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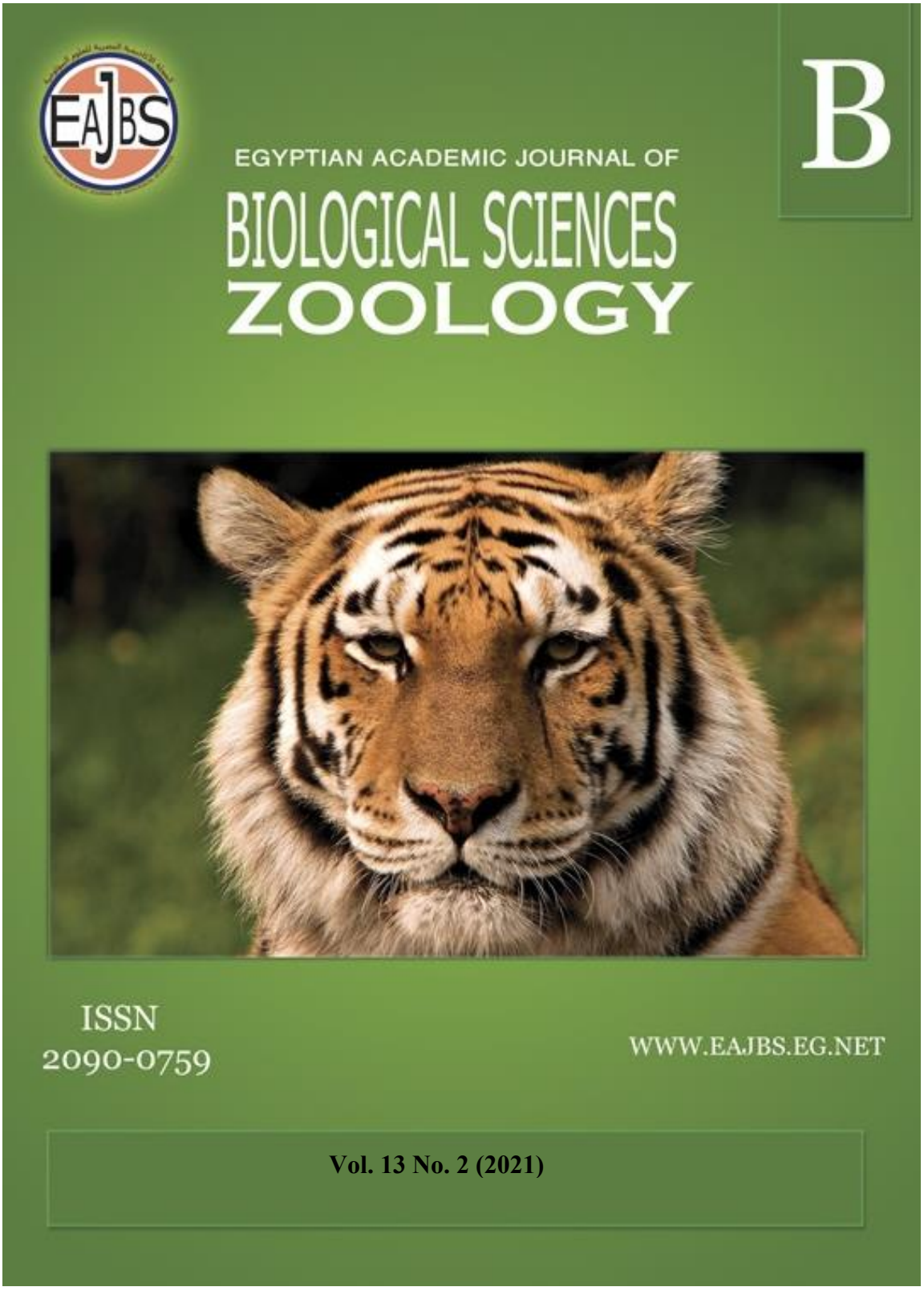

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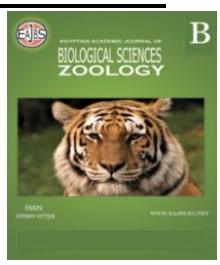

Modulatory Effect of *Camellia Sinensis* Extract on the Function of Hypothalamic-Pituitary-Testicular Axis in PTZ-Induced Epileptic Seizures in Rat Model

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ABSTRACT

Exposure to pentylenetetrazole (PTZ) is capable of inducing experimental epilepsy in rats. This study aimed to investigate the role of *Camellia sinensis* extract in ameliorating the toxicity induced by epilepsy. The treatment with the extract started orally in epileptic rats after intraperitoneal injection with PTZ for 30 days (300mg/kg). Induction of epilepsy resulted in a significant decrease in glutamate, aspartate, serine, 5-HT, DA, NE and a significant increase in GABA, glycine and taurine in the hypothalamus. In addition, it causes an increase in serum LH and FSH and a significant decrease in serum testosterone in addition to disturbance in oxidative and antioxidant system. Treatment with *Camellia sinensis* extract showed significant improvement in most of the studied parameters at $P<0.05$. In conclusion, it could be stated that *Camellia sinensis* extract exerts an ameliorative effect on the function of the hypothalamic-pituitary-testicular axis in the rat model of epilepsy.

