Evaluation of effect of sertraline on mitochondria-linked PI3K/Akt/GLUT-4 signaling in PTSD exposed rat brain

Introduction

Recently, it has been suggested that PTSD is also associated with metabolic disorders such as obesity, cardiometabolic risks and insulin resistance (IR; Farr et al., 2015; Farr et al., 2014; Rao et al., 2014). The data from prospective clinical study documents the fact that PTSD could be a marker of neuroendocrine and metabolic dysregulation which may lead to type-2 diabetes (Vaccarino et al., 2014). However, there is no experimental data to support the concept of existence of IR in PTSD condition.

The pharmacological management of PTSD comprises of selective serotonin (5-HT) reuptake inhibitors, monoamine oxidase inhibitors, anticonvulsants and other classes of drugs including antipsychotics (Kozaric-Kovacic, 2008). Sertraline (SER) and paroxetine are only two FDA approved drugs in the management of PTSD in the world market (Krishnamurthy et al., 2013). Clinical as well as preclinical studies report the therapeutic effectiveness of SER in the pharmacotherapy of PTSD (Panahi et al., 2011; Wilson et al., 2014; Zhang et al., 2015). Further, SER has also been reported to have anti-hyperglycemic activity against experimentally-induced diabetic animals (Erenmemisoglu et al., 1999; Khanam and Pillai, 2006; Mahmood et al., 2010). Hence, the present study was proposed to evaluate the therapeutic effectiveness of SER against PTSD-induced IR condition in the animals.

The concept of serotonergic dysregulation and mitochondrial dysfunction has been well established in the pathophysiology of PTSD and type-2 diabetes (Krishnamurthy et al., 2013; Garabadu and Krishnamurthy, 2013; Garabadu et al., 2015). Further, both serotonergic dysregulation and mitochondria dysfunction are considered as significant contributors in the pathogenesis of IR (Petersen et al., 2003; Lowell and Shulman, 2005; Johannsen and Ravussin,

2009; Garabadu and Krishnamurthy, 2013). It has been well documented that there is impairment in the activation of the insulin receptor substrate/phopstidylionositol-3 kinase/Akt (IRS/PI3K/Akt) signaling pathway in IR (Benomar et al., 2006). This leads to the reduction of the translocation of the glucose transporter-4 (GLUT-4) from the cytosol to the cell surface in several tissues (Furtado et al., 2002; Watson et al., 2004; Leney et al., 2009; Jurysta et al., 2013; Blázquezet al., 2014). It has also been suggested that PTSD animals exhibits IR in terms of reduced GLUT-4 level in the plasma membrane of hippocampal and hypothalamic tissues (Cohen et al., 2009).Thus, it is essential to understand the mitochondria-linked PI3K/Akt/GLUT-4 signaling in the pathogenesis of PTSD-induced IR.

Hence, the present study was proposed to evaluate the effectiveness of SER against IR in the modified SRS model of PTSD. Further, the effect of SER was evaluated on PTSD-induced alterations in serotonergic activity and mitochondria-linked PI3K/Akt/GLUT-4 signaling in discrete brain regions of the animals.

Materials and methods

Animals

Male adult Charles Foster rats (200 ± 20 g) were procured from the Central Animal House, Institute of Medical Sciences, Banaras Hindu University (BHU) and housed in polypropylene cages under controlled environmental conditions with a 12 hr light/dark cycle. The experimental animals had free access to food and water *ad libitum*. The experiment was conducted in accordance with the principles of laboratory animal care (2011) guidelines. Experiments on animals were approved by the Institutional Animal Ethics Committee of BHU, Varanasi, India (Protocol No: Dean/11-12/CAEC/328).

Chemicals and reagents

Sertraline was purchased from Sigma (St. Louis, MO, USA). Antibodies such as phosphor-Akt[ser473] (p-Akt), total Akt, GLUT-4 and beta-actin were purchased from Abcam Plc., Cambridge, USA. All other chemicals and reagents were available commercially from local suppliers and were of analytical grade.

Stress paradigm

Briefly, rats were individually exposed to 2 hr restraint in an animal holder, followed by 20 min forced swimming (25 °C) and halothane (0.8 ml of 4% halothane) anesthesia until the loss of consciousness on D-2. The rats were "re-stressed" by exposure to the forced swimming procedure for 20 min on D-8, D-14, D-20, D-26 and D-32 of the experimental protocol (Krishnamurthy et al., 2013). The immobility period was evaluated during last re-stress session as a measure of depression-like symptom. Immobility was defined as sustained immobility except for respiratory movements.

Experimental protocol

The experimental design consisted of three sub sets of experiments and was scheduled for 32 days. Briefly, animals were divided into six groups with five each and named control, modified stress re-stress (SRS), SER-0.1, SER-1.0 and SER-10.0. On Day-1 (D-1), all animals were exposed to trial session of elevated plus maze (EPM). Except control group animals, all other group rats were exposed to modified SRS paradigm. Sertraline (0.1, 1.0 and 10.0 mg/kg) was administered orally through oral gavage 1 hr after re-stress session to SER-0.1, SER-1.0 and SER-10.0 group animals respectively from D-8 to D-32 (Wilson et al., 2014). In experiment-1 and 2, all animals were subjected to oral glucose and insulin tolerance tests. In experiment-3, on D-32, after 2 hr of re-stress session the animals were subjected to EPM test followed by Y-maze

test. There was a lag of 5 min between the behavioral tests. All the behavioral observations were recorded and quantified with ANY-maze[TM] (Version-3.72; USA) video tracking system. Thereafter animals were killed and then plasma was collected and stored at -80°C for corticosterone estimation. The brain was microdissected (Palkovits and Brownstein, 1988) into hippocampus (HIP), hypothalamus (HYP), pre-frontal cortex (PFC) and amygdala (AMY) and the brain parts were stored at -80°C for further analysis.

Oral glucose tolerance test (OGTT)

Oral glucose tolerance test (OGTT) is considered as a classical and model-based estimate of beta-cell function (Rijkelijkhuizen et al., 2009). The OGTT was performed on overnight fasted rats on 13th day of the experimental schedule. Drug or vehicle was given 60 min prior to glucose administration (2 g/kg, i.g.). The blood samples were collected through retro-orbital puncture just before glucose load (0 min) and at 30, 60 and 120 min after glucose administration. Plasma glucose concentrations were determined with glucose GOD PAP kit (Priman Instrument Pvt. Ltd., India) based on glucose oxidase method (Wang et al., 2013).

Insulin tolerance test (ITT)

Insulin tolerance test (ITT) is a simple and reliable method of estimating insulin sensitivity (Duseja et al., 2007; Muniyappa et al., 2008). The ITT was performed on overnight fasted rats on 13th day of the experimental schedule. Drug or vehicle was given 60 min prior to insulin administration (0.4 IU/kg, s.c.). The blood samples were collected through retro-orbital puncture just before insulin load (0 min) and at 30, 60 and 90 min after insulin administration. Plasma glucose concentrations were determined with glucose GOD PAP kit (Priman Instrument Pvt. Ltd., India) based on glucose oxidase method (Wang et al., 2013).

Insulin resistance and β-cell function

Homoeostasis model assessment (HOMA) of IR (HOMA-IR)and HOMA of β-cell function (HOMA-B) were calculated by the HOMA method (Matthews et al., 1985) using the following equations: IR (HOMA-IR) = ¼ (fasting glucose (mmol/l) × fasting insulin (μIU/ml))/22·5, and β-cell function (HOMA-B) = ¼ (20 × fasting insulin (μIU/ml))/(fasting glucose (mmol/l) – 3·5).

Assessment of behavioral performance

Evaluation of anxiolytic activity in EPM

The open arm entries and time spent were estimated as indices of anxiety-like behaviors. Total arm entries were measured as an index of locomotor activity using EPM test (Itoh et al., 1991).

Evaluation of spatial recognition memory in Y-maze test

The total number of entries in all arms (for the 5 min of trial 1 and 2), % entries in known versus novel arm for the 5 min period of trial 2 and percentage of time spent in novel arm to time spent in all arms and in the center of the apparatus during trial 2 were estimated as indices of general exploratory behavior, spatial recognition memory and anxiety-like behavior respectively (Dellu et al., 1992; Krishnamurthy et al., 2013).

Estimation of neurotransmitters and their metabolites

The level of neurotransmitters and their metabolites such as 5-HT and 5-hydroxy indole acetic acid (5-HIAA) were estimated in discrete brain regions of control, modified SRS, SER (0.1, 1.0 and 10.0 mg/kg) treated modified SRS group animals using HPLC with electrochemical detector (Kim et al., 1987; Garabadu et al., 2011). The protein content was estimated colorimetrically (Lowry et al., 1951).

Assay of 5-HT$_{1A}$ and 5-HT$_{2A}$ receptor density and affinity:

In saturation binding assays, receptor density (B$_{max}$) and dissociation constant (K$_d$) were evaluated and expressed as fmol/mg of protein and nM, respectively. On the last day of the experimental protocol, animals were decapitated, brains were quickly removed and the HIP, HYP, PFC and AMY were dissected on ice. Tissues were fixed in liquid nitrogen (-196 °C) and stored at -80 °C until the assays were performed. On the day of assay, tissues were thawed at room temperature, weighed and were then suspended in 20 ml of ice-cold 50 mM Tris–HCl buffer containing 4 mM CaCl$_2$ with a pH of 7.7. Then they were homogenised with a Teflon glass homogenizer (setting 6, 5s) and centrifuged (18, 000×g, 10 min, 4 °C). The pellets were reconstituted with 20 ml of fresh buffer and homogenised for 5s and centrifuged (washed) to eliminate unwanted endogenous substances such as serotonin that may interfere with the radioligand binding procedures. The 'washing' procedure was performed by re-suspending the pelleted membrane fraction after which it was centrifuged. The procedure was repeated twice. The resultant pellet was reconstituted in 20 ml of Tris–HCl buffer, homogenised for 5 s and incubated in a shaking waterbath for 10 min at 37 °C. The incubation and shaking furthermore eliminated unwanted endogenous serotonin. After incubation the homogenate was centrifuged for the last time (18, 000 × g, 10 min, 4 °C). The final pellet was re-suspended in 20 volumes of ice-cold Tris–HCl buffer containing the 4 mM CaCl$_2$ and homogenised for the last time (setting 6, 5s).

