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INVESTIGATING THE ANTIDEPRESSANT EFFECT OF NATURAL BETAINE ANALOGUES IN RATS

Jyoti Bawa[1*], Love Goyal[1], Arun Kaura[2], Honey Goyal[2] and Deepak Kumar[3]

[1*]College of Pharmacy, AIMSR, Adesh University, Bathinda (Punjab) India.
[2]UIPSR, BFUHS, Faridkot (Punjab) India.
[3]Akal College of Pharmacy & Technical Education, Mastuana Sahib, Sangrur (Punjab) India.

Abstract

In the present study, we determined antidepressant potential of two choline derivatives (natural betaine analogues) BTH and TGH when given alone and in low dose combination. The antidepressant activity of BTH and TGH was determined using various well established behaviour animal models. BTH and TGH treatment reduced the immobility time in FST and TST experiments as compared to control and showed high antidepressant activity comparable to standard. Further, in locomotor activity, it was found that locomotor activity was enhanced more than twice as compared to control at BTH+TGH at low doses. After 7 days, drug administration rats were sacrificed and different biochemical parameters like GSH, catalase, MDA, and nitrite level were determined to evaluate the mechanism and biochemical pathways. These drugs significantly affected the levels of MDA, GSH, nitrite and catalase in whole region of rat brain. Further, the determination of concentration of neurotransmitters in brain neurons was carried out using HPLC method. It was found that BTH and TGH significantly enhanced the level of these neurotransmitters in brain as compared to control. Thus, it can be concluded from results that the antidepressant effect of drugs are due to enhanced concentration of monoamine neurotransmitters in synaptic cleft which leads to the elevation of the mood. In all groups, synergistic activity was observed when treated with low dose combination BTH+TGH at 50+30mg/kg. TGH (50mg/kg) also showed the significant activity in comparison to BTH group in some parameters (100mg/kg). Hence, it is concluded from the results that acute ingestion of BTH+TGH at 100+50mg/kg showed potent antidepressant effects. Further, it was also revealed from the study that low dose combination of BTH+TGH at 50+30mg/kg possessed potent synergistic antidepressant activity comparable to standard. Furthermore, the biochemical evaluation of monoamines suggested the vital role of 5-HT, DA and NE in the pathophysiology of depression.

Key words : Antidepressant, betaine, forced swim test, tail suspension test.

Introduction

Antidepressants hold the third rank among the most prescribed therapeutic agents world-wide. There are about two dozen antidepressants which act through nine different pharmacological targets (Stahl, 1998a). However, the synthetic antidepressant force pose serious challenge to human body in the form of adverse effects, eg. Tricyclic antidepressants inhibit sodium channels at high dose lead to cardiac arrhythmia, seizures, anxiety, sleep disturbances and sexual dysfunction (Trindade *et al.,* 1998), Selective serotonin reuptake inhibitors cause anxiety, insomnia and sexual dysfunction (Balon, 2006; Stahl, 1998b). Norepinephrine and Dopamine reuptake inhibitors cause dryness of mouth, constipation and sexual dysfunction. Monoamine oxidase produce weight gain, CNS stimulation, liver damage and convulsions. Lithium salts causes diarrhea, thyroid enlargement, hypothyroidism, tremor and renal effects (Balon, 2006).

Herbal medicines remained as an important part of the medicinal culture from ancient time with minimal side effects on human health. In modern world, 70% of world population is still dependent on herbal medicines for their health care needs. Natural betaine analogues such as Betaine hydrochloride (BTH) and Trigonelline hydrochloride (TGH) have been found to co-exist together and are found together in several plants like *Amaranthus spinosus, Areca catechu and Allivum sativum*.

Betaine Hydrochloride (BTH) has been reported as

Author for correspondence :

to improve fatty liver, hinder the accretion of triglyceride and cholesterol in the plasma and liver, boost the renewal of hepatocytes and avoid hypotension. Trigonelline Hydrochloride (TGH) is another choline family derivative, isolated from *Trigonella foenum-graecum* and is known for its mood enhancing properties and increasing body activity. TGH is known for its various protective physiological effects like memory-improving, antibacterial, antiviral, hypoglycemic, hypolipidemic, neuroprotective, antimigraine, sedative, and anti-tumor activities and antidiabetic. These compounds co-exist together in various plants like *Amaranthus spinosus*, *Allium sativum, and Areca catechu***.**

However, there are number of pharmacological effects and benefits of BTH and TGH, but exactly the mechanism of the effects of these have not been established yet, and the antidepressant activity of these drugs compounds, is still obscure. Thus, we designed this study to evaluate the antidepressant activity of the BTH and TGH in rats to get a better understanding of mechanism of these drugs. The present study deals with the determination of pharmacological investigation of antidepressant potential of two choline derivatives (natural betaines) BTH and TGH when given alone and in combination when given in low doses; oxidative damage: To determine the effect of BTH and TGH on oxidative damage of brain using various biochemical test like determination of concentration of MDA, GSH, nitrite and catalase; elucidation of biochemical pathways by analyzing monoamines 5-HT, DA and NE: To determine the effect of BTH and TGH on concentration of various monoamines like serotonin (5-HT), dopamine (DA) and norepinephrine (NE) using HPLC technique.

