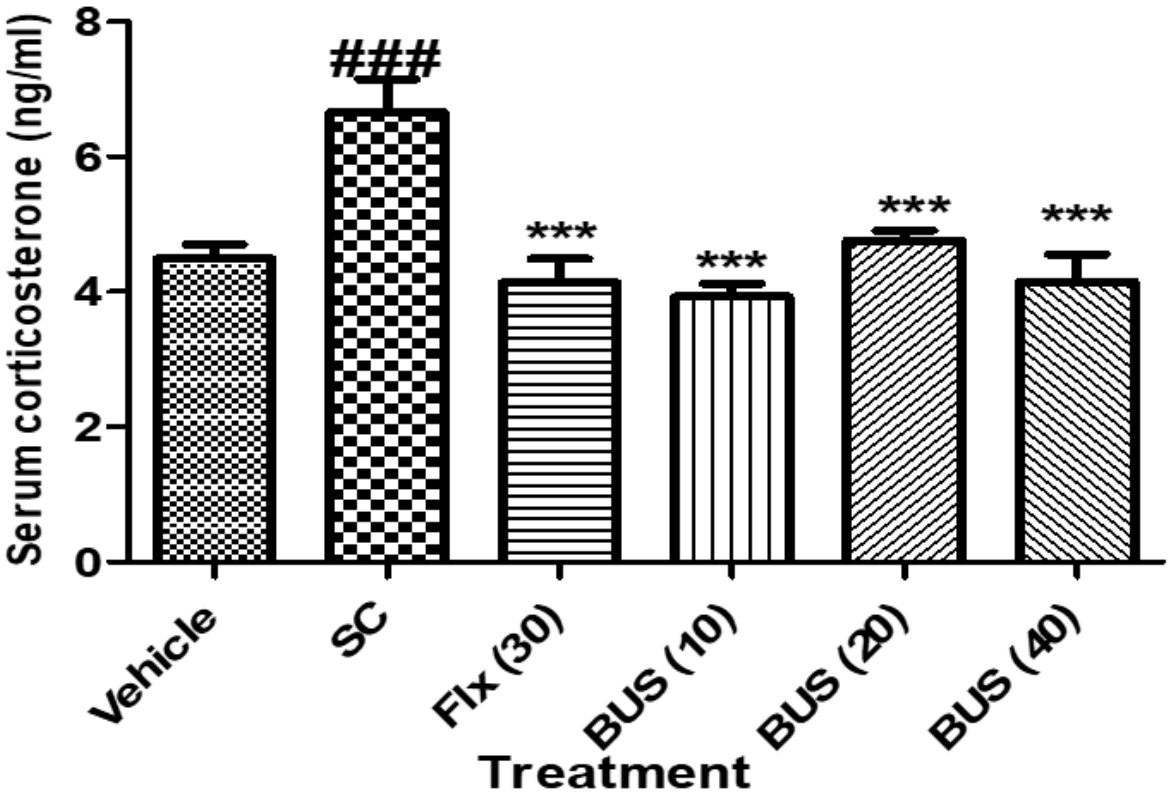


Figure 4

Effect of BUS (10, 20 and 40 mg/kg) on serum cortisol level. Data was expressed as mean ± SEM (n=6). Statistical significances were determined using one way ANOVA followed by Dunnett's post hoc test. ###P<0.01 compared to vehicle treated group, ***P<0.001 as compared to SC group. SC- Stress control, Flx- Fluoxetine, BUS- Buspirone.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- ExcelSheetSupplementarymaterialNSAP.xlsx