The assay of [^{3}H] 8-OH-DPAT binding to 5-HT -receptors was performed according to standard protocol (Jackson and Etgen, 2001). For determination of total binding, aliquots of 360 µl of the tissue preparation, containing 400–1200 µg protein/tube, were incubated with 10 concentrations (0.2–20 nM) of [^{3}H] 8-OH-DPAT (135 Ci/mmol) and buffer to a final volume of

400 µl. Non-specific binding was defined as that binding inhibited by 20 mM serotonin. After the radioligand had been added, the homogenate was mixed thoroughly using a vortex apparatus and incubated in a warmbath at 37 °C for 15 min. After incubation, the assay was terminated by rapid vacuum filtration through Whatman GF/C filters (Millipore, USA) pre-soaked in Tris–HCl buffer containing $CaCl_2$. The filters were washed rapidly with 3×5 ml ice-cold Tris–HCl. The filters were placed in polypropylene counting tubes (Packard) containing scintillation fluid (Filtercount). The tubes were left for 3 h in the scintillation counter (Model Wallac 1409, Turku, Finland) whereafter counting commenced. Radioactivity trapped on the filters was determined by liquid scintillation counting.

The assay of [^{3}H] ketanserin hydrochloride binding to 5-HT -receptors was performed according to standard protocol (Leysen et al., 1982). For determination of total binding, aliquots of 1 ml of the tissue preparation, containing 500–800 µg protein / tube, were incubated with 10 concentrations (0.2–3 nM) of [^{3}H] ketanserin hydrochloride (88 Ci /mmol) and buffer to a final volume of 1.1 ml. Non-specific binding was defined as that binding inhibited by 3 mMmethysergide. After the radioligand had been added, the homogenate was mixed thoroughly using a vortex apparatus and incubated in a warmbath at 37 °C for 15 min. After incubation, the assay was terminated by rapid vacuum filtration through Whatman GF/C filters pre-soaked in Tris-buffer. The filters were washed rapidly with 2×5 ml ice-cold Tris. The filters were placed in polypropylene counting tubes containing scintillation fluid. The tubes were left for 3 h in the scintillation counter where after counting commenced. Radioactivity trapped on the filters was determined by liquid scintillation. Specific binding was calculated as the total minus nonspecific binding. B_{max} and K_d were calculated from Scatchard plots by using Graph pad Prism software

(version 5.0). Concentration of protein was determined using Bovine serum albumin as standard (Lowry et al., 1951).

Assessment of mitochondrial integrity, function and oxidative stress

Isolation of mitochondria from discrete rat brain tissues

Mitochondria were isolated from each tissue by following standard protocol (Pedersen et al., 1978). The mitochondrial protein content was estimated using standard method (Lowry et al., 1951).

Estimation of mitochondrial bioenergetics

Mitochondrial respiration was measured polarographically using a Clark oxygen electrode (Hansatech Instruments Pvt. Ltd., USA).Isolated mitochondria (1 mg/ml) were incubated, at 30 °C, in the respiratory medium containing 125 mM sucrose, 65 mM KCl, 2.5 mM $MgCl_2$, 5 mM KH_2PO_4, 5 mM Hepes, pH 7.2. An initial rate of oxygen consumption (state 2 orV_2) was recorded following addition of glutamate plus malate (10 mM/5 mM), and the state 3 rate (V_3) was recorded following the subsequent addition of 250 nmol of ADP. After a measurable state 4 rate (V_4) (i.e., the rate after ADP phosphorylation) was obtained, a second pulse of ADP was added but the phosphorylative cycle was soon inhibited before its completion by adding 1 μg of oligomycin. After a measurable oligomycin oxygen consumption rate (V_{olig}) was obtained, a 1 μM concentration of the uncoupling agent FCCP was added to obtain a rate of oxygen consumption in the absence of coupled oxidative phosphorylation (V_{FCCP}). Further, to elaborate the respiratory complex-II activity, the oxygen consumption was recorded in presence of 15 mM succinate and 2.2 mM rotenone. Respiratory control ratios (RCR), respiratory states, and ADP/O ratios were determined according to the standard protocol (Chance and Williams, 1956).

Estimation of mitochondrial respiratory complex-I, II, IV and V activity

The activity of NADH dehydrogenase (complex-I) was measured by catalytic oxidation of NADH with potassium ferricyanide as an artificial electron acceptor at excitation and emission wavelengths for NADH were 350 nm and 470 nm, respectively (Shapiro et al., 1979). Activity of NADH dehydrogenase was expressed as nmole NADH oxidised/min/mg protein. The mitochondrial succinate dehydrogenase (SDH; complex-II) was determined by the progressive reduction of nitro blue tetrazolium (NBT) to an insoluble colored compound, diformazan at 570 nm (Sally et al., 1989). The SDH activity was expressed as micromole formazan produced/min/mg protein. The activity of cytochrome oxidase (complex-IV) was measured in mitochondrial fraction in presence of reduced cytochrome c at 550 nm for 3 min (Storrie and Madden, 1990). Results were expressed as nmole cytochrome c oxidized/min/mg protein (ε_{550} = 19.6 $mmol^{-1}cm^{-1}$). The F1-F0 synthase (complex-V) was measured by incubating mitochondrial suspension in ATPase buffer (Griffiths and Houghton, 1974) and the phosphate content was measured (Fiske and Subbarao, 1925). Results were expressed as nmole ATP hydrolyzed/min/mg protein.

Evaluation of MMP in discrete brain regions

The rhodamine dye taken up by mitochondria was measured in spectrofluorometer (Hitachi, F-2500) at an excitation λ of 535 ± 10 nm and emission λ of 580 ± 10 nm (Huang, 2002). The results were expressed as fluorescence intensity/mg protein.

Estimation of lipid peroxidation (LPO) and nitric oxide (NO) level

Mitochondrial malondialdehyde (MDA) content was measured as a marker of LPO at 532 nm (Ohkawa et al., 1979). The extent of LPO was expressed as micromoles of MDA/mg protein.

The NO level was estimated as a marker for nitrosative stress (Green et al., 1982) and expressed as nmoles of NO/mg protein.

Assessment of superoxide dismutase (SOD) and catalase (CAT) activity

Superoxide dismutase (SOD) activity was determined by the reduction of NBT in presence of phenazine-methosulphate and NADH at 560 nm using n-butanol as blank (Kakkar et al., 1984). A single unit of the enzyme was expressed as 50% inhibition of NBT reduction/minute/mg protein. Decomposition of hydrogen peroxide in presence of CAT was followed at 240 nm (Beers and Sizer, 1952). The results were expressed as units (U) of CAT activity/min/mg of protein.

Western blot analysis

The preparation of cellular membrane fraction was performed as described previously (Nishiumi and Ashida, 2007). In brief, tissues were lysed with buffer A [Tris, pH 8.0, 50 mM; dithiothreitol, 0.5 mM; NP-40, 0.1% (v/v); protease inhibitors (phenylmethylsulphonyl fluoride, 1 mM; leupeptin, 5 mg·mL^{-1}; and aprotinin, 5 mg·mL^{-1}) and phosphatase inhibitors (NaF, 10 mM and Na_3VO_4, 1 mM)] and centrifuged at 1000 × g for 10 min at 4 °C. Pellets were re-suspended in NP-40 free buffer A in ice for another 10 min and re-centrifuged at 1000 × g for 10 min at 4 °C. The pellets were re-suspended in buffer A and placed in ice for 1 h and centrifuged at 16 000 × g for 20 min at 4 °C. The supernatant was collected as the plasma membrane fraction and stored at -80 °C until use. The supernatants from the first and second spins at 1000 × g were combined and centrifuged at 16 000 × g for 20 min at 4 °C. The resultant supernatant was collected and used as the cytosol fraction. Protein concentrations were determined according to Bradford (1976) in each fraction. A standard plot was generated using bovine serum albumin. An aliquot of each sample were electrophoresed in 10% SDS-PAGE gels for Akt, p-Akt and GLUT-

4 proteins, transferred to polyvinylidene fluoride membranes and probed with specific antibodies. The membrane was incubated overnight with rabbit anti-Akt (Abcam Plc., Cambridge, USA), anti-p-Akt (Abcam Plc., Cambridge, USA) and anti-GLUT-4 (Abcam Plc., Cambridge, USA) polyclonal primary antibody at a dilution of 1:1000. After detection with the desired antibodies against the proteins of interest the membrane was stripped with stripping buffer (25 mM Glycine pH 2.0, 2% SDS for 30 min at room temperature) and re-probed overnight with rabbit anti β-actin (Santa Cruz Biotechnology Inc.; Santa Cruz, California, USA) polyclonal primary antibody at a dilution of 1:500 to confirm equal loading of protein. Further, membrane was probed with corresponding secondary antibodies. Immunoreactive band of proteins were detected by chemiluminescence using enhanced chemiluminescence (ECL) reagents (Amersham Bioscience, USA). Quantification of the results was performed by densitometric scan of films. The immunoreactive area was determined by densitometric analysis using Biovis gel documentation software.

Data analysis

All the data were mean ± standard error of the mean (S.E.M). The plasma glucose level at different time points in OGTT and ITT, and the data of total arm entries in trial-1 and 2, and percentage entries into known and novel arm in trial-2 in Y-maze in experiment-3 were evaluated by using repeated measure two-way analysis of variance (ANOVA) with Bonferroni Post-hoc test. Two-way ANOVA followed by Bonferroni Post-hoc test was performed to measure arm discrimination behavior between known and novel arm in Y-maze paradigm for each group. All other statistical analysis of data was done using one-way ANOVA with Newman-Keuls Post-hoc analysis to monitor significance among groups. $P < 0.05$ were considered as significant.

Results

Effect of SER (0.1, 1.0 and 10.0 mg/kg) on SRS-induced changes in the plasma glucose level in OGTT and ITT paradigm

Table-28 and 29 depicts the effect of SER (0.1, 1.0 and 10.0 mg/kg) on SRS-induced changes in the plasma glucose level of rats at different time points after administration of glucose and insulin respectively. Repeated measures of two-way ANOVA revealed that there were significant differences in plasma glucose level during OGTT and ITT among groups ($F_{(4, 80)} = 164.4$; $p<0.05$] and [$F_{(4, 80)} = 254.5$; $p<0.05$] respectively), time ([$F_{(3, 80)} = 3.5$; $p<0.05$] and [$F_{(3, 80)} = 0.6$; $p<0.05$] respectively) and there was a significant interaction between group and time ([$F_{(12, 80)} = 0.3$; $p<0.05$] and [$F_{(12, 80)} = 0.5$; $p<0.05$] respectively). Post-hoc analysis showed that before loading of either glucose or insulin to the animals (0 min), SRS paradigm caused a significant increase in the level of plasma glucose compared to vehicle treated rats. Only, SER (10.0 mg/kg) administration significantly decreased the plasma glucose level in SRS exposed animals at 0 min during both OGTT and ITT paradigms. This effect of SER (10.0 mg/kg) on SRS-induced increase in the plasma glucose level in animals was persistent even after either glucose or insulin administration.

