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$GABA_B$ Receptor Antagonist (CGP 55845) can be Intraperitoneally Supplemented as it does not Affect the Hematological and Serum Biochemical Profile in Adult Female Albino Mice

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Abstract.- $GABA_B$ receptor antagonists are known as memory enhancers in mammalian central nervous system. Present study was designed to demonstrate the effect of intraperitoneal injection 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 hematological and serum biochemical profile of adult female albino mice. Female albino mice (13 week old) were either injected with 1mg/Kg body weight/ml solvent of CGP 55845 (n=11) or saline solution (n=11) for 12 days. Blood was collected directly from cardiac puncture and various hematological (blood glucose, mean corpuscular volume, packed cell volume, total red and white blood cell count) and serum biochemical parameters (cholesterol, aspartate transaminase, alanine transaminase, high density lipoprotein, low density lipoprotein, total protein and triglycerides) were determined for both treatments. Our results indicated that total white blood cells ($p = 0.1$) had lower while total red blood cells ($p = 0.67$), packed cell volume ($p = 0.36$), glucose ($p = 0.43$) and MCV ($p = 0.88$) had higher values in CGP 55845 treated animals than in controls but the difference did not reached the statistical significance. Data analysis for studied serum biochemical parameters indicated that total protein ($p = 0.17$), triglycerides ($p = 0.76$), cholesterol ($p = 0.19$) and ALAT ($p = 076$) had higher values in CGP 55845 treated female albino mice than control group while ASAT ($p = 0.78$) and LDL ($p = 0.31$) were higher in control as compared to CGP 55845 treated mice but again none of the value varied significantly when compared between two treatments indicating that it is safe to inject CGP 55845 intraperitoneally to treat the neurological ailments as it do not affect the blood chemistry.

Key words. $GABA_B$ receptor antagonist, albino mice, hematology, serum biochemistry, CGP 55845.

INTRODUCTION

In the central nervous system of vertebrates, the gamma amino butyric acid (GABA) is the most widely distributed inhibitory neurotransmitter (Khadim *et al*., 2013), and it activates three structurally different classes of GABA receptors, $GABA_A$, $GABA_B$ and $GABA_C$ (Gillani *et al*., 2014a). $GABA_B$ receptor is a G-protein coupled receptor. It is a heterodimer, made up of two subnits $GABA_B$ 1 and $GABA_B$ 2, which are necessary for proper functioning of the $GABA_B$ receptor (Calver *et al.*, 2002). $GABA_B$ receptors are found on the presynaptic, postsynaptic and extra synaptic membranes. $GABA_B$ receptors are the drug specific for the treatment of anxiety, pain, epilepsy, drug drug addiction and depression (Bowery, 2006; Cryan and Kaupmann, 2005). $GABA_B$ receptor antagonist experiments on laboratory animals, both rodents and primates, suggested that it enhances learning and memory but these effects might vary under different conditions (Gillani *et al*., 2015). CGP 35348 and CGP 55845 are among the most extensively studied $GABA_B$ receptor antagonists (Gillani *et al*., 2014a). CGP 55845 was considered to be more active at presynaptic sites in the hippocampus although their receptors are present on both pre and post synaptic membranes (Gillani *et al*., 2014b).

The present study was designed to determine whether CGP 55845 can be intraperitoneally (i.p.) injected in adult female albino mice without affecting the complete blood count and selected parameters of the serum biochemical profile in order to use this drug delivery route for the treatment of neurological disorders affecting the learning and memory formation process in mammals.

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MATERIALS AND METHODS

Subjects

Adult female albino mice (13 week old) were used as subject during the present study. Breeding pairs were purchased from Veterinary Research Institute, Ghazi Road, Lahore. Mice were maintained in cages filled with wood chips at the Core Animal Facility, at Bio Park of Bahauddin Zakariya University, Multan, Pakistan. During the experiments, albino mice were housed in individual cages, standard mouse diet and water was available *at libitum*. 22±1°C room temperature was maintained, room was lighted at an intensity of about 200 x 1 Watt from 8 a.m. to 7 p.m. All the experimental protocol was approved by the ethical committee of Institute of Pure and Applied Biology at Bahauddin Zakariya University, Multan.

