Intraperitoneal $GABA_B$ Receptor Antagonist (CGP 55845) Injections Moderately Affect the Behavior of Male Albino Mice

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ABSTRACT

In order to determine the effect of $GABA_B$ receptor antagonist, CGP 55845 {3-N [1-(S)-(3,4-dichlorophenyl)ethyl]amino-2-(S)-hydroxypropyl-P-benzyl-phosphinic acid} on the selective aspects of male albino mice behavior, present study was designed consisting of series of neurological (Rota rod, Open field, Morris Water Maze, Light and dark transition, Elevated plus maze and Novel object) tests. Mice were intraperitoneally injected with 1mg CGP 5584/ Kg body weight/ ml of Dimethyl Sulfoxide or with equal volume of saline solution (0.9 % NaCl) for twelve days. Data analysis for various parameters for rota rod, open field, light and dark transition test, elevated plus maze and novel object test revealed that the neuromuscular coordination, locomotory and exploratory behavior of male albino mice was not significantly affected by the CGP 55845 injections ($P > 0.05$). The results for Morris water maze (MWM) probe trial 1 indicated that CGP 55845 treated animals took less time and visited the platform area more frequently than controls indicating improved spatial memory but this affect were neutralized during probe trial 2 indicating that applied dose of CGP 55845 was unable to induce long term memory formation in male albino mice. We recommend further studies with higher dose concentration of CGP 55845 to explore its effect on albino male mice behavior.

Key Words: CGP 55845, $GABA_B$ Receptor Antagonist, Behavior, Morris Water Maze

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Introduction

Glutamate and gamma-aminobutyric acid (GABA) is among the most abundant neurotransmitters in our central nervous system (CNS). Glutamate play role in excitatory responses while the GABA act as inhibitory stimuli in CNS (Pearl *et al*., 2006). Approximately 30% of neurons in the brain produces GABA and almost every neuron can respond to GABA (Rudolph *et al*., 2001). Action of GABA is mediated by three types of receptors $GABA_A$, $GABA_B$, $GABA_C$. They are categorized as fast-acting iono-tropic ($GABA_A$ and $GABA_C$) and slower-acting metabo-tropic ($GABA_B$) receptors (Bormann, 2000).

$GABA_B$ receptors mediate slow inhibitory transmissions, which appear to be important in memory, mood and pain (Meldrum and Chapman, 1999). $GABA_B$ receptors are metabotropic and produce many long-lasting changes in neurons, generally through activation of the Gαi protein complex, which results in the inhibition of adenylyl cyclase and decrease in cyclic adenosine monophosphate (a second-messenger) production, thus inducing long term potentiation (LTP) and enhancing memory (Morishita *et al*., 1990). In the CNS, $GABA_B$ receptors are expressed in the thalamus, cerebellum, hippocampus, cerebral cortex, dentate gyrus, interpenduncular nucleus and dorsal root ganglia (Bischoff *et al*.,

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1999). Therefore, $GABA_B$ receptors are known to be involved in a variety of brain disorders, mental and emotional states, epilepsy, spasticity nervousness, depression, gastroesophageal reflux disease, catatonia, obsessive compulsive disorder, dependence and pain (Bettler *et al*., 2004; Bowery *et al*., 2002; Chalifoux and Carter, 2011; Couve *et al*., 2000; Richter *et al*., 2012).

Several $GABA_B$ receptor antagonists have been reported to improve cognitive performance in a variety of animal models (Kerr and Ong, 1992). CGP 55845, (2S)-3-[[(1S)-1-(3, 4-dichlorophenyl) ethyl] amino-2-hydro-xypropyl] phosphinic acid, is a potent $GABA_B$ selective antagonist. CGP 55845 was amongst the first reported $GABA_B$ receptor antagonists that were able to cross the blood-brain barrier and remained active after peripheral administration (Frostel *et al*., 1992). The mechanism of action for CGP55845 involves decreasing the GABAergic tone in the structures it projects to, most prominently by preventing the action of GABA at the synapse by occupying the $GABA_B$ receptor on the postsynaptic neuron (LaSarge *et al*., 2007).

$GABA_B$ receptor antagonist experiments on laboratory animals, both rodents and primates, suggested that it enhance learning and memory but these effects might vary under different conditions (Frostel *et al*., 1995). CGP 5584, is known to be responsible for memory improvement and consciousness in rats (Hernandez *et al*., 2006) but limited information is available regarding its affects in albino mice. Aim of the present study was to demonstrate the effect of CGP 55845, a $GABA_B$ receptor antagonist, on neuromuscular coordination, exploratory and locomotory behaviour as well as on learning and memory formation in male albino mice, if any.

Methods

Subjects

Seven-week-old male albino mice were used as experimental subjects. The breeding pairs of mice were obtained from Zoology Department, University of the Punjab, Lahore. They were provided with standard mouse diet and water, housed in small rodent cages filled with wood chips and kept at Bio Park in Bahauddin Zakariya University Multan, Pakistan. Temperature of room was maintained at 22 ± 1°C and the light/dark rhythm was 14:10 hours. Rooms were enlightened with an artificial light that has an intensity of 200 Watt. All the experimental procedures and mouse handling protocols were approved by the ethical committee of Institute of Pure and Applied Biology, Bahauddin Zakariya University, Multan (Pakistan).

Experimental design

On postnatal days 22, male mice were separated from their parents and housed individually and fed on the normal rodent chew. During the seventh week of their life, accordingly to body weight, each mouse received intraperitoneal injection of 1mg CGP 55845 ($GABA_B$ receptor antagonist)/ Kg body weight/ ml of solvent for 12 days, thirty minutes before the start of experiment while the control animals received saline solution (0.9% NaCl) as per their body weight.

Rota rod

The rota rod apparatus comprised of locally manufactured rotating drum with uniform acceleration of 40 rpm to test the neuromuscular coordination and balance and in male albino mice. Each mouse was given three training trails before test. During the test, each had three trails and average time on the rotating drum was considered for analysis following Gillani *et al*. (2014b).

Open field

Exploratory behaviour of male albino mice was observed in an arena by using a computational tracking system Any Maze (Stoeling, USA) for 10 minutes. Parameters including total distance covered, mean speed, time mobile, time immobile, mobile episodes, immobile episodes, maximum speed and rotations, (clockwise and anti clockwise rotations) were considered according to Zahra *et al*. (2014).