Effect of SER (0.1, 1.0 and 10.0 mg/kg) on SRS-induced changes in depressive-like behavior during FST paradigm

Fig-35 depicts the effect of SER (0.1, 1.0 and 10.0 mg/kg) on SRS-induced changes in the depressive-like behavior in terms of immobility period in FST paradigm. Statistical analysis revealed that there were significant differences in the immobility period [$F_{(4, 20)} = 54.8$, $p<0.05$] among groups. Post-hoc test showed that SRS paradigm increased the depressive-like behavior in rats compared to control animals. SER (0.1 and 1.0 mg/kg) did not cause any change

in the SRS-induced increase in the depressive-like behavior in rats. However, SER (10.0 mg/kg) significantly decreased the SRS-induced increase in the depressive-like behavior in the animals.

Effect of SER (0.1, 1.0 and 10.0 mg/kg) on SRS-induced changes in anxiety-like behaviors during EPM test paradigm

The effect of SER (0.1, 1.0 and 10.0 mg/kg) on SRS-induced alterations in the anxiety-like behaviors in terms of percentage of entries and time spent into open arm is depicted in Fig-36. Statistical analysis revealed that there were significant differences in the percentage of entries [Fig-2 (A); $F_{(4, 20)} = 68.4$, $p<0.05$] and time spent [Fig-2 (B); $F_{(4, 20)} = 30.2$, $p<0.05$] into open arm among groups. However, there were no significant differences in the total arm entries [Fig-2 (C); $F_{(4, 20)} = 0.05$, $p>0.05$] during EPM test paradigm. Post-hoc test showed that SRS exposure caused a significant increase in the anxiety-like behaviors in the animals compared to vehicle administered rats. Only, SER at highest dose level was able to reduce the SRS-induced increase in the anxiety-like behaviors in the animals.

Effect of SER (0.1, 1.0 and 10.0 mg/kg) on SRS-induced alterations in the behaviors during Y-maze test paradigm

Fig-37 illustrates the effect of SER (0.1, 1.0 and 10.0 mg/kg) on SRS-induced alterations in the total arm entries in trial-1 and 2 (curiosity; A), spatial recognition memory (B) and coping behavior to novel arm (anxiety-like behavior; C) in Y-maze test paradigm. Statistical analysis revealed that there were significant differences in curiosity among groups [$F_{(4, 40)} = 36.6$, $p<0.05$] and trials [$F_{(1, 40)} = 189.8$, $p<0.05$], a significant interaction between group and trial [$F_{(4, 40)} = 3.4$, $p<0.05$]. Post-hoc test showed that the curiosity was higher in SRS subjected animals compared to control group rats. SER at highest dose level significantly decreased the SRS-induced increase in the level of curiosity in the rats. There were significant differences for

arm discrimination behavior between known and novel arm among groups [F (4, 40) = 1.3, p<0.05] and arms [F (1, 40) = 228.3, p<0.05], and a significant interaction between group and arm [F (4, 40) = 70.4, p<0.05]. Post-hoc test showed that the control animals exhibited significantly higher arm discrimination to novel arm as compared to known arm, indicating a gain in spatial recognition memory. SRS exposed rats exhibited a loss in spatial recognition memory. SER (10.0 mg/kg) treatment caused a significant reduction in the SRS-induced loss in spatial recognition memory. Further, one-way ANOVA revealed that there were significant differences among groups in known [F (4, 20) = 17.9, p<0.05] and novel [F (4, 20) = 92.6, p<0.05] arm entries. Post-hoc analysis showed that SRS exposed rats showed significant increase and decrease in the percentage entries into known and novel arm compared to vehicle administered animals respectively. SER (10.0 mg/kg) treatment attenuated the SRS-induced increase and decrease in the percentage entries into known and novel arm respectively. Moreover, statistical analysis revealed that there were significant differences in anxiety-like behavior among groups [F (4, 20) = 8.3, p<0.05]. Post-hoc test showed that SRS exposure significantly increased the anxiety-like behavior in the animals compared to vehicle treated rats. SER administration at highest dose level to the rats caused a significant reduction in anxiety-like behavior in SRS exposed animals.

Effect of SER (0.1, 1.0 and 10.0 mg/kg) on SRS-induced alterations in the levels of plasma glucose, insulin HOMA-IR and HOMA-B

The effect of SER (0.1, 1.0 and 10.0 mg/kg) on SRS-induced alterations in the levels of fasting blood glucose and insulin, and the extent of IR (HOMA-IR), and the function of pancreatic β-cell (HOMA-B) of rats are depicted inTable-30. Statistical analysis revealed that there were significant differences in the levels of fasting blood glucose [F (4, 20) = 82.0; p<0.05], insulin [F

(4, 20) = 8.0; p<0.05], HOMA-IR [F (4, 20) = 50.0; p<0.05] and HOMA-B [F (4, 20) = 128.3; p<0.05] among groups. Post-hoc test showed that the levels of fasting blood glucose, insulin and HOMA-IR were significantly increased and the level of HOMA-B was significantly reduced in SRS exposed rats compared to control group animals. SER (10.0 mg/kg) attenuated the SRS-induced changes in these levels.

Effect of SER (0.1, 1.0 and 10.0 mg/kg) on SRS-induced changes in the density and affinity of 5-HT$_{1A}$ and 5-HT$_{2A}$ receptors in discrete brain regions

Fig-38 depicts the effect of SER (0.1, 1.0 and 10.0 mg/kg) on SRS-induced alterations in the density and affinity of 5-HT$_{1A}$ and 5-HT$_{2A}$ in discrete brain regions. Statistical analysis showed that there were significant differences in the density and affinity of 5-HT$_{1A}$ in HIP ([F (4, 20) = 108.1; p<0.05] and [F (4, 20) = 24.7; p<0.05] respectively), HYP ([F (4, 20) = 98.0; p<0.05] and [F (4, 20) = 6.7; p<0.05] respectively), PFC ([F (4, 20) = 98.2; p<0.05] and [F (4, 20) = 19.3; p<0.05] respectively) and AMY ([F (4, 20) = 74.0; p<0.05] and [F (4, 20) = 33.2; p<0.05] respectively) among groups. Further, there were significant differences in the density and affinity of 5-HT$_{2A}$ in HIP ([F (4, 20) = 47.6; p<0.05] and [F (4, 20) = 12.4; p<0.05] respectively), HYP ([F (4, 20) = 106.1; p<0.05] and [F (4, 20) = 9.0; p<0.05] respectively), PFC ([F (4, 20) = 179.0; p<0.05] and [F (4, 20) = 24.6; p<0.05] respectively) and AMY ([F (4, 20) = 183.0; p<0.05] and [F (4, 20) = 42.6; p<0.05] respectively) among groups. Post hoc test showed that SRS paradigm caused a significant increase in the density (B$_{max}$) and decrease in the affinity (increase in K$_d$) of 5-HT$_{1A}$ receptors in all the brain regions of the animals compared to vehicle treated rats. None of the doses of SER caused a significant change in the density as well as affinity of 5-HT$_{1A}$ receptors in any brain regions of the SRS exposed rats. Moreover, SRS paradigm caused a significant increase in the density (B$_{max}$) and affinity (decrease in the K$_d$) of 5-HT$_{2A}$ receptors in

all the brain regions of rats compared to control animals. SER (10.0 mg/kg) attenuated the SRS-induced increase in the density of 5-HT$_{2A}$ receptors in all the brain regions.

Effect of SER (0.1, 1.0 and 10.0 mg/kg) on SRS-induced changes in the levels of 5-HT and 5-HIAA, and their ratio (5-HIAA/5-HT) in discrete brain regions

The effect of SER (0.1, 1.0 and 10.0 mg/kg) on SRS-induced changes in the levels of 5-HT and 5-HIAA, and their ratio (5-HIAA/5-HT) are depicted in Table-31. Statistical analysis revealed that there were significant differences in the levels of 5-HT in HIP [F (4, 20) = 9.7; p<0.05], HYP [F (4, 20) = 5.8; p<0.05] and AMY [F (4, 20) = 12.1; p<0.05], 5-HIAA in HIP [F (4, 20) = 6.8; p<0.05] and AMY [F (4, 20) = 23.4; p<0.05] and the ratio of 5-HIAA/5-HT in HYP [F (4, 20) = 13.2; p<0.05] and AMY [F (4, 20) = 5.7; p<0.05] among groups. However, there were no significant differences in the level of 5-HT in PFC [F (4, 20) = 1.2; p>0.05], 5-HIAA in HYP [F (4, 20) = 0.1; p>0.05] and PFC [F (4, 20) = 1.0; p>0.05], and the ratio of 5-HIAA/5-HT in HIP [F (4, 20) = 0.2; p>0.05] and PFC [F (4, 20) = 0.2; p>0.05] among groups. Post-hoc test showed that SRS paradigm caused a significant increase in the levels of both 5-HT and 5-HIAA in HIP of animals compared to vehicle treated rats. None of the doses of SER caused any change in the level of either 5-HT or 5-HIAA in HIP of SRS exposed rats. The levels of 5-HT and 5-HIAA, and the ratio of 5-HIAA/5-HT of AMY were significantly higher in SRS exposed rats compared to control animals. None of the dose of SER caused any change in the levels of 5-HT and 5-HIAA, and the ratio of 5-HIAA/5-HT in AMY of SRS exposed rats. On contrary, the level of 5-HT and the ratio of 5-HIAA/5-HT were significantly decreased and increased in HYP of SRS exposed rats compared to vehicle treated animals respectively. It is interesting to note that SER at highest dose level was able to mitigate the changes in the level of 5-HT and ratio of 5-HIAA/5-HT in HYP of SRS exposed animals.