Materials and Methods

Animals

In present study, male Wistar rats *(Rattus norvigecus)* weighing 200-300g were used. The animals were obtained from the animal house of the ISF College of Pharmacy, Moga, India under protocol number 266. Animals were housed in group of six, under the standard laboratory conditions with standard rats food and water ad libitum. All animals were adjusted to laboratory conditions before starting the test. All the behavior assessments were carried out between 9.00 and 17.00 h. Rats were housed with a 12-h light/dark cycle of animal house. The experimental protocol was approved by the Institutional Animal Ethical Committee (IAEC) of ISF College of Pharmacy, Moga, India and was carried out in accordance with the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) for the use and care of experimental animals.

Drugs and treatment schedule

Drugs used in the present study includes BTH and TGH (Sigma Chemicals Co. St, Louis MO, USA) administered orally in divided doses. BTH, TGH and its combination (BTH+TGH) was dissolved in 0.5% w/v of sodium carboxymethylcellulose (Na-CMC). IMP was administered one hour prior to BTH treatment from day 7 to 14 *i.e.* for 7 days. All drugs were given in constant volume according to body weight (b.wt). For behavioural assessment locomotor activity was assessed by actophotometer, forced swim test using rectangular glass jar and tail suspension test. On day 14th all animals were sacrificed and brain were removed for biochemical estimation (reduced glutathione, lipid peroxidation. catalase, nitrite, protein estimation).

Experimental design

The experimental protocol was divided into five groups. Group 1 receive 0.5% of Sodium carboxymethylcellulose as control; group 2 receive 10mg/kg, *i.p* imipramine as standard; group 3 receive 100mg/kg, *p.o.* BTH; group 4 receive 50mg/kg, *p.o.* TGH and group 5 receive 50mg/kg, *p.o*. BTH + 30mg/kg/b.wt, *p.o.* TGH.

Behavioral assessment

The antidepressant activity of target drugs was investigated by using well-established animal protocol such as tail suspension test (TST) (Steru *et al.,* 1985) and forced swimming test (FST) (Arya and Preeti, 2012). The locomotor activity was investigated using Actophotometer (Kumar *et al.,* 2007; Kumar *et al.,* 2011).

Measurement of oxidative stress parameters

Proteins level was estimated in brain because they serve as catalysts that maintain metabolic processes in the cell and as signals secreted by one cell or deposited in the extracellular matrix that are recognized by other cells. The protein was measured by the Biuret method using bovine serum albumin (BSA) as a standard.

Estimation of lipid peroxidation

The malondialdehyde (MDA) content is a measure of lipid peroxidation, was assayed in the form of thio-barbituric acid reacting substances (TBARS). The thio-barbituric acid reactive substances (TBARs) assay is the colorimetric method used for the detection of lipid peroxidation in biological materials. MDA reacts with thio-barbituric acid at high temperature (90-100^{0}C) in acidic conditions by using the Wills Method.

0.5ml of post mitochondrial supernatant and 0.5ml of Tris HCl were incubated at 37ºC for 2 hr. After incubation, 1ml of 10% trichloroacetic acid (TCA) was added and centrifuged at 1000×g for 10 min. To 1ml of supernatant, 1ml of 0.67% thio-barbituric acid was added and the tubes were kept in boiling water for 10 min. After cooling 1ml double distilled water was added and absorbance was measured at 532 nm using UV Spectrophotometer (UV-1700 Spectrophotometer, Shimadzu, Japan). TBARS were quantified using an extinction coefficient of $1.56\times105\ M^{-1}cm^{-1}$amount was expressed as n mol/ mg pr (pr: protein) (Wills, 1966).

Estimation of nitrite

Nitric oxide (NO) is a molecule that acts as a neurotransmitter in the central and peripheral nervous systems and, therefore, is critical in the pathogenesis neurodegenerative disorders. NO is an activator of soluble guanylylcyclase, which converts guanosine triphosphate (GTP) to cyclic guanosine monophosphate (cGMP) and plays critical roles in the maintenance of vascular homeostasis. In the cell, NO undergoes a series of reactions with several molecules present in biological fluids and is eventually metabolized to nitrite (NO^{2-}) and nitrite (NO^{3-}). Thus, the best index of total NO production is the sum of both nitrite and nitrite (Stocker *et al.,* 2003). The accumulation of nitrite in the supernatant, an indicator of the production of nitric oxide (NO) was determined by a colorimetric assay with Greiss reagent (0.1% N-(1-naphthyl) ethylene diamine dihydrochloride, 1% sulfanilamide and 2.5% phosphoric acid) as described by Green *et al.,* 1982.

Equal volumes of supernatant and Greiss reagent were mixed, and this mixture was incubated for 10 min at room temperature in the dark. Absorbance at 540 nm was measured with a UV spectrophotometer. The concentration of nitrite in the supernatant was determined from a sodium nitrite standard curve and expressed as expressed as µ mol/l (Green *et al.,* 1982).