Morris water maze (MWM)

The apparatus consists of a circular pool (76cm depth, 122cm diameter) in which mice were trained to swim to locate an unseen platform (1.5 cm beneath surface of water) that could be located merely by identifying the distal extra-maze cues of different colors and dimensions attached to the walls of the pool. Throughout the experiment shades and dimensions of iconic cues were kept constant. Water temperature was sustained at 21 ± 1°C. A computerised tracking/image analyzer system (video camcorder, add link Barcelona) attached to computational tracking system: Any Maze (Stoeling, USA) divided the spherical pool in four equal quarters (compass locations: NE, NW, SW and SE). The platform occupied the middle area in the SE quadrant throughout the experiment.

The training phase comprised of 16 trials: Each day consist of 4 training trials per day for 4 days with an inter-trial interval of 30 mins. Mice were discharged at random in a way that their heads remained towards the wall of the pool from the four compass positions and were given 120 sec to move in water and trace the platform. If after 120 sec mice failed to trace the platform, mice were put on the platform by hand and allowed to stay there for 30 sec. The time to reach the platform, time immobile throughout the test, time mobile and mean speed was considered.

After the acquisition phase, mice had two probe trials, one on 5th day and second on 12th day for analysis of spatial memory and long term potentiation (LTP). The probe trials were carried out in the absence of platform inside the pool. From the start point of NE quadrant, mice were discharged and were allowed to move freely in water for 1 min. The pathway exercised by mice throughout swimming was followed and analyzed for the fraction of distance to reach platform, time mobile, time immobile, total latency, average speed was recorded according to Kipnis *et al*. (2004).

Elevated plus maze

The elevated plus maze apparatus comprised of two closed arms (Length 25 x Width 5 x Height 16 cm) across from each other and vertical to two open arms (Length 25 x Width 5 x Height 0.5 cm) joining a platform (Length5 x Width 5 x Height 0.5 cm) in the centre making a "+" sign. A mouse with its head towards the closed arms was positioned on the platform in the central area of the maze. Mice were given 5 min to move about the maze. Each mouse had one trial. The entrances into the closed and open arms, and the time consumed in both arms, head dipping, urination and defecation were counted during a five-minute test trial with the help of mouse tracking software (Anymaze, USA). The entries in-the open-arm and time that is consumed in the open arms were used to indicate anxiety Kulkarni, (2002).

Light and Dark Transition Test

The light and dark transition test apparatus comprised of plywood box (Length 45 x Width 27 x Height 27 cm) and divided into two chambers that are linked by a small opening (Height 7.5 x Width 7.5 cm) situated at floor altitude in the center of the wall that divided the two chambers. The base of the box was separated into 9 x 9 cm squares and was covered with Plexiglas. The small compartment of the box (Length 18 x Width 27 cm) was colored black and the larger chamber (Length 27 x Width 27 cm) was painted white. Both chambers were covered with lids of clear Plexiglas. Mice were carried into the test room in their home cages and positioned in the middle of the white chamber with their heads towards the opening and were let free to search the chambers for 5 minutes. An observer sitting quietly recorded the behavior of the animals (transition frequency, rearing frequency, stretch attends frequency, time spend in dark portion and time spend in light portion, urination and defecation) in the box (Bourin and Hascoet, 2003; Bouwknecht and Paylor, 2002).

Novel Object Recognition Test

The test was carried out in the open field box. Testing consisted of two trials for each mouse. In the trial 1, two objects (A and B) were positioned in diagonal corners opposed to each other. The mouse was carried from its home cage and released in the middle of the open field area. Each mouse was allowed to search both the object and area for 5 min. The behavior such as line crossing, rearing, stretch attend reflex, approaches to object A and B, time spent with object A and B were recorded. At the end of the trial the animal was taken out from the apparatus and put back to its cage. After a 20 min inter-trial gap the mouse was again positioned in open field for trial 2. The arena this time had the common object (B from trial 1) in the similar position as trial 01 and a novel object (N) instead of object A. The same behavior recorded in trial 1were recorded for 5 min in trial 2 in order to compare the novel object recognition ability of mice (Podhorna and Brown, 2001).

Statistical Analysis

All the data is presented as mean ± standard error (SE) of mean. For analyzing results, Minitab 16 (Minitab, Inc.), statistical package was used. 2 sample t-tests was used for the comparison of various parameters of rota rod, open field, Morris water maze test, light and dark transition test, elevated plus maze and novel object recognition test between CGP 55845 and saline injected male albino mice.

Results

Rota Rod

Results of rota rod test showed that the time consumed on rotating rod by male albino mice injected with CGP 55845 was non-significantly (P = 0.3) lower than mice treated with saline solution

demonstrating that CGP 55845 had no influence on neuro-muscular coordination of male albino mice (Fig. 1).

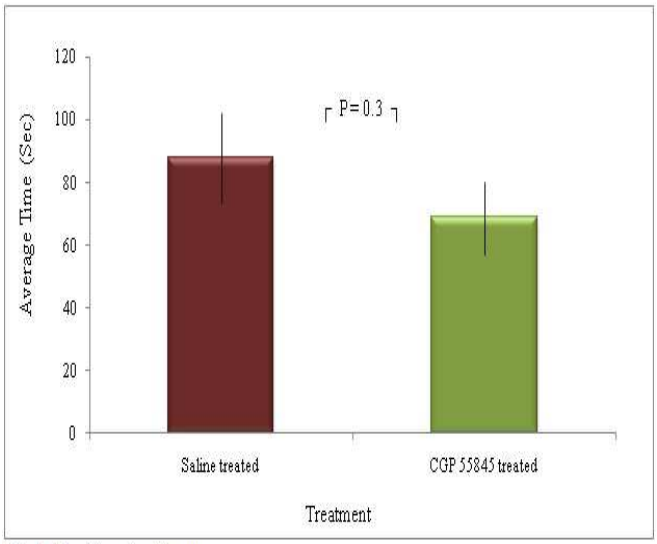


Figure 1. Comparison of rota rod test results between the GABA$_B$ receptor antagonist (CGP 55845) and saline treated male albino mice. *Data is expressed as mean ± standard error. P-value indicates the results of 2 sample t-test*

Open Field

Results of open field test revealed that although GABA$_B$ receptor antagonist injected male albino mice were more mobile and remained immobile for less time but all other studied parameters had higher values for saline treated male albino mice though the difference in test performance did not reached the statistical significance for any of the parameters studied (Table 1) representing that exploratory and locomotory conduct of male albino mice stayed unaltered upon the GABA$_B$ receptor antagonist treatment.