Effect of SER (0.1, 1.0 and 10.0 mg/kg) on SRS-induced changes in mitochondrial function, integrity, oxidative stress and bioenergetics in discrete brain regions

The effect of SER (0.1, 1.0 and 10.0 mg/kg) on SRS-induced changes in the mitochondrial function in terms of mitochondrial complex-I, II, IV and V activity and integrity in terms of MMP are depicted in Table-32. Statistical analysis revealed that there were significant differences in mitochondrial complex-I, II, IV and V activity and the level of MMP in HIP ([$F_{(4, 20)}$ = 20.2; $p < 0.05$], [$F_{(4, 20)}$ = 24.3; $p < 0.05$], [$F_{(4, 20)}$ = 35.5; $p < 0.05$], [$F_{(4, 20)}$ = 11.6; $p < 0.05$] and [$F_{(4, 20)}$ = 13.9; $p < 0.05$] respectively), HYP ([$F_{(4, 20)}$ = 18.8; $p < 0.05$], [$F_{(4, 20)}$ = 50.0; $p < 0.05$], [$F_{(4, 20)}$ = 43.9; $p < 0.05$], [$F_{(4, 20)}$ = 12.0; $p < 0.05$] and [$F_{(4, 20)}$ = 58.6; $p < 0.05$] respectively), PFC ([$F_{(4, 20)}$ = 24.5; $p < 0.05$], [$F_{(4, 20)}$ = 38.9; $p < 0.05$], [$F_{(4, 20)}$ = 69.9; $p < 0.05$], [$F_{(4, 20)}$ = 22.2; $p < 0.05$] and [$F_{(4, 20)}$ = 25.6; $p < 0.05$] respectively) and AMY ([$F_{(4, 20)}$ = 5.8; $p < 0.05$], [$F_{(4, 20)}$ = 8.1; $p < 0.05$], [$F_{(4, 20)}$ = 60.6; $p < 0.05$], [$F_{(4, 20)}$ = 14.3; $p < 0.05$] and [$F_{(4, 20)}$ = 8.6; $p < 0.05$] respectively) among groups. Post-hoc test showed that SRS paradigm caused a significant increase in the activity of all mitochondrial complex enzymes and decrease in mitochondrial integrity in all the brain regions of animals compared to control rats. SER (10.0 mg/kg) attenuated the SRS-induced increase and decrease in the mitochondrial function and integrity in all the rat brain regions respectively.

Table-33 depicts the effect of SER (0.1, 1.0 and 10.0 mg/kg) on SRS-induced changes in the mitochondrial oxidative stress in terms of the extent of LPO, level of NO and the activity of SOD and CAT in discrete rat brain regions. Statistical analysis showed that there were significant differences in the extent of mitochondrial LPO, level of NO, and activity of SOD and CAT in HIP ([$F_{(4, 20)}$ = 18.4; $p < 0.05$], [$F_{(4, 20)}$ = 29.9; $p < 0.05$], [$F_{(4, 20)}$ = 27.3; $p < 0.05$] and [$F_{(4, 20)}$ = 16.0; $p < 0.05$] respectively), HYP ([$F_{(4, 20)}$ = 57.1; $p < 0.05$], [$F_{(4, 20)}$ = 58.3;

$p < 0.05$], [$F_{(4, 20)} = 110.3$; $p < 0.05$] and [$F_{(4, 20)} = 7.9$; $p < 0.05$] respectively), PFC ([$F_{(4, 20)} = 19.5$; $p < 0.05$], [$F_{(4, 20)} = 33.9$; $p < 0.05$], [$F_{(4, 20)} = 63.9$; $p < 0.05$] and [$F_{(4, 20)} = 20.4$; $p < 0.05$] respectively) and AMY ([$F_{(4, 20)} = 36.5$; $p < 0.05$], [$F_{(4, 20)} = 59.6$; $p < 0.05$], [$F_{(4, 20)} = 106.8$; $p < 0.05$] and [$F_{(4, 20)} = 31.5$; $p < 0.05$] respectively) among groups. Post-hoc test showed that SRS paradigm increased the level of LPO and NO, and decreased the activity of SOD and CAT in all the rat brain regions compared to control animals. SER (10.0 mg/kg) attenuated the SRS-induced changes in all these parameters in all the rat brain regions.

Fig-39 demonstrates the representative pattern of mitochondrial respiration in HIP (A), HYP (B), PFC (C) and AMY (D) of each animal of each group. The effect of SER (0.1, 1.0 and 10.0 mg/kg) on SRS-induced changes in the mitochondrial bioenergetics in terms of the level of RCR (A) and the ratio of ADP/O (B) are depicted in Fig-40. Statistical analysis revealed that there were significant differences in the level of RCR and the ratio of ADP/O in HIP ([$F_{(4, 20)} = 25.5$; $p < 0.05$] and [$F_{(4, 20)} = 19.1$; $p < 0.05$] respectively), HYP ([$F_{(4, 20)} = 47.5$; $p < 0.05$] and [$F_{(4, 20)} = 24.5$; $p < 0.05$] respectively), PFC ([$F_{(4, 20)} = 49.1$; $p < 0.05$] and [$F_{(4, 20)} = 17.1$; $p < 0.05$] respectively) and AMY ([$F_{(4, 20)} = 30.1$; $p < 0.05$] and [$F_{(4, 20)} = 37.3$; $p < 0.05$] respectively) among groups. Post-hoc test showed that SRS paradigm caused a significant decrease in the level of RCR and the ratio of ADP/O in all the brain regions of rats compared to vehicle administered animals. SER at highest dose level mitigated SRS-induced decrease in the mitochondrial bioenergetics in all the rat brain regions.

Effect of SER (0.1, 1.0 and 10.0 mg/kg) on SRS-induced alterations in the extent of phosphorylation of Akt and translocation of GLUT-4 in discrete brain regions

Fig-41 depicts the effect of SER (0.1, 1.0 and 10.0 mg/kg) on SRS-induced alterations in the extent of phosphorylation of Akt in terms of the level of expression of Akt (A) and p-Akt (C) in

discrete rat brain regions. Statistical analysis showed that there were significant differences in the level of expression of p-Akt (D) and the ratio of p-Akt/Akt (E) in HIP ([$F_{(4, 10)} = 36.4$; $p<0.05$] and [$F_{(4, 10)} = 43.5$; $p<0.05$] respectively), HYP ([$F_{(4, 10)} = 6.0$; $p<0.05$] and [$F_{(4, 10)} = 10.3$; $p<0.05$] respectively), PFC ([$F_{(4, 10)} = 36.4$; $p<0.05$] and [$F_{(4, 10)} = 43.5$; $p<0.05$] respectively) and AMY ([$F_{(4, 10)} = 28.6$; $p<0.05$] and [$F_{(4, 10)} = 52.6$; $p<0.05$] respectively) among groups. However, there were no significant differences in the level of expression of Akt (B) in HIP [$F_{(4, 10)} = 0.1$; $p>0.05$], HYP [$F_{(4, 10)} = 0.2$; $p>0.05$], PFC [$F_{(4, 10)} = 0.1$; $p>0.05$] and AMY [$F_{(4, 10)} = 0.7$; $p>0.05$] among groups. Post-hoc analysis revealed that the SRS paradigm caused a significant decrease in the level of expression of p-Akt and the ratio of p-Akt/Akt in all the brain regions of the animals compared to control rats, indicating a loss in the extent of phosphorylation of Akt in all the brain regions with SRS exposure. SER (10.0 mg/kg) treatment attenuated SRS-induced decrease in the extent of phosphorylation of Akt in all the brain regions of the rats.

The effect of SER (0.1, 1.0 and 10.0 mg/kg) on SRS-induced alterations in the extent of translocation of GLUT-4 in terms of the levels of expression of GLUT-4 in membranous (A) and cytoplasmic (C) fraction of discrete rat brain regions is depicted in Fig-42. Statistical analysis revealed that there were significant differences in the levels of membranous (B) and cytoplasmic (D) GLUT-4, and their ratio (E; membranous/cytoplasmic) in HIP ([$F_{(4, 10)} = 28.4$; $p<0.05$], [$F_{(4, 10)} = 90.9$; $p<0.05$] and [$F_{(4, 10)} = 17.4$; $p<0.05$] respectively), HYP ([$F_{(4, 10)} = 29.3$; $p<0.05$], [$F_{(4, 10)} = 40.0$; $p<0.05$] and [$F_{(4, 10)} = 9.5$; $p<0.05$] respectively), PFC ([$F_{(4, 10)} = 39.2$; $p<0.05$], [$F_{(4, 10)} = 90.9$; $p<0.05$] and [$F_{(4, 10)} = 17.4$; $p<0.05$] respectively) and AMY ([$F_{(4, 10)} = 47.6$; $p<0.05$], [$F_{(4, 10)} = 42.9$; $p<0.05$] and [$F_{(4, 10)} = 21.0$; $p<0.05$] respectively) among groups. Post-hoc test showed that SRS paradigm significantly decreased the

extent of translocation of GLUT-4 to membrane from cytoplasm in all the rat brain regions compared to vehicle administered animals. SER at highest dose level attenuated these changes in all the brain regions of SRS exposed animals.

Table-28: Effect of SER (0.1, 1.0 and 10.0 mg/kg) on the SRS-induced increase in the plasma glucose level of rats in OGTT.

Groups	Plasma glucose level (mmol/L)			
	0 min	30 min	60 min	120 min
Control	3.5 ± 0.15	6.7 ± 0.07	7.3 ± 0.08	5.3 ± 0.13
SRS	20.3 ± 1.22 [a]	21.8 ± 1.17 [a]	22.2 ± 1.29 [a]	22.4 ± 1.33 [a]
SER-0.1	20.9 ± 1.09 [a]	22.3 ± 1.12 [a]	22.1 ± 1.32 [a]	22.3 ± 1.27 [a]
SER-1.0	20.5 ± 1.19 [a]	21.1 ± 1.31 [a]	21.9 ± 1.13 [a]	21.7 ± 1.14 [a]
SER-10.0	14.3 ± 1.07 [a, b, c, d]	15.7 ± 1.22 [a, b, c, d]	16.1 ± 1.21 [a, b, c, d]	16.4 ± 1.27 [a, b, c, d]

All values are mean ± SEM (n = 5). [a]$p<0.05$ compared to control, [b]$p<0.05$ compared to SRS, [c]$p<0.05$ compared to SER-0.1 and [d]$p<0.05$ compared to SER-1.0 (repeated measures of two-way ANOVA followed by Bonferroni post hoc test).

Table-29: Effect of SER (0.1, 1.0 and 10.0 mg/kg) on the SRS-induced increase in the plasma glucose level of rats in ITT.

Groups	Plasma glucose level (mmol/L)			
	0 min	30 min	60 min	90 min
Control	4.2 ± 0.15	2.3 ± 0.21	2.9 ± 0.34	2.9 ± 0.28
SRS	20.1 ± 1.13 [a]	20.7 ± 1.17 [a]	21.9 ± 1.04 [a]	22.1 ± 1.22 [a]
SER-0.1	20.3 ± 1.25 [a]	20.5 ± 1.21 [a]	21.3 ± 1.14 [a]	21.9 ± 1.18 [a]
SER-1.0	20.7 ± 1.09 [a]	20.1 ± 1.11 [a]	20.9 ± 1.04 [a]	21.1 ± 1.03 [a]
SER-10.0	12.3 ± 1.13 [a, b, c, d]	11.2 ± 1.13 [a, b, c, d]	10.8 ± 1.02 [a, b, c, d]	11.1 ± 1.09 [a, b, c, d]

All values are mean ± SEM (n = 5). [a]$p<0.05$ compared to control, [b]$p<0.05$ compared to SRS, [c]$p<0.05$ compared to SER-0.1 and [d]$p<0.05$ compared to SER-1.0 (repeated measures of two-way ANOVA followed by Bonferroni post hoc test).