Experimental design

Female albino mice were maintained on normal rodent diet until they were 13 weeks old. The treated group (n=11) received i.p. injection of 1mg/kg body weight/ml solvent $GABA_B$ receptor antagonist (CGP 55845) for 12 days. Control group (n=11) received the same concentration of saline solution as treated group for 12 days.

Blood and serum collection

After receiving the i.p. injections for 12 days, blood was sampled through either cardiac puncture or from retro-orbital sinus under Isoflurane (3%) inhalation. For the analysis of serum biochemical and haematological profiling of female albino mice, blood was separated into two parts respectively.

Hematological and serum biochemical profiling

Hematological parameters *viz*., blood glucose level, mean corpuscular volume (MCV), packed cell volume (PVC), total red (TRBC) and white blood cell (TWBC) count and serum biochemical parameters such as cholesterol, aspartate transaminase (ASAT), alanine transaminase (ALAT), high density lipoprotein (HDL), low density lipoprotein (LDL), total protein and triglycerides were determined in blood samples by using Hitachi 902 automatic analyzer (Japan) following Khadim *et al*. (2013, 2014).

Statistical analysis

All the data are presented as Mean ± SD. Statistical package Minitab (version 16, USA) was used for the analysis of results. Two sample t- tests was applied to compare various parameters of haematology and serum biochemical profile of female albino mouse between CGP55845 treated and untreated controls.

RESULTS

Analysis of the results revealed that TWBC ($P = 0.1$), TRBC ($P = 0.67$), PCV ($P = 0.36$), glucose ($P = 0.43$), and MCV ($P = 0.88$) did not vary significantly when compared with CGP 55845 treated and untreated female albino mice (Table I). All studied parameters had higher values in CGP 55845 treated female albino mice except TWBC indicating no infection in the subjects following CGP 55845 treatment (Table I). When various parameters of serum biochemical profile of adult female albino mice were compared between CGP 55845 treated and untreated control groups, it was observed that total protein ($P = 0.17$), triglycerides ($P = 0.76$), cholesterol ($P = 0.19$) and ALAT ($P = 0.76$) had higher values in CGP 55845 treated female albino mice, while ASAT ($P = 0.78$), LDL ($P = 0.31$) had higher serum concentrations in control mice when compared with mice treated with $GABA_B$ receptor antagonist (Table I) but none of the studied parameters reached the statistical significance (Table I).

DISCUSSION

Blood is a connective tissue which carries oxygen, hormones and other useful substances to the body tissues and remove waste substances and carbon dioxide from the body, help in the regulation of body temperature and involved in the protection of body from germs (Robert *et al.,* 2006; Estridge and Reynolds, 2011). Hence blood analysis is an indicator for the diagnosis of diseases (Anderson, 2003; Aleman *et al.,* 2000). This study was conducted to determine the effect of $GABA_B$ receptor antagonist 55845 on the hematology and serum biochemical parameters of adult female albino mice.

Table I.- Comparison of various haematological and biochemical parameters between female albino mice treated with saline solution (control) and CGP 55845, GABA $_B$ receptor antagonist. Data is presented as Mean ± Standard deviation. P-value indicates the results of 2 sample t-test calculated for each studied parameter.