Table 1. Comparison of Open field test parameters between GABA$_B$ receptor antagonist and saline treated mice.

Parameters	Saline Treated (N= 10)	CGP 55845 Treated (N= 10)	P-Value
Total Distance (m)	19.71 ± 1.1	18.0 ± 0.7	0.52
Mean Speed (m/Sec)	0.03 ± 0.001	0.03 ± 0.001	0.51
Time mobile (Sec)	412.8 ± 13.7	437.8 ± 9.5	0.46
Time immobile (Sec)	187.2 ± 13.7	162.2 ± 9.5	0.46
Mobile episodes(Sec)	34.2 ± 2.4	31.9 ± 1.1	0.67
Immobile episodes (Sec)	33.4 ± 2.5	31.1 ± 1.1	0.68
Max speed (m/Sec)	0.3 ± 0.01	0.3 ± 0.01	0.2
Rotations	21.8 ± 0.9	19.3 ± 0.7	0.29
Clockwise rotations	10.9 ± 0.8	9.3 ± 0.6	0.42
Anti clockwise rotation	10.9 ± 0.7	10 ± 0.5	0.6

P > 0.05 = Non significant
N = 10 for both treatments. Values are expressed as mean ± standard error of mean. P-values indicate the results of 2 sample t-test

Dark and Light Box Test

Analysis of the results revealed that all the studied parameters (transition frequency, rearing frequency, stretch attend frequency, time in light, time in dark, urination and defecation) remained unaffected when compared between the CGP 55845 and saline injected animals indicating that CGP 55845 treatment did not affected the exploratory behaviour in mice (Table 2).

Table 2. Comparison of results between CGP 55845 and saline treated mice in Light and Dark test.

Parameters	Saline treated	CGP 55845 Treated	P- Value
Line cross	13.2 ± 0.5	14.0 ± 1.1	0.7
Rearing frequency	1.3 ± 0.2	2.3 ± 0.3	0.13
Stretch attend frequency	13 ± 0.1	1.2 ± 0.4	0.83
Time in dark (Sec)	195.8 ± 5.8	199.5 ± 9.6	0.85
Time in light (Sec)	104.2 ± 5.8	100.5 ± 9.6	0.85
Urination	0.0 ± 0.0	0.2 ± 0.1	#
Defecation	0.8 ± 0.3	0.2 ± 0.1	0.23

P > 0.05 = Non significant; # = value cannot be calculated
N = 10 for both treatments. Values are expressed as mean ± standard error of mean. P-values indicate the results of 2 sample t-test

Novel Object Test

Results for novel object test trial 1 indicated that the time spend with object A (P = 0.03) and object B (P = 0.01) were significantly different when compared between the two treatments with control mice spending more time with both objects than CGP 55845 treated male albino mice indicating better exploratory behaviour. While all other parameters remained unaffected when compared between the two treatments (Table 3A).

Table 3A. Comparison of results between the GABA$_B$ receptor antagonist (CGP 55845) and saline treated male albino mice in the novel object test (Trial 1).

Parameters	Saline treated	CGP 55845 Treated	P- Value
Line cross	17.7 ± 1.4	23.7 ± 1.2	0.09
Stretch attend reflex	1.7 ± 0.2	4.8 ± 1.5	0.27
Approaches object A	6.7 ± 0.5	4.5 ± 0.8	0.22
Approaches object B	10.2 ± 0.8	7.0 ± 0.8	0.12
Time object A (Sec)	28.8 ± 1.9	15.5 ± 2.4	0.03*
Time object B (Sec)	52.3 ± 4.9	21.4 ± 3.7	0.01**

P > 0.05 = Non significant; P < 0.05 = least significant (*); P < 0.01 = Significant (**)
N = 10 for both treatments. Values are expressed as mean ± standard error of mean. P-values indicate the results of 2 sample t-test

Results for novel object test trial 2 indicated that all the parameters varied non significantly when compared between GABA$_B$ receptor antagonist and saline injected mice although there was a trend that control mice visited more frequently the novel object as compared to CGP 55845 injected mice (Table 3B).

Table 3B. Comparison of results between (CGP 55845) and saline treated mice in the novel object test (Trial 2).

Parameters	Saline treated	CGP 55845 Treated	P-Value
Line cross	8.6 ± 1.4	10.3 ± 2.6	0.73
Stretch attend reflex	2.0 ± 0.3	0.8 ± 0.2	0.1
Approaches object novel	3.1 ± 0.5	3.3 ± 0.6	0.9
Approaches object B	6.7 ± 0.7	3.1 ± 1.0	0.75
Time object novel (Sec)	16.9 ± 2.9	16.8 ± 4.5	0.99
Time object B (Sec)	16.3 ± 3.2	10.4 ± 3.9	0.5

P > 0.05 = Non significant
N = 10 for both treatments. Values are expressed as mean ± standard error of mean. P-values indicate the results of 2 sample t-test

Elevated Plus Maze Test

Results for the elevated plus maze test indicated that all the studied parameters remained unaltered upon comparison between CGP 55845 and saline injected mice (Table 4). Overall CGP 55845 treated animals performed better as they covered more distance (P = 0.3), with higher mean speed (P = 0.3), were mobile for long time (P = 0.4), with lower urination and defecation frequency than control mice indicating better exploration of plus maze arena.

Table 4. Comparison of results between (CGP 55845) and saline treated mice in the elevated plus maze test.