Table-30: Effect of SER (0.1, 1.0 and 10.0 mg/kg) on the SRS-induced changes in fasting blood glucose and insulin level, HOMA-IR and HOMA-B indices of rats.

Groups	Fasting blood Glucose (mmol/L)	Fasting blood Insulin (pmol/L)	HOMA-IR	HOMA-B
Control	4.3 ± 0.22	74.4 ± 3.42	2.6 ± 0.51	169.5 ± 12.23
SRS	25.5 ± 1.29[a]	123.4 ± 10.41[a]	21.3 ± 1.49[a]	15.8 ± 1.86[a]
SER-0.1	25.3 ± 1.17[a]	123.4 ± 8.73[a]	20.8 ± 0.53[a]	15.3 ± 1.15[a]
SER-1.0	25.5 ± 1.08[a]	124.3 ± 9.85[a]	20.5 ± 1.37[a]	14.9 ± 1.11[a]
SER-10.0	16.1 ± 1.03[a, b, c, d]	84.8 ± 8.89[a, b, c, d]	11.7 ± 1.43[a, b, c, d]	93.9 ± 5.53[a, b, c, d]

All values are mean $\pm$ SEM (n = 5). [a]$p<0.05$ compared to control, [b]$p<0.05$ compared to SRS, [c]$p<0.05$ compared to SER-0.1 and [d]$p<0.05$ compared to SER-1.0 (one-way ANOVA followed by Student–Newman–Keuls test).

Table-31: Effect of SER (0.1, 1.0 and 10.0 mg/kg) on the SRS-induced alterations in the level of 5-HT, 5-HIAA and the ratio of 5-HIAA/5-HT in discrete brain regions.

Group	5-HT	5-HIAA	5-HIAA/5-HT
HIP			
Control	4.11±0.37	2.13±0.12	0.54±0.05
SRS	8.34±0.65[a]	4.16±0.40[a]	0.59±0.07
SER-0.1	7.45±0.82[a]	4.14±0.48[a]	0.53±0.05
SER-1.0	7.27±0.35[a]	4.16±0.31[a]	0.52±0.09
SER-10.0	7.73±0.23[a]	3.96±0.28[a]	0.53±0.03
HYP			
Control	12.31±0.63	2.18±0.21	0.17±0.02
SRS	8.42±0.46[a]	2.26±0.22	0.34±0.03[a]
SER-0.1	8.81±1.31[a]	2.23±0.12	0.33±0.02[a]
SER-1.0	9.31±0.32[a]	2.13±0.31	0.31±0.02[a]
SER-10.0	11.58±0.43[b,c,d]	2.03±0.33	0.19±0.02[b,c,d]
PFC			
Control	4.53±0.45	1.48±0.14	0.33±0.03
SRS	5.13±0.34	1.83±0.14	0.34±0.02
SER-0.1	4.46±0.32	1.83±0.13	0.35±0.02
SER-1.0	4.53±0.53	1.63±0.12	0.35±0.02
SER-10.0	5.53±0.52	1.82±0.23	0.33±0.01
AMY			
Control	17.31±0.42	2.12±0.22	0.13±0.01
SRS	26.71±1.20[a]	3.43±0.12[a]	0.16±0.01[a]
SER-0.1	27.23±1.54[a]	4.13±0.17[a]	0.19±0.01[a]
SER-1.0	27.57±1.52[a]	4.14±0.13[a]	0.14±0.01[a]
SER-10.0	26.52±1.24[a]	4.17±0.24[a]	0.17±0.01[a]

All values are mean ± SEM (n = 5). [a]$p < 0.05$ compared to control, [b]$p < 0.05$ compared to SRS, [c]$p < 0.05$ compared to SER-0.1 and [d]$p < 0.05$ compared to SER-1.0 (one-way ANOVA followed by Student–Newman–Keuls test).

Table-32: Effect of SER (0.1, 1.0 and 10.0 mg/kg) on the SRS-induced alterations in the activity of mitochondrial complex-I, II, IV and V, and MMP in discrete brain regions.

Groups	HIP	HYP	PFC	AMY
Complex-I (nmol NADHoxidized/min/mg protein)				
Control	5.3 ± 0.4	5.4 ± 0.3	4.6 ± 0.4	6.5 ± 0.4
SRS	10.4 ± 0.7 [a]	10.7 ± 0.9 [a]	9.7 ± 0.5[a]	10.3 ± 0.6[a]
SER-0.1	10.5 ± 0.5 [a]	10.3 ± 0.7 [a]	9.1 ± 0.7 [a]	10.5 ± 0.9 [a]
SER-1.0	10.3 ± 0.7 [a]	10.1 ± 0.5 [a]	9.8 ± 0.4 [a]	10.3 ± 1.1 [a]
SER-10.0	6.5 ± 0.4[b,c,d]	5.9 ± 0.4[b,c,d]	5.4 ± 0.3[b,c,d]	7.5 ± 0.7[b,c,d]
Complex-II (μmolformazan produced/min/mg protein)				
Control	0.5 ± 0.02	0.5 ± 0.01	0.5 ± 0.02	0.5 ± 0.03
SRS	1.0 ± 0.05 [a]	0.7 ± 0.02 [a]	1.5 ± 0.08 [a]	0.7 ± 0.06 [a]
SER-0.1	1.1 ±0.07 [a]	0.8 ± 0.02 [a]	1.3 ± 0.09 [a]	0.7 ± 0.05 [a]
SER-1.0	1.2 ± 0.09 [a]	0.7 ± 0.03 [a]	1.1 ± 0.06 [a]	0.8 ± 0.05 [a]
SER-10.0	0.7 ± 0.04[b,c,d]	0.4 ± 0.03[b,c,d]	0.7 ± 0.06[b,c,d]	0.5 ± 0.04[b,c,d]
Complex-IV(nmol cytochrome coxidized/min/mg protein)				
Control	0.8 ± 0.05	1.1 ± 0.06	1.3 ± 0.07	1.3 ± 0.05
SRS	1.6 ± 0.07 [a]	2.3 ± 0.05[a]	3.1 ± 0.13 [a]	2.3 ± 0.09 [a]
SER-0.1	1.5 ± 0.04 [a]	2.4 ± 0.14 [a]	2.9 ± 0.11 [a]	2.3 ± 0.08 [a]
SER1.0	1.6 ± 0.08 [a]	2.3 ± 0.12 [a]	2.8 ± 0.12 [a]	2.2 ± 0.09 [a]
SER-10.0	1.1 ± 0.05[b,c,d]	1.2 ± 0.09[b,c,d]	1.4 ± 0.08[b,c,d]	1.1 ± 0.06[b,c,d]
Complex-V(nmol ATPhydrolyzed/mg protein)				
Control	7.5± 0.4	16.8 ± 0.6	12.5 ± 0.7	12.3 ± 0.6
SRS	16.6 ± 1.9 [a]	37.2 ± 3.0 [a]	29.4 ± 2.3 [a]	30.5 ± 2.4 [a]
SER-0.1	16.3 ± 1.3 [a]	36.3 ± 3.5 [a]	28.5 ± 2.1 [a]	31.3 ± 3.2 [a]
SER-1.0	15.9 ± 1.2 [a]	36.3 ± 3.1 [a]	28.9 ± 2.3 [a]	31.5 ± 2.6 [a]
SER-10.0	9.9 ± 0.9[b,c,d]	21.3 ± 2.9[b,c,d]	13.3 ± 1.4[b,c,d]	18.5 ± 2.1[b,c,d]
MMP (Intensity/mg protein)				
Control	463.7±6.0	457.8±10.0	478.5±16.8	456.4±16.5
SRS	314.6±23.4 [a]	332.4±8.2 [a]	316.8±17.1 [a]	325.8±26.4 [a]
SER-0.1	312.4 ± 21.5 [a]	329.4 ± 7.8 [a]	312.4 ± 14.5 [a]	323.5 ± 23.5 [a]
SER-1.0	317.3 ± 23.1 [a]	327.7 ± 6.7 [a]	315.6 ± 13.3 [a]	321.6 ± 23.4 [a]
SER-10.0	415.5 ± 14.3[b,c,d]	411.4 ± 5.6[b,c,d]	432.1 ± 15.6[b,c,d]	423.6 ± 19.5[b,c,d]

All values are mean ± SEM (n = 5). [a]p<0.05 compared to control, [b]p<0.05 compared to SRS, [c]p<0.05 compared to SER-0.1 and [d]p<0.05 compared to SER-1.0 (one-way ANOVA followed by Student–Newman–Keuls test).

Table-33: Effect of SER (0.1, 1.0 and 10.0 mg/kg) on the SRS-induced alterations in the level of mitochondrial LPO and NO, and activity of mitochondrial SOD and catalase in discrete brain regions.