Catalase estimation

Hydrogen peroxide (H_2O_2) is a harmful by-product of many normal metabolic processes: to prevent damage to cells and tissues, it must be quickly converted into other, less dangerous substances. To this end, catalase is frequently used by cells to rapidly catalyze the decomposition of hydrogen peroxide into less reactive gaseous oxygen and water molecules. The reaction of catalase in the decomposition of hydrogen peroxide is Catalase activity was assayed by the method of Luck 1971, in which the breakdown of hydrogen peroxide (H_2O_2) is measured at 240 nm (Murphy and Sies, 1990).

The assay mixture consisted of 12.5 mM H_2O_2 in phosphate buffer (50 mM phosphate buffer, pH 7.0) and 0.05 ml of supernatant from the tissue homogenate (10%) and the change in absorbance was recorded at 240 nm. The results were expressed as expressed as µ mol of H_2O_2 decomposed per milligram of protein/min (Bernt *et al.,* 1974; Luck, 1971).

Glutathione estimation

It is an antioxidant, preventing damage to important cellular components caused by reactive oxygen species such as free radicals and peroxides (Pompella *et al.,* 2003). In the process, glutathione is converted to its oxidized form glutathione disulfide, also called L (-)-Glutathione. Once oxidized, glutathione can be reduced back by glutathione reductase, using NADPH as an electron donor. The ratio of reduced glutathione to oxidized glutathione within cells is often used as a measure of cellular toxicity (Clementi *et al.,* 1999).

Reduced glutathione can be estimated using Ellman's reagent which consist of 0.1mM DTNB in 0.1M phosphate buffer (pH 8.0). In 0.1ml of homogenate of sample, 1ml of 4% sulphosalicylic acid (SSA) was added and then incubated at 40ºC for 1hr. After incubation, sample was centrifuged at 1200g for 15min at 40ºC and 0.1ml of supernatant was separated. In the supernatant, 2.7ml of phosphate buffer and Ellman's reagent was added; change in absorbance was recorded at 412 nm. The results were expressed as µ mol of GSH/mg protein (Ellman, 1959).

Calculation of Dopamine (DA), Serotonin (5-HT) and Nor-epinephrine (NE)

HPLC method was used to determine the concentration of biogenic amines, nor epinephrine (NE), dopamine (DA) and serotonin (5-HT) in cortex, striatum and hippocampus of rats brain after treatment with target drugs BTH, TGH in combination and alone along with standard antidepressant imipramine using method described by Reipschlager in 1997.

The estimation of brain catecholamines was done by method described by Patel *et al.,* 2005 with slight modifications. Catecholamine's (DA, 5-HT and NE) levels in rat brain were estimated by HPLC using electro chemical detector. Waters standard system consisting of a high pressure isocratic pump, a 20µl manual sample injector valve, C_{18} reverse phase column and electrochemical detector were used in the study. Mobile phase consisted of sodium citrate buffer (pH 4.5) – Acetonitrile (87:13, v/v). Sodium citrate buffer consisted of 10 mM citric acid, 25 mM NaH_2HPO_4, 25 mM EDTA, and 2 mM of 1-heptane sulfonic acid. Electrochemical

conditions for the experiment were +0.75 V, sensitivity ranges from 5 to 50 nA. Separation was carried out at a flow rate of 0.8 ml/min. Samples (20 µl) were injected manually. On the day of experiment frozen brain samples were thawed and homogenized in homogenizing solution containing 0.2 M per chloric acid. After that samples were centrifuged at 12,000g for 5 min. The supernatant was filtered through 0.22 mm nylon filters before injecting in the HPLC sample injector. Data were recorded and analysed with the help of breeze software. Concentrations of neurotransmitters were calculated from the standard curve generated by using standard in a concentration range of 10–100 ng/ml.

Statistical analysis

Statistical analysis results were expressed as standard error of mean (SEM). The statistical analysis was performed using the Prism software v.6.0 (R) (Graph Pad Inc., U.S.A). The data was considered significant at ($P < 0.05$). Separate Two-Way Analysis of Variance, (ANOVA) followed by Bonferroni Post-hoc test was used for each parameters. Separate One-Way ANOVA followed by Dunnett's test was used for each parameters.

Results and Discussion

Effect of BTH, TGH and its combination on immobility of rats in FST

The effect of natural betaines BTH, TGH and its combination was investigated for two week intervals in a rat FST model for depression as shown in Fig. 1.

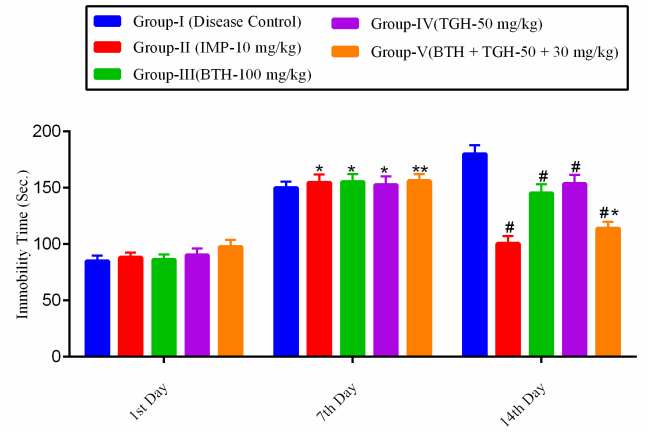

Fig. 1: Effect of BTH, TGH and its combination on immobility time of rats in FST.