Parameters	Saline treated	CGP 55845 Treated	P Value
Distance (m)	8.6 ± 0.9	20.1 ± 9.6	0.31
Mean Speed (m/Sec)	0.02 ± 0.003	0.06 ± 0.03	0.31
Time mobile (Sec)	174.8 ± 11.3	192.5 ± 16.6	0.43
Time immobile (Sec)	125.1 ± 11.3	107.5 ± 16.6	0.43
Mobile episodes	16 ± 1.4	13.8 ± 1.5	0.35
Immobile episode	15.6 ± 1.3	13.3 ± 1.4	0.29
Max speed (m/Sec)	9.0 ± 1.02	8.8 ± 1.6	0.94
Rotations	0.2 ± 0.03	1.02 ± 0.4	0.19
Clockwise rotations	8 ± 1.5	6.8 ± 1.1	0.58
Anti clockwise Rotation	4.1 ± 0.8	4 ± 1.0	0.91
Urination	0.1 ± 0.1	0 ± 0	#
Defecation	1.3 ± 0.8	0.1 ±0.1	0.25
Head dipping	8.1 ± 1.8	9 ± 3.1	0.84

P > 0.05 = Non significant; # = value cannot be calculated
N = 10 for both treatments. Values are expressed as mean ± standard error of mean. P-values indicate the results of 2 sample t-test

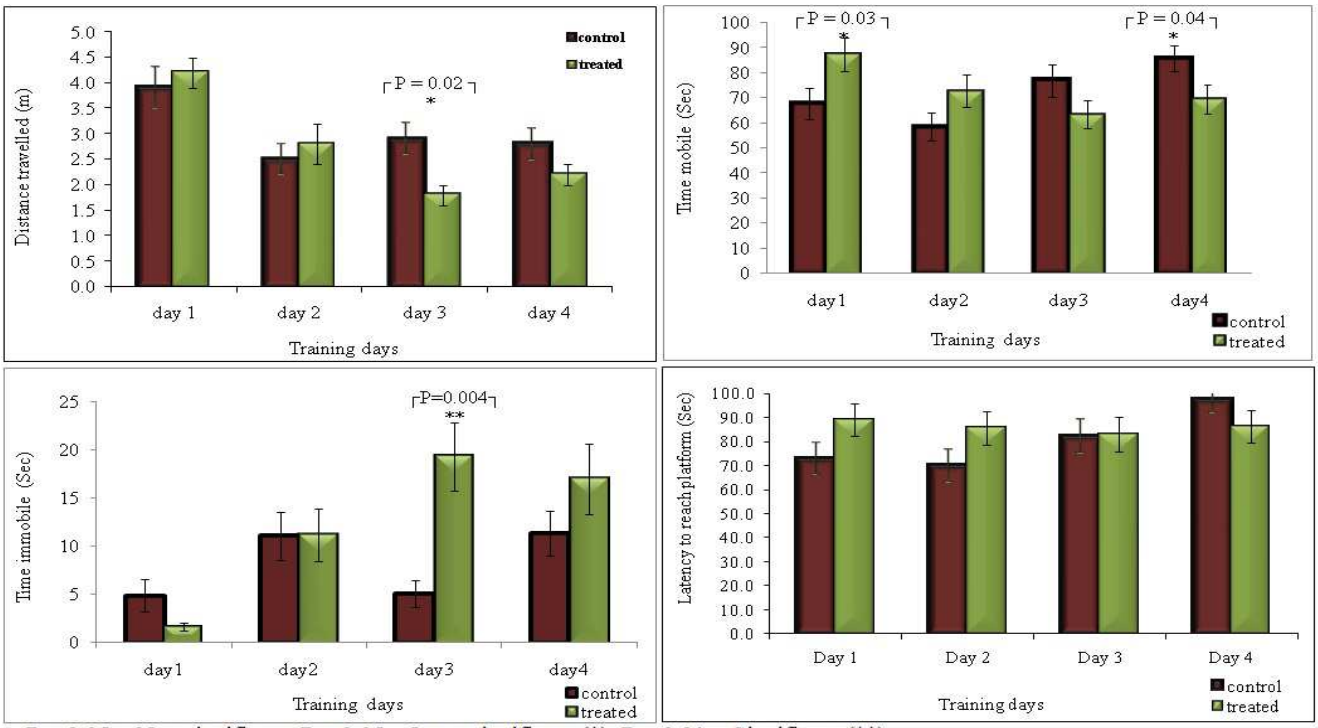
Morris Water Maze

During the acquisition phase the total distance travelled was significantly different between CGP 55845 treated and untreated male albino mice on training day 3 when $GABA_B$ receptor antagonist treated mice travelled significantly lower (P = 0.02) distance as compared to control mice (Supplementary Fig 1A).The parameter time mobile had interesting variations during acquisition phase. This parameter was significantly different on training day 1 (P = 0.03) when CGP 55845 treated mice were more active while the situation was opposite on last training day (P = 0.04) when saline treated animals were more mobile indicating a decreasing trend in neuro muscular coordination of CGP 55845 treated male albino mice (Supplementary Fig 1B). Time immobile had complementary results to the parameter time mobile during the training days. This parameter was only significantly different on training day 3 (P = 0.004) when CGP 55845 treated mice were immobile for longer time then control group. The parameter varied not significantly between the two treatments on remaining training days with an overall trend that mobility of CGP 55845 treated mice was decreasing over time (Supplementary Fig 1C). The parameter latency to reach platform remained unaffected throughout the training session (Supplementary Fig 1D).

The results for the probe trial 1 indicated that the CGP 55845 had lower latency (P = 0.11), covered more distance (P = 0.05) (Fig 2) with lower speed (P = 0.81) and had visited the platform area more frequently (P =0.84) then saline treated male albino mice indicating an overall effect of spatial memory enhancement.