Groups	HIP	HYP	PFC	AMY
LPO level(µM MDA/mg protein)				
Control	0.4 ± 0.02	0.4 ± 0.03	0.5 ± 0.03	0.5 ± 0.03
SRS	0.8 ± 0.05 [a]	0.8 ± 0.02 [a]	0.9 ± 0.07 [a]	0.8 ± 0.02 [a]
SER-0.1	0.8 ± 0.04 [a]	0.8 ± 0.03 [a]	0.9 ± 0.06 [a]	0.8 ± 0.04 [a]
SER-1.0	0.8 ± 0.07 [a]	0.8 ± 0.04 [a]	0.9 ± 0.05 [a]	0.8 ± 0.02 [a]
SER-10.0	0.5 ± 0.03 [b,c,d]	0.4 ± 0.02 [b,c,d]	0.5 ± 0.02 [b,c,d]	0.5 ± 0.02 [b,c,d]
Nitrite level (nM NO/mg protein)				
Control	1.1 ± 0.07	0.8 ± 0.04	1.5 ± 0.07	1.4 ± 0.08
SRS	2.5 ± 0.15 [a]	2.1 ± 0.07 [a]	2.8 ± 0.06 [a]	2.8 ± 0.09 [a]
SER-0.1	2.3 ± 0.16 [a]	2.1 ± 0.09 [a]	2.7 ± 0.13 [a]	2.8 ± 0.13 [a]
SER-1.0	2.4 ± 0.12 [a]	2.3 ± 0.15 [a]	2.7 ± 0.17 [a]	2.9 ± 0.11 [a]
SER-10.0	1.2 ± 0.11 [b,c,d]	1.1 ± 0.05 [b,c,d]	1.6 ± 0.09 [b,c,d]	1.5 ± 0.07 [b,c,d]
SOD activity (Units/min/mg protein)				
Control	0.4 ± 0.02	0.4 ± 0.002	0.4 ± 0.003	0.6 ± 0.011
SRS	0.2 ± 0.02 [a]	0.2 ± 0.011 [a]	0.2 ± 0.017 [a]	0.3 ± 0.015 [a]
SER-0.1	0.2 ± 0.01 [a]	0.2 ± 0.013 [a]	0.2 ± 0.018 [a]	0.3 ± 0.014 [a]
SER-1.0	0.2 ± 0.02 [a]	0.2 ± 0.015 [a]	0.2 ± 0.014 [a]	0.3 ± 0.013 [a]
SER-10.0	0.4 ± 0.03 [b,c,d]	0.4 ± 0.005 [b,c,d]	0.4 ± 0.011 [b,c,d]	0.5 ± 0.015 [b,c,d]
CAT activity(Units/min/mg protein)				
Control	2.3 ± 0.13	3.5 ± 0.05	2.5 ± 0.15	2.5 ± 0.11
SRS	1.4 ± 0.14 [a]	2.4 ± 0.14 [a]	1.3 ± 0.13 [a]	1.1 ± 0.05 [a]
SER-0.1	1.3 ± 0.13 [a]	2.5 ± 0.19 [a]	1.1 ± 0.14 [a]	1.3 ± 0.09 [a]
SER-1.0	1.3 ± 0.12 [a]	2.4 ± 0.21 [a]	1.2 ± 0.15 [a]	1.3 ± 0.07 [a]
SER-10.0	2.2 ± 0.11 [b,c,d]	3.4 ± 0.31 [b,c,d]	2.5 ± 0.21 [b,c,d]	2.4 ± 0.21 [b,c,d]

All values are mean ± SEM (n = 5). [a]p<0.05 compared to control, [b]p<0.05 compared to SRS, [c]p<0.05 compared to SER-0.1 and [d]p<0.05 compared to SER-1.0 (one-way ANOVA followed by Student–Newman–Keuls test).
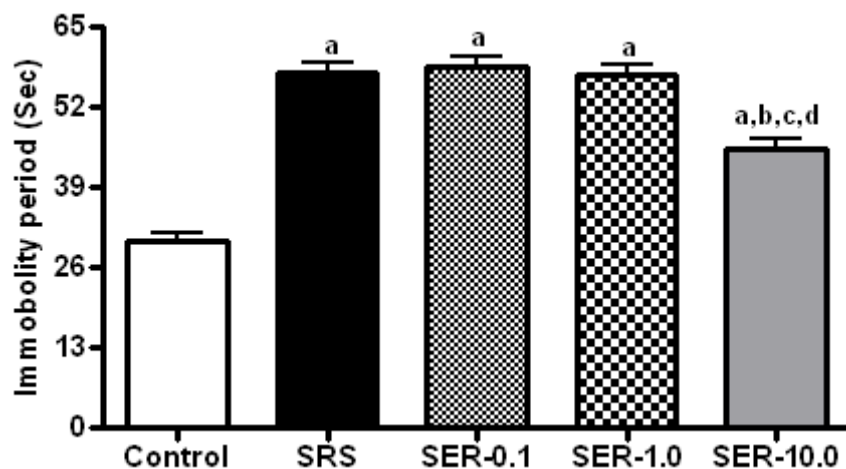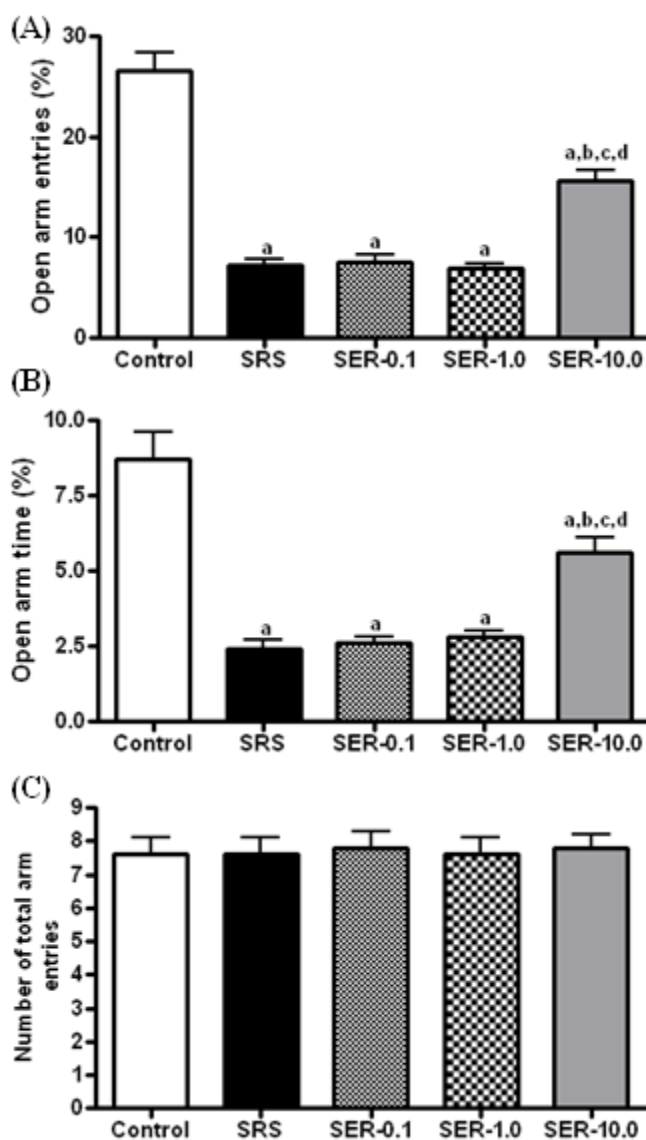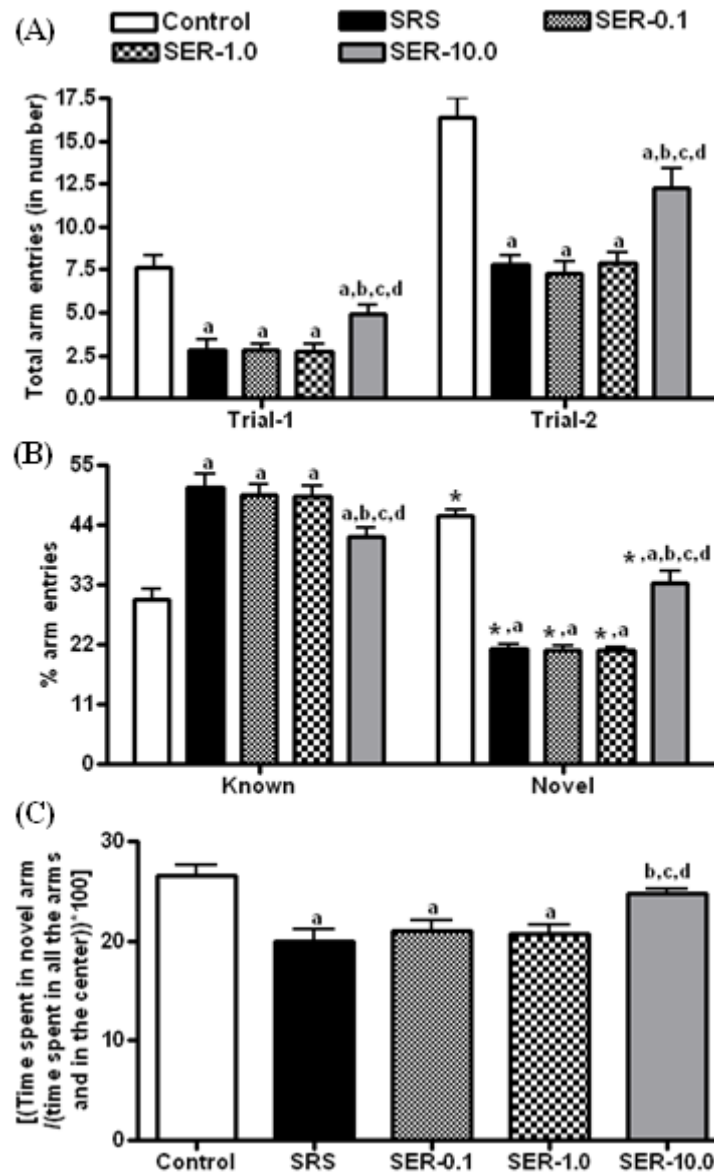
Figure 35: The effect of sertraline (0.1, 1.0 and 10.0 mg/kg) on SRS-induced changes in the immobility period in FST. All values are mean ± SEM (n = 6). [a]$p<0.05$ compared to control, [b]$p<0.05$ compared to SRS, [c]$p<0.05$ compared to SER-0.1 and [d]$p<0.05$ compared to SER-1.0 (one-way ANOVA followed by Student–Newman–Keuls test).