Data values are expressed as mean ± S.E.M, n =6 rats/group. *P<0.05 vs. disease control, **P<0.05 vs. day 1st, #P<0.05 vs. respective groups day 7, #*P<0.05 vs. disease control and standard group.

During the initiation of the protocol, immobility time of all the groups (n=6) was noted down. All the groups showed no significance difference in the immobility time as shown in Fig. 1. Further, it was observed that the immobility time of all the groups was significantly increased after the 6th day of the FST protocol. Group-I (Disease Control) showed significantly increase in immobility time with respect to normal control rats ($P<0.05$) after the completion of the 6 days. Groups (II, III and IV) showed also significantly higher immobility time in comparison to normal control rats ($P<0.05$) respectively. At 7th day of the protocol, all the groups were simultaneously treated with standard and test regimens for depression. Group-I was administered with vehicle (0.5%Na-CMC) only. In group II, the standard therapeutic regimen of (IMP-10 mg/kg/b.wt, *i.p.*) was followed. The test groups- III and IV were treated with individually with (BTG at 100 mg/kg and TGH at 50 mg/kg) respectively. Further, a combination therapy (BTG + TGH) at low doses was given in case of group V to study the effect of adjuvant therapy of natural betaines. On the 14th day, the standard drug (IMP) treated group showed significant reduction in immobility time with respect to disease control group ($P<0.05$). Further, in case natural betaines, BTH treated group (group-III) and TGH treated group (group-IV) revealed significant reduction in comparison to their respective groups at the 7th day of the protocol. Furthermore, lower dose combination therapy *i.e.* (BTH + TGH) showed marked decrease in immobility time in comparison to their respective groups on 7th day of the protocol. Hence, it was concluded that combination therapy showed synergistic antidepressant activity at low dose in a despair based model of depression.

Effect of BTH, TGH and its combination on immobility of rats in TST

The effect of natural betaines and its combination was also evaluated in depression induced rats in TST for two weeks as shown in Fig. 2. The treatment and protocol was followed similar to FST. At 1st day, the immobility time of all the animals was observed. It was found that no significance difference was observed among the respective groups. Group-I (disease control) was administered with vehicle (0.5% Na-CMC) only. Group-II was treated with standard antidepressant therapy of IMP at (10mg/kg/b.wt) to compare the antidepressant activity with respect to natural betaine analogues and its combination. It was observed that during 1-6 days, all the groups were induced with acute state of depression using TST.

At 7th day, the immobility time of all the groups were observed. It was found that all the groups showed significant rise in the immobility time ($P<0.05$) with respect to the animals at 1st day. This indicated that all the animals exhibited acute state of depression. Therefore, all the

groups were investigated for the antidepressant activity of natural betaines along with its combination. From 7-14 days, groups III and IV were administered with BTH at dose of (100mg/kg/b.wt),

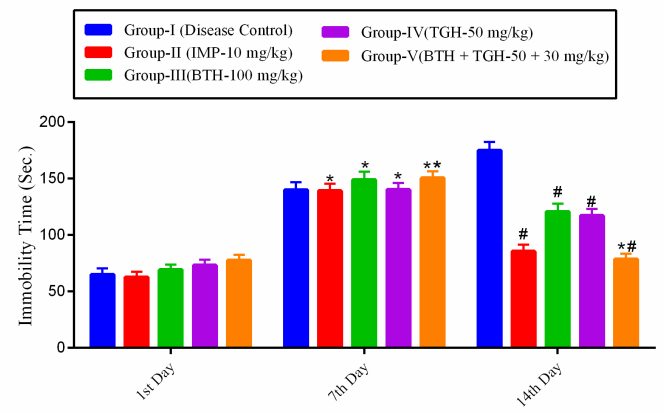

Fig. 2: Effect of BTH, TGH and its combination on immobility time of rats in TST.

Data values are expressed as mean ± S.E.M, n =6 rats/group. *P<0.05 vs. disease control, **P<0.05 vs. day 1st, #P<0.05 vs. respective groups day 7, #*P<0.05 vs. disease control and standard group.

TGH at dose of (50mg/kg/b.wt) and (BTH and TGH) therapy at dose of (50+30mg/kg/b.wt). It was found that group-II showed significant reduction in immobility time with respect to groups at 7th day. Further, the BTH and TGH treated groups also showed significant decrease in immobility time with respect to disease control group (P<0.05). However, individually treated BTH and TGH groups exhibited showed lesser anti-depressant action in comparison to standard treatment of IMP. However, the combination therapy (BTH + TGH) at a dose (50+30 mg/kg/b.wt) exhibited marked reduction in immobility time with respect to group-II (P<0.05) and standard treatment group-III (P<0.05) respectively as shown in Fig. 2. Hence, it was observed that combination therapy of natural betaines possessed potent anti-depressant activity in TST model of depression.