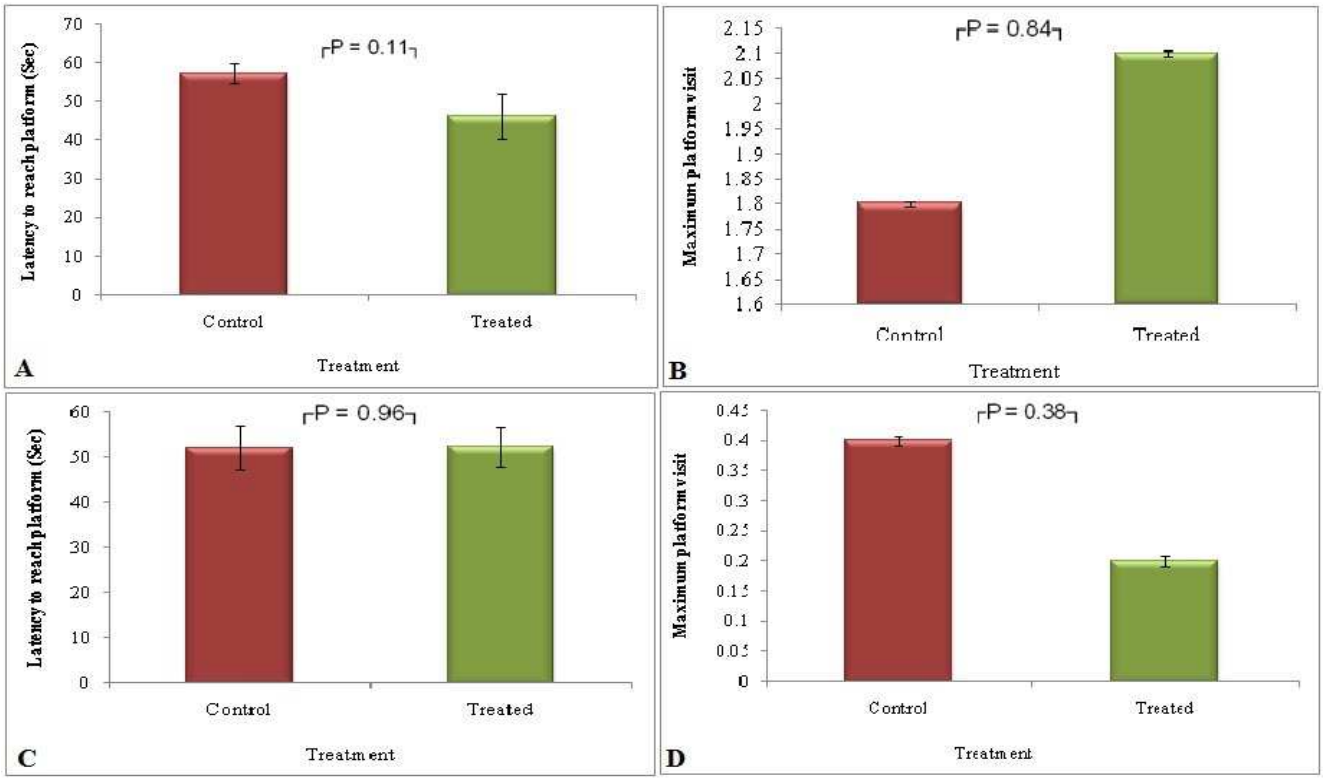
The results of the probe trial 2 indicated that the effect of CGP 55845 for memory enhancement were temporary as the CGP 55845 treated mice covered more distance (P = 0.32), with less speed (P = 0.39) and visited the platform area lesser then saline treated male albino mice (P = 0.38).

Comparison of swim patterns used throughout the trails by saline treated male albino mice indicated that direct approaches to the platform increased while focal incorrect approaches decreased over time indicating memory formation but at the same time increasing trend of wall hugging was also observed in these animals (Supplementary Fig 2A). On the other hand CGP 55845 treated male albino mice had improved focal search and decreased wall hugging strategies but direct approaches were decreasing while incorrect focal search for platform was increasing over time indicating overall poor memory formation in $GABA_B$ receptor antagonist, CGP 55845 treated male albino mice (Supplementary Fig 2B).



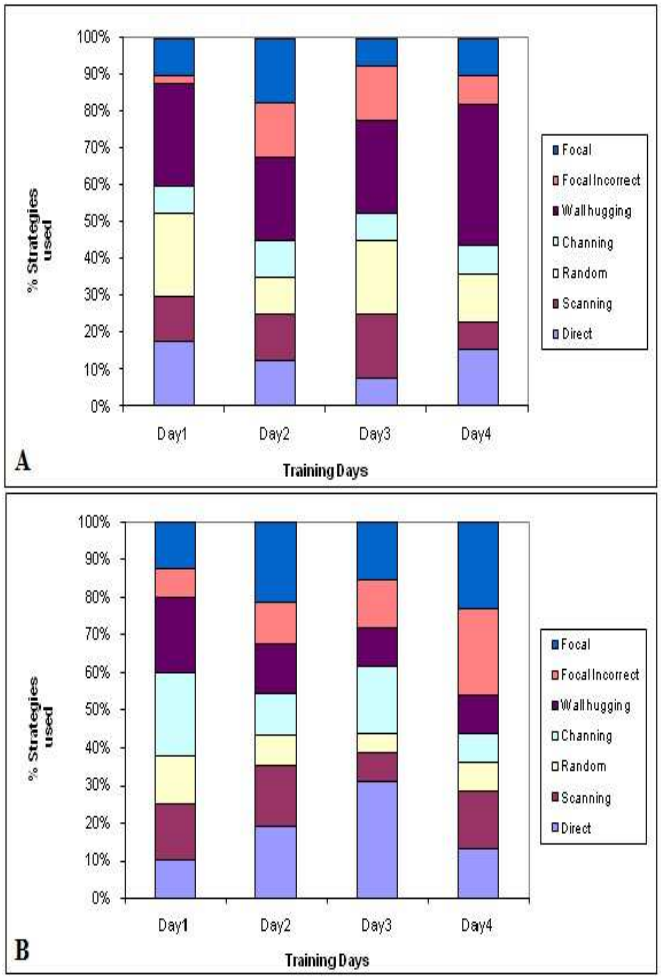
P > 0.05 = Non significant; P < 0.05 = Least significant (*); P < 0.01 = Significant (**).

Supplementary Figure 1. Comparison of results between the GABA$_B$ receptor antagonist (CGP 55845) and saline treated male albino mice for the parameter A. distance travelled to reach platform, B. time mobile, C. time immobile during training session and D. latency to reach platform area during the acquisition phase of Morris Water Maze test.
Data is presented as mean ± standard error of mean. P-value indicates the result of 2 sample t-test



P > 0.05 = Non significant

Figure 2. Comparison of results between intraperitoneally CGP 55845 and saline injected male albino mice for the latency to reach platform area (Sec) (A and C) and number of entries in platform area (B-D) during Morris Water Maze probe trial 1 and 2.
P-value represents the results of 2 sample t-test



Supplementary Figure 2. Comparison of results for swim strategies during training days in Morris Water Maze following intraperitoneal injection of A. saline B. $GABA_B$ receptor antagonist (CGP 55845) for 12 days in male albino mice
Acquisition phase, Female Albino Mouse (CGP 55845 Treated)

Discussion

GABA, the inhibitory neurotransmitter γ-aminobutyric acid, is richly distributed throughout the central nervous system of vertebrates (Hollis and Boyd, 2005). Pre-synaptic activation of $GABA_B$ receptor inhibits neurotransmitter release through decrease in Ca^{+2} conductance, blockade of these receptors enhances hippocampal signaling (Sherif, 1994). Hippocampal $GABA_B$ receptors activation in post-synaptic terminal activation enhances K^+ conductance and activates CREB2, a transcription factor that regulates the memory suppressor gene (Bettler *et al*., 2004; Emson *et al*., 2007).