Parameters	Saline treated female albino mice (n = 11)		CGP55845 treated female albino mice (n = 11)		P-value
	Mean ± SE	Range	Mean ± SE	Range	
Haematological					
TWBC count(µlit)	472.2 ± 132.1	145 – 1487	236 ± 22.3	127 – 393	0.11
TRBC count (µlit)	169.8 ± 18.3	101 -280	182.4 ± 23	93– 347	0.67
PCV (%)	44.4 ± 4.1	20 – 67	50.1 ± 4.6	33– 83	0.37
Glucose (mg/dl)	191.5 ± 14.9	120–180	211.2 ±19.5	127 -318	0.43
MCV (fp)	3.1 ± 0.5	0.7–6.5	3.2 ± 0.5	1.4–6	0.87
Biochemical					
Total protein (g/dl)	2.5 ± 0.2	1.3 - 1.9	4.4 ± 1.2	0.1 – 10.5	0.17
Triglycerides (mg/dl)	443.7 ± 47.3	179 – 551	485.4 ± 109.2	137 -1106	0.76
Cholesterol (mg/dl)	127.7 ± 35.9	103 - 315	130.7 ± 17.5	38 - 170	0.19
HDL (mg/dl)	21.5 ± 3.5	18 – 25	21.7 ± 2.2	19 - 26	0.97
LDL (mg/dl)	95.0 ± 33.8	24.8 – 204.8	26.5 ± 1.5	25 – 28	0.31
ASAT (µ/l)	116.5 ± 113.8	3 - 230	69.5± 67.7	2 - 137	0.78
ALAT (µ/l)	78.3 ± 34.7	15- 134	92.3 ± 26.7	14 - 130	0.76

TWBC, total white blood cell count; TRBC, total red blood cell count; PCV, packed cell volume; MCV, mean corpuscular volume.
P > 0.05 = Non significant

CGP 55845 is among the most widely studied commercially available GABA$_B$ receptor (Hopkins and Groom, 2002). Blocking of GABA$_B$ receptors with CGP 55845 results in the decrease of GABA release and is involved in the activation of silent synapses, while opposite effect was shown with the baclofen (Safiulina and Cherubini, 2009). Slattery *et al.* (2005) have reported enhanced learning and swimming time in rats following GABA$_B$ receptor antagonist, CGP 55845 treatment in modified forced swim test. Wang *et al*. (2010) has documented that changes in the degree of sympathetic neuromodulation by CGP 55845 can affect the homeostatic parameters such as blood pressure, heart rate and gut motility.

In our results, it was observed that TWBC count was decreased in female albino mice treated with CGP 55845 (P = 0.1) (Table I) when compared with saline treated group (control). Khadim *et al*. in 2013 also reported the decreased level of WBC in adult female albino mice treated with CGP 55845 following hypoxia ischemia encephalopathy when they had injected i.p. CGP 55845 to female albino mice indicating that CGP 55845 injection in the animal body did not result into any sort of infection.

In the present study the concentration of glucose was higher in the CGP 55845 treated mice when compared with the saline treated (control) female albino mice (P = 0.43) (Table I). Nourian *et al.* (1996) reported that blood glucose level is influenced by GABA through the regulation of pancreatic secretion and inhibit the secretion of glucagon and its antagonist CGP 35348 reverse the inhibition caused by GABA and thus results in the increased level of blood glucose. Khadim *et al.* (2013) determined significantly lower level of glucose in blood of both male and female when compared between CGP 55845 and saline treated (control) albino mice following neonatal asphyxia. The contradictory results are due to the different experimental designs as Khadim *et al.* (2013) had determined the blood chemistry in albino mice following the application of hypoxic ischemic insult applied which was missing in our experiments.

Data analysis indicated that PCV and RBC levels were higher in female mice with CGP 55845 treatment when compared with saline treated (control) female mice. while Khadim *et al.* (2103) had reported that PCV (%) value reduced significantly when compared between CGP 55845 and saline treated male and female albino mice (P = 0.008). The difference is again due to the different

experimental design.

It has been established that triglyceride concentrations in plasma has inverse correlation with the HDL cholesterol level. High level of triglyceride is associated with low level of HDL in plasma (Phillips *et al.*, 1981). We have observed higher triglyceride concentration in $GABA_B$ receptor antagonist subjects when comparison was made between CGP 55845 treated and saline treated control female albino mice. Khadim *et al.* (2013) also observed significantly high level of triglyceride in plasma in both male and female CGP 55845 and saline treated albino mice indicating that this parameter was not affected upon intraperitoneal CGP 55845 injection.

CONCLUSIONS

In conclusion, we have determined that intraperitoneal injection of 1mg/kg body weight/ml of solvent of CGP 55845 did not affect the blood chemistry of female albino mice and as it is known to be used as memory enhancer in mammals so intraperitoneal injection is a safe route for CGP 55845 delivery in mammalian system.

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