Effect of BTH, TGH and its combination on locomotor activity in rats

The effect of natural betaine analogues and its combination were also evaluated for behavioral assessment in an anxiety based depression model (locomotor activity) for two weeks with the help of actophotometer. The locomotor activity was analyzed in terms of no of crossings in a digital activity cage (actophotometer). At 1st day, all the groups showed increased locomotor activity. Further, anxiety based acute state of depression was induced among all the groups for 1-6 days using actophotometer. At 7th day, it was observed that locomotor activity was severely reduced among all the groups. Groups (II to V) showed significant decrease in the number of crossings (P<0.05) with respect to groups at 1st day. Therefore, the animals were administered with standard therapy (group-II) and test therapies (groups III, IV and V) to study the antidepressant effect of natural betaines and its combination. In the present study, group-II showed significant increase in the locomotor activity with respect to group-I (disease control) after chronic administration of standard drug (imipramine) in rats (P<0.05). BTH treated or TGH treated animals also showed relatively increase in the locomotor activity in comparison to disease control (P<0.05). However, natural betaines BTH or TGH alone were not able to mimic the antidepressant activity of standard therapy (IMP) as shown in Fig. 3.

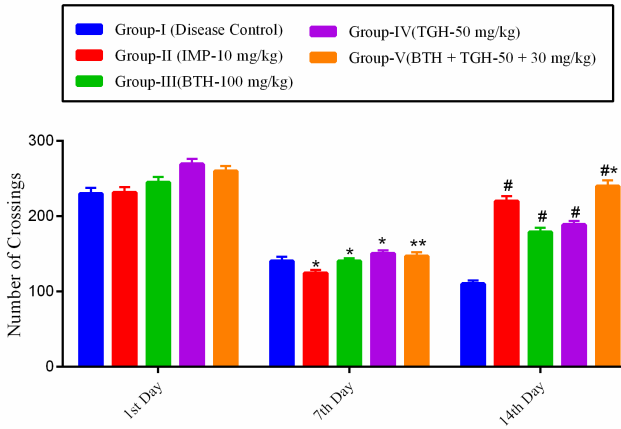

Fig. 3: Effect of BTH, TGH and its combination on locomotor activity in rats measured on actophotometer.

Data values are expressed as mean ± S.E.M, n =6 rats/group. *P <0.05 vs. disease control, **P<0.05 vs. day 1st, #P<0.05 vs. respective groups day 7, #*P<0.05 vs. disease control and standard group.

Further, a combination therapy of natural betaines (BTH + TGH) showed a significant enhancement in the locomotor activity (*i.e.* increased no of crossings) with respect to group-II (disease control) and group III (standard therapy). This study indicated potent synergistic effect of combination therapy of natural betaines (BTH and TGH) at low doses in comparison to standard therapy.

Investigation of oxidative stress markers in depression

It is a well-known fact that enhanced oxidative stress play a vital role in the modulations of neurochemical pathways of the brain. Lipid peroxidation is considered an important mechanism of injury occurring in cells during oxidative stress. An initial formation of large amounts of reactive oxygen species (ROS) during stress may also initiate lipid peroxidation to occur in brain. Emotional stress, which accompanies severe depression, may enhance lipid peroxidation and clinical studies have

directly demonstrated higher levels of MDA (an end product of lipid peroxidation), nitrites and lower levels of glutathione (GSH) and catalase enzyme respectively in patients with depression. In addition, several studies have demonstrated that the restrained stress significantly elevated lipid peroxidation level in rat brain. In order to study the role of oxidative stress in depression, animals were sacrificed on 14th day following drug treatment. Animals were killed by decapitation and brains were removed and were rinsed immediately with ice-cold isotonic saline. Whole brains were separated out. Brain tissue samples were then homogenized with ice-cold 0.1M phosphate buffer (pH 7.4; 10% w/v). The homogenate was centrifuged at 10,000 g for 15 min and aliquots of supernatant were separated and used for biochemical estimation.

Effect of BTH, TGH and its combination on malonyldialdehyde (MDA) enzyme concentration

In the present study, group-I (disease control) showed increase MDA levels in the whole rat brain. Group-II (standard therapy) significantly showed decrease in MDA levels with respect to group I ($P<0.05$). BTH treated (100mg/kg/b.wt) or TGH (50mg/kg/b.wt) treated groups caused a significant reduction in the lipid peroxidation in whole rat brain as evidenced by decreased amount of MDA levels (an oxidative stress marker) in treated rats. Further, TGH at a dose of 50mg/kg/b.wt also showed significant decrease in MDA level in comparison to individual BTH therapy at (100mg/kg/b.wt) ($P<0.05$).

Furthermore, the combination therapy of natural betaines (BTH-50mg/kg/b.wt + TGH- 30mg/kg/b.wt) significantly attenuated the MDA levels in rat's brain as compared to group I ($P<0.05$) and standard treatment ($P<0.05$) respectively as shown in Fig. 4.