$GABA_B$ antagonists are known to have antidepressant activity (Cryan and Kaupmann, 2005) and are reported to play role in cognition improvement (Froestl *et al*., 2004) by inhibiting the mechanism that suppresses memory (Helm *et al*., 2005; Kandel *et al*., 2000). It was reported that $GABA_B$ antagonists elevates the levels of the brain-derived neurotrophic factor (BDNF) in the hippocampus that is needed to bring normal cognition (Heese *et al*., 2000; Helm *et al*., 2005). Suppression of $GABA_B$ receptors restores the short and long-term forms of memory (Egan *et al*., 2003). Enhancement of long and short-term memory indicates that $GABA_B$ receptor antagonists may act by enhancing long-term synaptic plasticity (Mombereau *et al*., 2004).

In this study, we have reported the impact of CGP 55845, $GABA_B$ receptor antagonist, on the behaviour of the male albino mice through their performance in different neurological test batteries i.e. Rota rod, Open field, Morris water maze, Elevated plus maze, Light and dark transition test and Novel object test.

Rota rod is widely used to test the balance and neuromuscular coordination (Helm *et al*., 2005). Our results indicated that time spent on rota rod by mice treated with CGP 55845 was lower than mice treated with saline solution (P = 0.3) indicating that CGP 55845 had no influence on neuro-muscular coordination of mice (Fig. 1). This result is in accordance with Olpe *et al*. (1990) and Gillani *et al*. (2014a) who had demonstrated that $GABA_B$ receptor antagonist is not able to induce effects on motor execution throughout the rota rod experiments. Our findings are conflicting to Malcangio *et al*. (1991) who had reported that throughout the rota rod test $GABA_B$ receptor antagonist, CGP 35348, had antagonized the baclofen evoked motor incoordination in rats. The contradiction is probably due to the different $GABA_B$ receptor antagonist used and experimental design of the two studies.

While analyzing the exploratory and locomotory behavior of male albino mice through open field test, our results indicated all the studied parameters did not reached the statistical significance when evaluated among the two treatments indicating that locomotory and exploratory behavior of male mice remained unaltered after CGP 55845 treatment. Our findings are in accord with that of Partyka *et al*. (2007) who had demonstrated that $GABA_B$ receptor antagonist did not influenced the exploratory behavior in mice. Our results are also in accordance with Gillani *et al*. (2014a). Who had reported that there was no effect of $GABA_B$ receptor antagonist, CGP 35348, supplementation on the studied open field parameters in male albino mice.

To examine learning that depends on hippocampus, involving acquisition of spatial memory and learning in mice, the Morris Water

Maze (MWM) was applied. Analysis of result indicated that during the acquisition phase the total distance travelled (P = 0.02) was significantly different between the CGP 55845 and saline treated mice with CGP 55845 treated male albino mice traveled significantly lower on training day 3 as compared to saline treated male albino mice that covered more distance to reach platform (Supplementary Fig 1) indicating the increased spatial learning in CGP 55845 treated male albino mice. Evaluation of result for mobility during training phase between saline and CGP 55485 injected male albino mice revealed considerable difference on first day (P = 0.03) when CGP 55845 treated male albino mice were more active (Supplementary Fig 1) but that mobility decreased with time on other training days indicating a decreasing trend in neuromuscular coordination of CGP 55845 mice. Our results are in accord with Gillani *et al*. (2014a) who demonstrated that saline treated male albino mice remained active for longer time and covered more distance in MWM than the $GABA_B$ receptor antagonist treated male albino mice. Results of time immobile (P = 0.004) indicated that this parameter remained significantly different on training day 3 with CGP 55845 treated male albino mice remained immobile for longer time (Supplementary Fig 1) indicating the overall trend that mobility of CGP55845 treated was decreasing over the period of time. So our results revealed that CGP 55845 had enhanced the learning and memory but not the neuromuscular coordination in male albino mice. These findings are in accordance with that of Forestl *et al*. (2004) and Gillani *et al*. (2014a) who had reported that CGP 35348 is able to improve learning and memory in mice.

Results of the probe trial 1 indicated that CGP 55845 treated male albino mice had lower latency but covered more distance to reach hidden platform with lower speed and had visited the platform area more frequently then saline treated male albino mice indicating an overall positive effect on spatial memory enhancement. Our results are in accord to Gillani *et al*. (2014a) who had demonstrated that CGP 35348, treatment led to the improved learning in male albino mice. Results of probe trial 2 indicated that the effect of CGP 55845 in memory enhancement in male albino mice were temporary as they covered more distance to reach platform with less speed and visited platform area lesser than saline treated male albino mice indicating there is no effect of CGP 55845 treatment on long term memory. Our findings are contradictory to those of Forestl *et al*. (2004); Gillani *et al*. (2014a); Chan *et al*. (2006) and who had reported that CGP 35348 was able to improve learning and memory in test of learning and perception functions and LTP formation in mice. The contradiction in the results can be justified by the fact we are comparing two different $GABA_B$ receptor antagonists having different mechanism of action. In above mentioned studies CGP 35348 was used that is capable of blocking post synaptic $GABA_B$ receptors which results in enhancing the memory retention and LTP formation [10].While we have used CGP 55845 that affects the functioning of presynaptic $GABA_B$ receptors leading to the learning and memory formation but cannot play role in memory retention (Fukuda *et al*., 1993).