Figure 36: The effect of sertraline (0.1, 1.0 and 10.0 mg/kg) on SRS-induced changes in the percentage open arm entries (A), time spent (B) and total arm entries (C) in EPM. All values are mean ± SEM (n = 6). [a]$p<0.05$ compared to control, [b]$p<0.05$ compared to SRS, [c]$p<0.05$ compared to SER-0.1 and [d]$p<0.05$ compared to SER-1.0 (one-way ANOVA followed by Student–Newman–Keuls test).
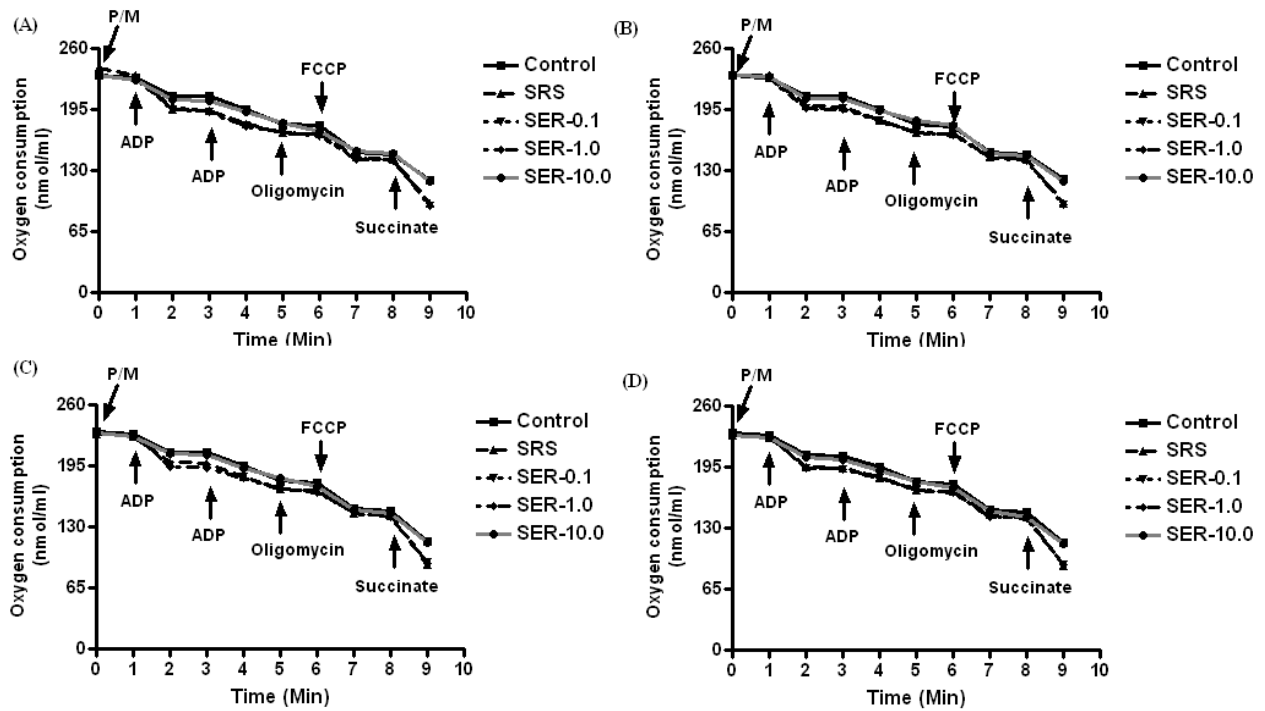
Figure 37: The effect of SER (0.1, 1.0 and 10.0 mg/kg) on the total arm entries in trial-1 and 2 (curiosity; A), spatial recognition memory (B) and coping behavior to novel arm (anxiety-like behavior; C) in Y-maze test paradigm. All values are mean ± SEM (n=5). [a]$P<0.05$ compared to control, [b]$P<0.05$ compared to SRS, [c]$P<0.05$ compared to SER-0.1 and [d]$P<0.05$ compared to SER-1.0 [Repeated measure two-way ANOVA followed by Bonferroni test for curiosity analysis and, percentage entries into known and novel arm. One-way ANOVA followed by Student Newmann-Keuls test was performed for the analysis of anxiety-like behavior]. [*]$P<0.05$ compared to known arm entries [Two-way ANOVA followed by Bonferroni test].
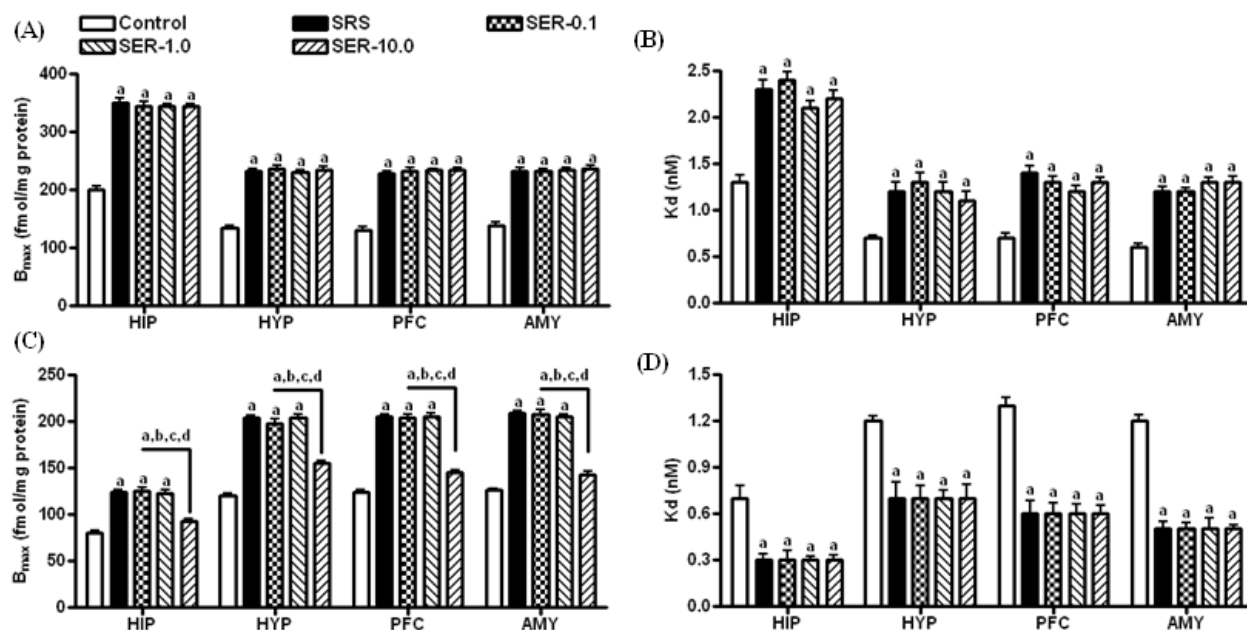
Figure 38: The effect of SER (0.1, 1.0 and 10.0 mg/kg) on the SRS-induced changes in Bmax (A) and Kd (B) for 5-HT$_{1A}$ and Bmax (C) and Kd (D) for 5-HT$_{2A}$ in HIP, HYP, PFC and AMY of rats. All values are mean ± SEM (n = 6). [a]p<0.05 compared to control, [b]p<0.05 compared to SRS, [c]p<0.05 compared to SER-0.1 and [d]p<0.05 compared to SER-1.0 (one-way ANOVA followed by Student–Newman–Keuls test).