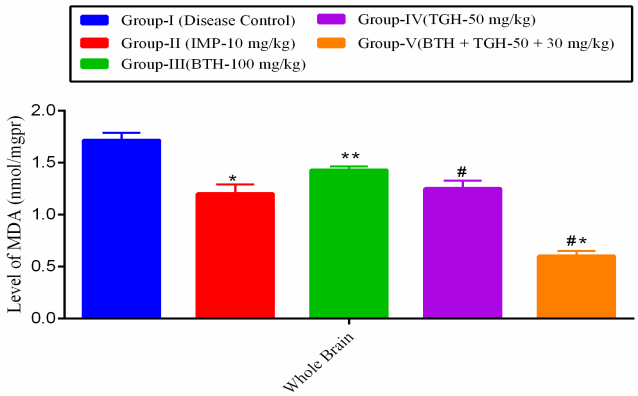

Fig. 4: Effect of BTH, TGH and its combination on concentration of MDA in rat brain.

Data values are expressed as mean ± S.E.M, n =6 rats/group. *P<0.05 vs. disease control, **P<0.05 vs. group-II, #P<0.05 vs. group-III, *#P<0.05 vs. disease control and group II.

Effect of BTH, TGH and its combination in glutathione (GSH) level

The glutathione (GSH) system is an important tool mediating protection several (pro- oxidant) molecules in the brain and alterations in this system are involved in several neuropath logical conditions. Several studies have indicated that the central administration of GSH elicits antidepressant response in the FST and TST models of depression. In this study, GSH levels in rat brain were assessed to evaluate the effect of natural betaine therapy along with its combination. It was observed that oral administration of BTH or TGH in rats significantly enhanced the GSH levels in rat brain as compared to disease control animals (group-I) as shown in Fig. 5.

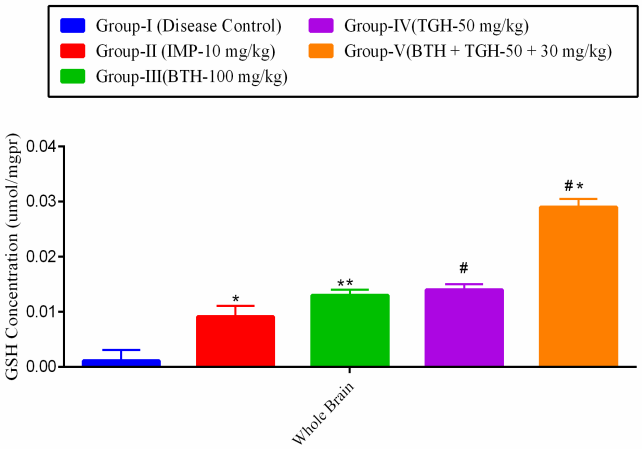

Fig. 5: Effect of BTH, TGH and its combination on concentration of GSH in rat brain.

Data values are expressed as mean ± S.E.M, n =6 rats/group. *P<0.05 vs. disease control, **P<0.05 vs. group-II, #P<0.05 vs. group-III, *# P<0.05 vs. disease control and group II.

However, no significant difference in the GSH content was found among the BTH or TGH treated groups. Further, group-II also showed increased GSH levels in comparison to group-I. Moreover, GSH level of rat brain was most significantly ($P<0.05$) effected in case of group-V (BTH + TGH; at 0.029 µmol GSH/ mg pr.) *i.e.* when treated a low dose in combination as compared to group-I (0.0011 µmol GSH/ mg pr.). These results indicated potent synergistic effect of the combination therapy eliciting an antidepressant response in depressed rats.

Effect of BTH, TGH and its combination on nitrite levels in rat brain

The accumulation on nitrite in the supernatant is an indicator of production nitric oxide (NO), which has produced due to oxidative stress occurring in the brain. The nitrite level in the rat brain were evaluated to study the oxidative stress as shown in Fig. 6.

In the present study, higher levels of nitrite were

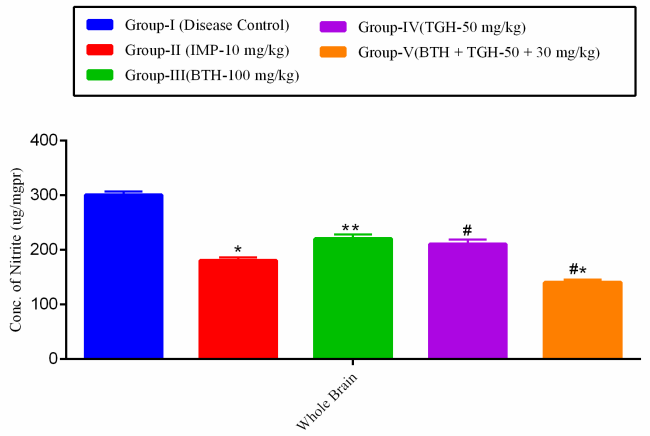

Fig. 6: Effect of BTH, TGH and its combination on concentration of nitrite in rat brain.