Analysis of search strategies used by mice during navigation helped us to explain further behavioral nature of the cognitive status of the mice in MWM test. Comparison of swim strategies used during training days by CGP 55845 treated male albino mice indicated improved focal search and decreased wall hugging strategies but direct approaches were decreased while incorrect focal search for platform increased over time that indicated over all poor memory development in $GABA_B$ receptor antagonist injected mice. Our results are contradictory to the findings of Gillani *et al*. (2014a) who had reported that $GABA_B$ receptor antagonist, CGP 35348, had more pronounced effects on learning and memory of male albino mice (Supplementary Fig 2).

Results for Dark and Light Box test exposed that all the parameters studied, when evaluated among the two treated groups, remained unaffected indicating that $GABA_B$ receptor antagonist, CGP 55845, had no effects on the exploratory behavior in male albino mice (Bischoff *et al*., 1999). Our results are in accord with Mombereau *et al*. (2004) who had demonstrated that the $GABA_B$ receptors was unable to change anxiety-associated conduct in the light-dark box.

Results for novel object recognition test indicated that the CGP 55845 treated male albino mice spent significantly less time with object A (P = 0.03) and object B (P = 0.01) in trial 1 as compared to saline treated male albino mice indicating poor exploratory behavior [Table 3A-B]. Our results are in accord with Kleschevnikov *et al*. (2012) who showed that CGP 55845 had no influence on memory of novel object recognition in mouse model of Down's syndrome.

Elevated plus maze was employed as model for evaluation of learning, memory and exploratory behavior. Results of elevated plus maze indicated that over all CGP 55845 treated mice performed better as they covered more distance, with higher mean speed, remained mobile for longer time, with more rotations and head dips with lower urination and defecation frequency than control mice that indicated better exploration of elevated plus maze but none of these parameters reached the statistical significance (Table 4). Our findings are in accord with the findings of Zarrindast *et al*. (2001) where $GABA_B$ receptor antagonist, CGP 35348, was reported to improve performance of rats in the elevated plus-maze test.

It is concluded that (1 mg/ ml of solvent/ Kg body weight) of CGP 55845, is unable to enhance the neuromuscular co-ordination in mice. It did not affect the locomotory and exploratory behaviour in CGP 55845 injected mice during open field, light and dark transition test, novel object recognition test and in elevated plus maze. CGP 55845 treated mice showed developed spatial memory in Morris water maze in contrast to saline injected controls in probe trial 1but in probe trial 2, for nearly all the parameters studied, the distinction did not reached the statistical significance representing that CGP 55845 was most effective at presynaptic sites, it is not able to enhance the formation of long term memory in male albino mice. The dosage of $GABA_B$ receptor antagonist (CGP 55845) used in current study (1mg/ ml of solvent/ Kg body weight) is possibly too small for male albino mice as only few parameters of a variety of neurological tests were influenced at this concentration. Application of high dose of CGP 55845 is recommended to demonstrate its effects on long term potentiation and behaviour.

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Conflict of interest

Authors declare that they do not have conflict of interest of any sort with anyone.

References

Bettler B, Kaupmann K, Mosbacher J, Gassmann M. Molecular structure and physiological functions of GABAB receptors. Physiological Reviews 2004; 84(3): 835-67.

Bischoff S, Leonhard S, Reymann N, Schuler V, Shigemoto R, Kaupmann K, Bettler B. Spatial distribution of GABABR1 receptor mRNA and binding sites in the rat brain. Journal of Comparative Neurology 1999; 412(1): 1-6.

Bormann J. The 'ABC'of GABA receptors. Trends in Pharmacological Sciences 2000; 21(1): 16-19.

Bourin M, Hascoët M. The mouse light/dark box test. European Journal of Pharmacology 2003; 463(1-3): 55-65.

Bouwknecht JA, Paylor R. Behavioral and physiological mouse assays for anxiety: a survey in nine mouse strains. Behavioural Brain Research 2002; 136(2): 489-501.

Bowery NG, Bettler B, Froestl W, Gallagher JP, Marshall F, Raiteri M, Bonner TI, Enna SJ. International Union of Pharmacology. XXXIII. Mammalian γ-aminobutyric acidB receptors: structure and function. Pharmacological Reviews 2002; 54(2): 247-64.

Chalifoux JR, Carter AG. GABAB receptor modulation of synaptic function. Current Opinion in Neurobiology 2011; 21(2): 339-44.

Chan KF, Burnham WM, Jia Z, Cortez MA, Snead III OC. GABAB receptor antagonism abolishes the learning impairments in rats with chronic atypical absence seizures. European Journal of Pharmacology 2006; 541(1-2): 64-72.

Couve A, Moss SJ, Pangalos MN. GABAB receptors: a new paradigm in G protein signaling. Molecular and Cellular Neuroscience 2000; 16(4): 296-312.

Cryan JF, Kaupmann K. Don't worry 'B'happy!: a role for GABAB receptors in anxiety and depression. Trends in Pharmacological Sciences 2005;26(1):36-43.

Deisz RA, Billard JM, Zieglgänsberger W. Presynaptic and postsynaptic GABAB receptors of neocortical neurons of the rat in vitro: differences in pharmacology and ionic mechanisms. Synapse 1997; 25(1): 62-72.

Egan MF, Kojima M, Callicott JH, Goldberg TE, Kolachana BS, Bertolino A, Zaitsev E, Gold B, Goldman D, Dean M, Lu B. The BDNF val66met polymorphism affects activity-dependent secretion of BDNF and human memory and hippocampal function. Cell 2003; 112(2): 257-69.

Emson PC. GABAB receptors: structure and function. Progress in Brain Research 2007;160: 43-57.

Froestl W, Gallagher M, Jenkins H, Madrid A, Melcher T, Teichman S, Mondadori CG, Pearlman R. SGS742: the first GABAB receptor antagonist in clinical trials. Biochemical Pharmacology 2004;68(8): 1479-87.

Froestl W, Mickel SJ, von Sprecher G, Diel PJ, Hall RG, Maier L, Strub D, Melillo V, Baumann PA. Phosphinic acid analogs of GABA. 2. Selective, orally active GABAB antagonists. Journal of Medicinal Chemistry 1995; 38(17): 3313-31.

Frostel W, Mickel S. Mondadori C, Olpe H, Bittiger H. GABAB receptors in memory process. Nucleic Aids 1992; 15: 30-31.

Fukuda AT, Mody IS, Prince DA. Differential ontogenesis of presynaptic and postsynaptic GABAB inhibition in rat somatosensory cortex. Journal of Neurophysiology 1993; 70(1): 448-52.