Figure 39: Representative figures for mitochondrial respiration pattern in HIP (A), HYP (B), PFC (C) and AMY (D) of control, SRS and SER (0.1, 1.0 and 10.0 mg/kg) treated PTSD-like rats.
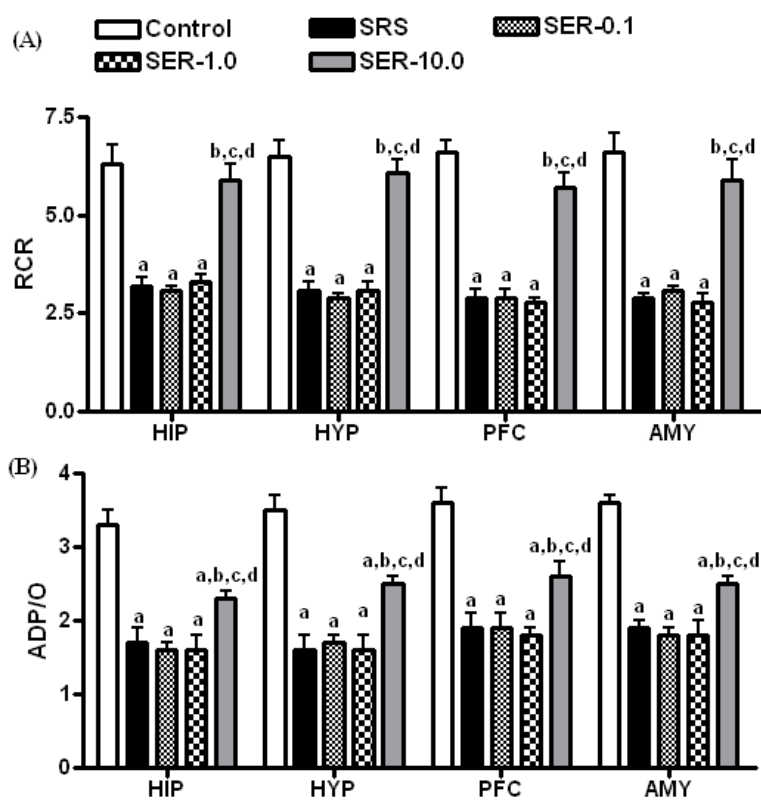
Figure 40: The effect of SER (0.1, 1.0 and 10.0 mg/kg) on the SRS-induced changes in RCR (A) and ADP/O (B) in HIP, HYP, PFC and AMY of rats. All values are mean ± SEM (n = 6). [a]p<0.05 compared to control, [b]p<0.05 compared to SRS, [c]p<0.05 compared to SER-0.1 and [d]p<0.05 compared to SER-1.0 (one-way ANOVA followed by Student–Newman–Keuls post-hoc test).
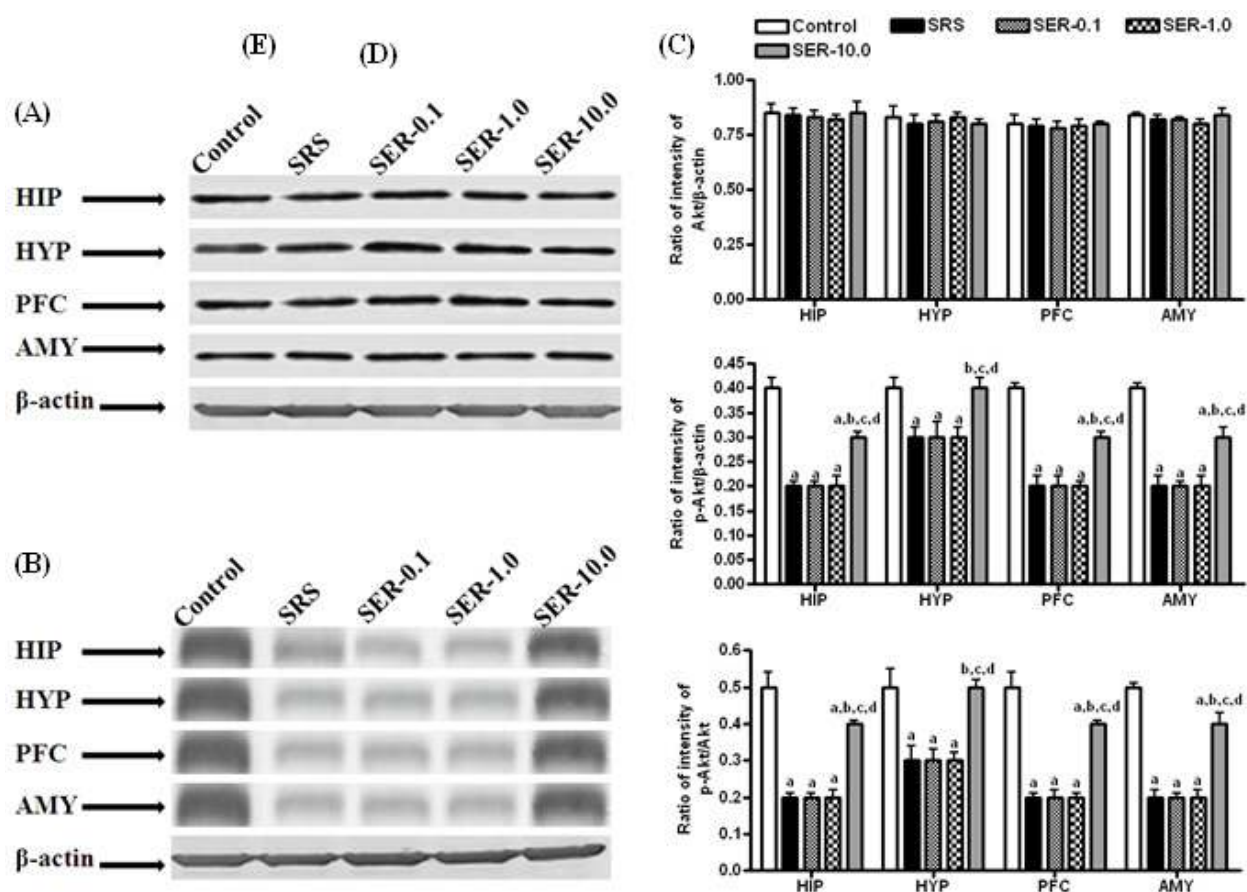
Figure 41: The effect of SER (0.1, 1.0 and 10.0 mg/kg) on the level of expression of Akt (C) and p-Akt (D), and ratio of p-Akt to Akt (E) in HIP, HYP, PFC and AMY of control and T2DM rats. The blots are representative of Akt (A) and p-Akt (B) in HIP, HYP, PFC and AMY. The results in the histogram are expressed as the ratio of relative intensity of levels of expression of Akt or p-Akt to β-actin. All values are mean ± SEM of three separate sets of independent experiments. [a]p<0.05 compared to control, [b]p<0.05 compared to SRS and [c]p<0.05 compared to SER-0.1 and [d]p<0.05 compared to SER-1.0 [One-way ANOVA followed by Student Newmann-Keuls post-hoc test].
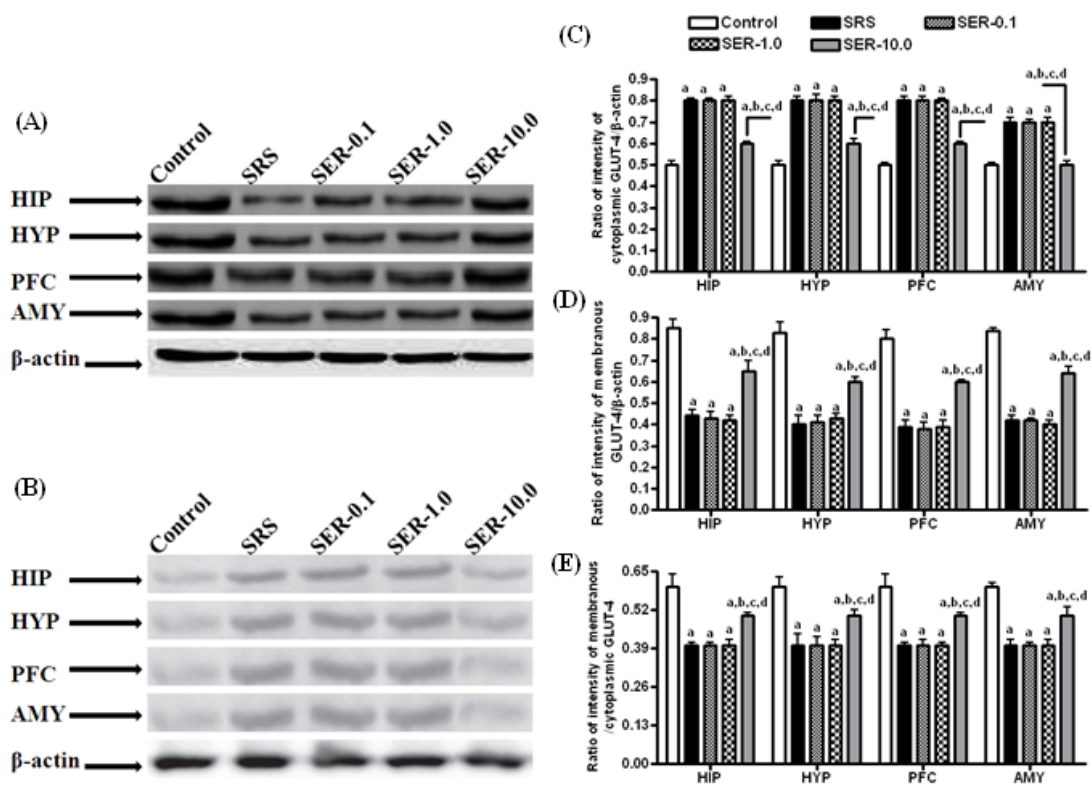
Figure 42: The effect of SER (0.1, 1.0 and 10.0 mg/kg) on the level of expression of cytoplasmic (C) and membranous (D), and ratio of membranous to cytoplasmic (E) GLUT-4 in HIP, HYP, PFC and AMY of SRS rats. The blots are representative of cytoplasmic (A) and membranous (B) GLUT-4 in HIP, HYP, PFC and AMY. The results in the histogram are expressed as the ratio of relative intensity of levels of expression of cytoplasmic or membranous GLUT-4 to β-actin.   All values are mean ± SEM of three separate sets of independent experiments. [a]$p<0.05$ compared to control, [b]$p<0.05$ compared to SRS, [c]$p<0.05$ compared to SER-0.1 and [d]$p<0.05$ compared to SER-0.1 [One-way ANOVA followed by Student Newmann-Keuls Post-hoc test].

Discussion

The present study for the first time demonstrates the condition of IR in PTSD-like rats in the modified SRS paradigm. SER at highest dose level attenuated IR in addition to depression, anxiety and cognitive impairment in PTSD-like animals.SER attenuated mitochondria dysfunction and PI3K/Akt/GLUT-4 signaling in discrete brain regions of PTSD-like rats. Further, SER mitigated SRS-induced aberrant serotonergic activity in these animals. Hence, it can be presumed that SER could be a potential candidate in the management of PTSD-induced IR.

The simultaneous existence of IR and beta-cell dysfunction is considered as the primary events in the pathogenesis of type-2 diabetes (Tahara et al., 2008). In the present study, SRS paradigm caused a significant IR, hyperglycemia, hyperinsulinemia and beta-cell dysfunction in the animals. Similar to our results, others have reported the cardinal attributes of type-2 diabetes in the PTSD-like animals (Cohen et al., 2009). SER at the highest dose level mitigated all these metabolic disturbances in the PTSD-like animals. The effectiveness of SER against altered metabolic parameters in diabetic animals is well established (Erenmemisoglu et al., 1999; Khanam and Pillai, 2006; Mahmood et al., 2010). However, this is the first report of SER as an effective agent against PTSD-induced IR. Further, similar to our earlier reports SRS exposed animals exhibited depressive-like, anxiety-like and cognitive impairment manifestations (Krishnamurthy et al., 2013; Garabadu et al., 2015). SER attenuated the SRS-induced behavioral manifestations in the animals similar to that of earlier studies (Panahi et al., 2011; Wilson et al., 2014; Zhang et al., 2015). These results clearly demonstrate the fact that the SRS exposed rats exhibited IR in addition to psychological manifestations and SER was able to ameliorate these changes in the PTSD-like animals.

In the present study, SRS paradigm caused a significant mitochondrial dysfunction in terms of increase in the mitochondrial function and oxidative stress, and decrease in the mitochondrial integrity, activity of anti-oxidative enzymes and bioenergetics in all the brain regions. Earlier study suggests that there was significant increase in the mitochondrial complex activities in addition to oxidative stress in SRS exposed animals. Further, this paradigm decreased the mitochondrial integrity in all the brain regions (Garabadu et al., 2015). It is interesting to note that the present study for the first time demonstrates the reduction in the mitochondrial bioenergetics in terms of decrease in the level of RCR and ratio of ADP/O in all the brain regions. It has been already reported that SER mitigates the mitochondrial dysfunction and oxidative stress in discrete brain regions of Huntington disorder-like animals (Kumar et al., 2010). Further, SER attenuated the SRS-induced decrease in the phosphorylation of Akt and translocation of GLUT-4 in all the brain regions. Based on these observations, it can be assumed that SER may exhibits anti-PTSD like effect perhaps through mitochondria-linked PI3K/Akt/GLUT-4 signaling mechanism in these animals.

The serotonergic dysfunction is well characterized in the pathophysiology of PTSD and it is well evident from several animal models of PTSD (Krishnamurthy et al., 2013). In the present study, SER at highest dose level attenuated the SRS-induced alteration in the serotonergic activity in HYP of the rats. The ameliorative effect of SER on PTSD-induced change in the serotonergic activity is well documented in other experimental animal models of PTSD (Wilson et al., 2014). We for the first time report that SER significantly attenuated the SRS-induced increase in the density of 5-HT_{2A} receptors in all the brain regions. It has been well reported that the time-dependent stress paradigm, an animal model of PTSD, caused a significant increase in the affinity of 5-HT_{2A} receptors in PFC, suggesting the fact that these receptor activity

encourages the PTSD-like symptoms (Harvey et al., 2003). Another study has reported that 5-HT_{2A} receptor antagonist facilitates the phosphorylation of Akt and thus stimulates the insulin signaling pathway in adipocytes (Li et al., 2013). However, SER indirectly showed the effect through decreasing the density of 5-HT_{2A} receptors in all the brain regions. These results culminate to a presumption that SER may also exert 5-HT_{2A}-mediated effect in addition to mitochondria-linked PI3K/Akt/GLUT-4 signaling in this animal model of PTSD.

In conclusion, SER mitigated SRS-induced IR in PTSD-like animals. Further, SER attenuated the altered mitochondria-linked PI3K/Akt/GLUT-4 signaling in PTSD-like rats. Further, SER also exhibited 5-HT_{2A}-dependent mechanism to mitigate PTSD-like manifestations in the animals. Hence, SER could be a potential candidate in the management of PTSD.