Data values are expressed as mean ± S.E.M, n =6 rats/group. *P <0.05 vs. disease control, **P<0.05 vs. group-II, #P<0.05 vs. group-III, *# P<0.05 vs. disease control and group I.

observed in the brain of disease control animals (group-I). However, animal group treated with standard therapy of IMP (10mg/kg/b.wt) showed significant reduction in the nitrite levels with respect to group-I (P<0.05). Further, BTH and TGH treated animals revealed significant decrease in brain nitrite levels with comparison to group-I (P<0.05). There was no significant difference found in the nitrite levels of brain among the BTH or TGH administered therapy groups. But, the decrease in the nitrite levels was comparatively lower than the IMP treated group. Therefore, the adjuvant therapy of natural betaines, BTH or TGH was also assessed. Hence, it was observed that the combined therapy of natural betaines (BTH+TGH) exhibited synergistic effect and showed significant decrease in the rat brain nitrite levels in comparison to group-I (P<0.05) and group-II (P<0.05) respectively.

Effect of BTH, TGH and its combination on catalase levels in rat brain

Levels of catalase enzyme is another prognostic bio marker used to study the oxidative stress. The present investigation showed highly suppressed catalase levels in the rat brain in group-I (disease control). Further, catalase levels in the rat brain was significantly increased in the IMP treated group (P<0.05). Groups-III, IV and V showed significant rise in the catalase levels in comparison to group-I (P<0.05). Furthermore, significant difference was also observed in groups-IV and V in terms of high catalase enzyme levels in the rat brain with comparison to BTH treated group as shown in Fig. 7.

Effect of natural betaines on monoamine concentration in rat brain

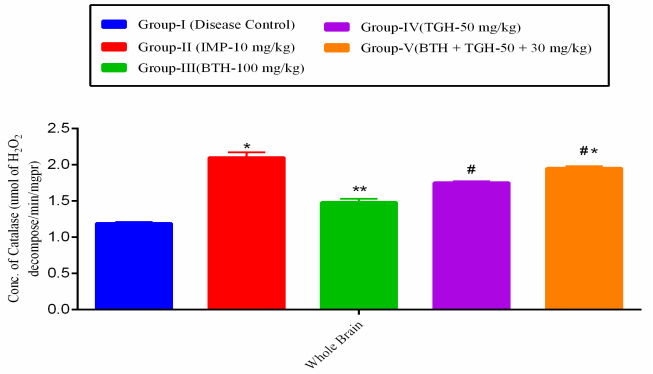

Fig. 7: Effect of BTH, TGH and its combination on concentration of catalase of rat brain.

Data values are expressed as mean ± S.E.M, n =6 rats/group. *P<0.05 vs. disease control, **P<0.05 vs. group-II, #P<0.05 vs. group-III, *# P<0.05 vs. disease control and group II.

The effect of natural betaines and its combination on the monoamine concentration in the rat brain was analyzed by reverse phase HPLC technique equipped with electro chemical detector (ECD). The analytical assessment of biogenic amines (*i.e.* Dopamine, Serotonin, and Norepinephrine) was carried out in the different regions (striatum, cortex and hippocampus) of rat brain.

Effect of BTH, TGH and its combination on dopamine levels (DA) in rat brain

In the present investigation, the concentration dopamine was prominently affected in the striatum, cortex and hippocampal region of brain in group-I (disease control) as shown in Fig. 8. The standard therapy of IMP showed significantly increase in the brain DA levels (P<0.05) with respect to group-I in all the three regions (striatum, cortex and hippocampus) of rat brain. Further, BTH and TGH treated animals showed enhanced dopamine levels, but to a lesser extent in comparison to standard therapy. Furthermore, combination therapy of natural betaines (BTH+TGH) showed marked increase in dopamine levels in comparison to group-1 (P<0.05). Hence, the combination of natural betaines showed a promising antidepressant approach for the management of major depressive disorder (MDD).

Effect of BTH, TGH and its combination on concentration of serotonin (5-HT) in rat brain

The serotonin (5-HT) levels were also assessed in different regions (striatum, cortex, and hippocampus) of the rat brain for all the groups as shown in Fig. 9. It was found that the 5-HT levels were highly suppressed in the rat brain of group-I animals. The IMP treated animals showed significant enhancement in 5-HT levels in all the regions of brain in comparison to disease control rats (P<0.05). Further, BTH treated showed significant

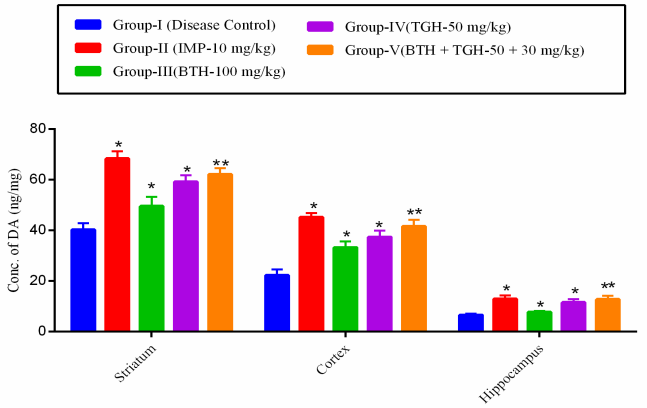

Fig. 8: Effect of BTH, TGH and its combination on concentration of dopamine (DA) of rat brain.