Gillani Q, Ali M, Iqbal F. CGP 35348, GABAB receptor antagonist, has a potential to improve neuromuscular coordination and spatial learning in albino mouse following neonatal brain damage. BioMed Research İnternational 2014b; 2014.

Gillani Q, Iqbal S, Arfa F, Khakwani S, Akbar A, Ullah A, Ali M, Iqbal F. Effect of receptor antagonist (CGP35348) on learning and memory in albino mice. The Scientific World Journal 2014; 2014.

Heese K, Otten U, Mathivet P, Raiteri M, Marescaux C, Bernasconi R. GABAB receptor antagonists elevate both

mRNA and protein levels of the neurotrophins nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF) but not neurotrophin-3 (NT-3) in brain and spinal cord of rats. Neuropharmacology 2000; 39(3): 449-62.

Helm KA, Haberman RP, Dean SL, Hoyt EC, Melcher T, Lund PK, Gallagher M. GABAB receptor antagonist SGS742 improves spatial memory and reduces protein binding to the cAMP response element (CRE) in the hippocampus. Neuropharmacology 2005; 48(7): 956-64.

Hernandez CM, Gearhart DA, Parikh V, Hohnadel EJ, Davis LW, Middlemore ML, Warsi SP, Waller JL, Terry AV. Comparison of galantamine and donepezil for effects on nerve growth factor, cholinergic markers, and memory performance in aged rats. Journal of Pharmacology and Experimental Therapeutics 2006; 316(2): 679-94.

Hollis DM, Boyd SK. Distribution of GABA-like immunoreactive cell bodies in the brains of two amphibians, Rana catesbeiana and Xenopus laevis. Brain, Behavior and Evolution 2005; 65(2): 127-42.

Kandel E, Schwartz JH, Jessell TM. Principles of neural science. USA: McGraw-Hill Companies, 2000.

Kerr DI, Ong J. GABA agonists and antagonists. Medicinal Research Reviews 1992; 12(6): 593-636.

Kipnis J, Cohen H, Cardon M, Ziv Y, Schwartz M. T cell deficiency leads to cognitive dysfunction: implications for therapeutic vaccination for schizophrenia and other psychiatric conditions. Proceedings of the National Academy of Sciences 2004; 101(21): 8180-85.

Kleschevnikov AM, Belichenko PV, Mobley WC. Deficits in cognition and synaptic plasticity in a mouse model of Down syndrome ameliorated by GABAB receptor antagonists. Journal of Neuroscience 2012; 32(27): 9217-27.

Kulkarni SK. Animals behavioural models for testing anti-anxiety agents. In hand book of experimental pharmacology, 3rd edn. Delhi: Vallabh Prakashan, 2002: 27-37.

LaSarge CL, Montgomery KS, Tucker C, Slaton GS, Griffith WH, Setlow B, Bizon JL. Deficits across multiple cognitive domains in a subset of aged Fischer 344 rats. Neurobiology of Aging 2007; 28(6): 928-36.

Malcangio M, Ghelardini C, Giotti A, Malmberg-Aiello P, Bartolini A. CGP 35348, a new GABAB antagonist, prevents antinociception and muscle-relaxant effect induced by baclofen. British Journal of Pharmacology 1991; 103(2): 1303-08.

Meldrum BS, Chapman AG. Basic mechanisms of gabitril (tiagabine) and future potential developments. Epilepsia 1999; 40: S2-6.

Mombereau C, Kaupmann K, Froestl W, Sansig G, van der Putten H, Cryan JF. Genetic and pharmacological evidence of a role for GABA B receptors in the modulation of anxiety-and antidepressant-like behavior. Neuropsychopharmacology 2004; 29(6): 1050-62.

Morishita R, Kato K, Asano T. GABAB receptors couple to G proteins G_o, G^*_o and G_{i1} but not to G_{i2}. FEBS Letters 1990; 271(1-2): 231-35.

Nagahara AH, Tuszynski MH. Potential therapeutic uses of BDNF in neurological and psychiatric disorders. Nature Reviews Drug Discovery 2011; 10(3): 209–19.

Olpe HR, Karlsson G, Pozza MF, Brugger F, Steinmann M, Van Riezen H, Fagg G, Hall RG, Froestl W, Bittiger H. CGP 35348: a centrally active blocker of GABAB receptors. European Journal of Pharmacology 1990; 187(1): 27-38.

Partyka A, Klodzinska A, Szewczyk B, Wieroñska JM, Chojnacka-Wójcik E, Librowski T, Filipek B, Nowak G, Pilc A. Effects of GABAB receptor ligands in rodent tests of anxiety-like behavior. Pharmacological Reports 2007; 59(6): 757-62.

Pearl PL, Hartka TR, Cabalza JL, Taylor J, Gibson MK. Inherited disorders of GABA metabolism. Future of Neurology 2006; 1(5): 631-36.

Podhorna J, Brown RE. Strain differences in activity and emotionality do not account for differences in learning and memory performance between C57BL/6 and DBA/2 mice. Genes, Brain and Behavior 2002; 1(2): 96-110.

Richter MA, De Jesus DR, Hoppenbrouwers S, Daigle M, Deluce J, Ravindran LN, Fitzgerald PB, Daskalakis ZJ. Evidence for cortical inhibitory and excitatory dysfunction in obsessive compulsive disorder. Neuropsychopharmacology 2012; 37(5): 1144–51.

Rudolph U, Crestani F, Möhler H. GABAA receptor subtypes: dissecting their pharmacological functions. Trends in Pharmacological Sciences 2001; 22(4): 188-94.

Sherif FM. GABA-transaminase in brain and blood platelets: basic and clinical aspects. Progress in Neuro-Psychopharmacology and Biological Psychiatry 1994; 18(8): 1219-33.

Zahra K, Khan MA, Iqbal F. Oral supplementation of Ocimum basilicum has the potential to improves the locomotory, exploratory, anxiolytic behavior and learning in adult male albino mice. Neurological Sciences 2015; 36(1): 73-78.

Zarrindast MR, Rostami P, Sadeghi-Hariri M. GABAA but not GABAB receptor stimulation induces antianxiety profile in rats. Pharmacology Biochemistry and Behavior 2001; 69(1-2): 9-15.