Data values are expressed as mean ± S.E.M, n =6 rats/group. *Significant at P<0.05 vs. disease control, **Highly significant at P<0.05 vs. disease control.

increase in brain 5-HT levels in comparison to group-I, however the therapy showed relatively lesser increase in the serotonin levels with respect to TGH or combination therapy. Furthermore, the combination therapy (BTH+TGH) showed marked rise in the serotonin levels in striatum, cortex and hippocampal regions of the brain with respect to group-I (P<0.05).

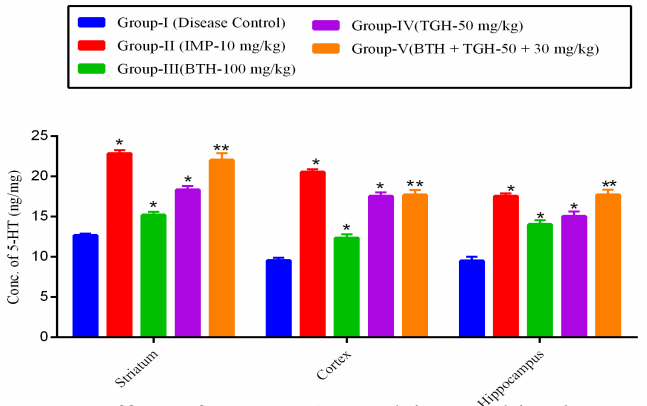

Fig. 9: Effect of BTH, TGH and its combination on concentration of serotonin (5-HT) of rat brain.

Data values are expressed as mean ± S.E.M, n =6 rats/group. *Significant at P<0.05 vs. disease control, **Highly significant at P<0.05 vs. disease control.

Effect of TGH, BTH and its combination on norepinephrine (NE) in rat brain

The norepinephrine (NE) levels in the rat brain were also determined in the striatum, cortex and hippocampal regions of the rat brain as shown in Fig. 10. The NE levels were significantly increased in the IMP administered group with respect to disease control animals (P<0.05). Although, BTH treated animals showed significant increase in NE levels when compared to group-I but, it was observed that individual therapy of BTH was not able to enhance the NE levels in all the regions of brain to the extent as that of TGH therapy. Further, combination therapy of (BTH + TGH) showed highly significant increase in NE levels in all the regions of the brain with comparison to disease control group (P<0.05).

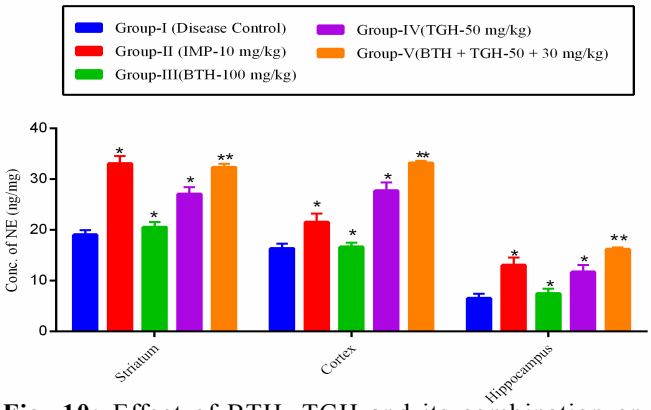

Fig. 10: Effect of BTH, TGH and its combination on concentration of norepinephrine (NE) of rat brain.

Data values are expressed as mean ± S.E.M, n =6 rats/group. *Significant at P<0.05 vs. disease control, **highly significant at P<0.05 vs. disease control.

Conclusion

BTH and TGH treatment reduced the immobility time in FST and TST experiments as compared to disease control group and showed high antidepressant activity comparable to those of standard treatment. Further, in determination of locomotor activity, it was found that when rats were treated with BTH + TGH at low doses, the locomotor activity was enhanced more than twice as compared to disease control group treated with vehicle only. After 7 days, drug administration rats were sacrificed and different biochemical parameters like GSH, catalase, MDA, and nitrite level were determined to evaluate the mechanism and biochemical pathways of these drugs. These drugs significantly affected the levels of MDA, GSH, nitrite and catalase in whole region of rat brain. Further, the determination of concentration of neurotransmitters in brain neurons was carried out using HPLC method. It was found that BTH and TGH significantly enhanced the level of these neurotransmitters in brain as compared to disease control group. Thus, it can be concluded from these studies that the antidepressant like effect of these drugs are due to the enhanced concentration of monoamine neurotransmitters in synaptic cleft which leads to the elevation of the mood. In all groups, synergistic activity was observed in group-V *i.e.* when treated with BTH (50mg/kg) and TGH (30mg/kg) in combination at low doses. Group-IV *i.e.* TGH (50mg/kg) also showed the significant antidepressant activity in comparison to BTH group in some parameters (100mg/kg). Hence, it is concluded from the results that

acute ingestion of natural betaines (BTH at 100mg/kg; TGH at 50mg/kg) showed potent antidepressant like effects. Further, it was also revealed from the study that low dose combination of natural betaines (BTH+TGH at 50+30mg/kg) possessed potent synergistic antidepressant activity comparable to those of IMP and better efficacy than individual treatment at high doses respectively. Furthermore, the biochemical evaluation of monoamines suggested the vital role of 5-HT, DA and NE in the pathophysiology of depression. Hence, the validation of this preclinical assessment is further warranted in clinical studies.

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