INTRODUCTION

Epilepsy is a neurodevelopmental disorder that is hypothesized to arise due to exposure to a broad range of toxicants or alternation in a constellation of genetic factors. These etiological factors can disrupt neuronal development leading to the manifestation of epileptic symptoms. Epilepsy is growing at an alarming pace with 50 million people being affected globally. Abnormalities in several brain regions and pathways could be responsible for this disorder (Devinsky *et al.,* 2018). Epileptic seizures can be produced by the disturbance in neuronal excitation and inhibition with amino acid neurotransmitters (Meldrum *et al.,* 1999; Morimoto *et al.,* 2004). Several works have focused on changes in the function of excitatory and inhibitory neurotransmitters and morphological adaptive processes, including changes in the synaptic efficacy and growth of new inter-neuronal connections (Freichel *et al.,* 2006; Park *et al.,* 2006). Treatment of epilepsy was improved by several third-generation of antiepileptic drugs during the past decades. Nevertheless, opposition to antiepileptic drugs as well as the inability to resist in 20-30% of the patients led to developing new drugs or strategies for epilepsy treatment (Longhi *et al.,* 2005; Azari *et al.,* 2010). Valproate is one of the most widely accepted antiepileptic drugs which is also utilized for managing bipolar disorder (Natasha *et al.,* 2008). Many reports suggest that the usual anti-epileptic drugs can

produce behavioural abnormalities, difficulties in learning and multiple birth defects (Štefánik *et al.*, 2015).

Camellia sinensis (Green tea) is rich in polyphenols and L-theanine contained in the leaves and stems of the tea plant. Green tea polyphenols are the secondary metabolites in tea plants and account for 30% to 36% weight of the water-extractable materials in tea leaves. The main polyphenolic components in green tea are epigallocatechin gallate (EGCG), epicatechin (EC), epigallocatechin (EGC) and epicatechin gallate (ECG) (Weinreb *et al.*, 2004). Polyphenols are the most active component of green tea catechins, acts as an antioxidant in the biological system (Choi *et al.*, 2001) and are rapidly absorbed and distributed mainly into the mucous membranes of the small intestine and the liver; more interestingly, they can cross the blood-brain barrier. The polyphenols in green tea can neutralize free radicals and may reduce or even help to prevent some of the damage caused by reactive oxygen species (ROS). Long-term intake of green tea catechins may be important because cells are constantly exposed to oxidative stress. It has been reported that, in addition to directly quenching reactive oxygen species, tea polyphenols have the ability to participate in vitamin E recycling (Zhang *et al*., 2021).

The present study aims to investigate the possible modulatory effects of *Camellia sinensis* extracts on the function of the Hypothalamic-Pituitary-Testicular axis in the PTZ-induced epileptogenesis rat model.

MATERIALS AND METHODS

Chemicals:

Pentylenetetrazole (PTZ, Sigma Aldrish Co. PVT Ltd, USA) was injected intraperitoneal at a dose (60mg/kg) according to Arafa *et al.* (2013).

Plant Extract:

Green tea was obtained from the Egyptian Herbal Market, Cairo, Egypt. The watery extract of the plants was performed according to the method described by Dulloo *et al.*, (1999). The animals received daily oral administration at a dose of (300mg/kg) according to Banji *et al.* (2011).

Animals and Experimental Design:

Forty adult male rats were obtained from the Holding Company for Biological Products and Vaccines (VACSERA, Cairo, Egypt). The rats were housed in polypropylene cages and maintained at room temperature (22 ± 3 ℃) on a 12-h light/12-h dark cycle throughout the experiment. The rats were provided with water and a balanced diet ad libitum. They allowed adapting to the housing conditions for one week before starting the experiment. All protocols and animal handling approved by the Committee on Research Ethics for Laboratory Animal Care at the Department of Zoology, Faculty of Science, Helwan University, and were in accordance with the National Institutes of Health (NIH) Guidelines for the Care and Use of Laboratory Animals, 8th edition (NIH Publication No. 85-23 revised 1985).

The animals were classified randomly into four groups (n= 10) as follows: Control group (Ctrl), the animals were i.p injected with a single dose of normal saline, epileptic group (pentylenetetrazole; PTZ): The animals of this group were i.p injected with a single dose of PTZ (60 mg/kg b.wt.). The third group green tea extract group (GTE): the normal rats received daily oral administration of GTE for 30 days, and finally, PTZ+GTE group: The animals of this group were i.p injected with a single dose of PTZ (60 mg/kg), then after 48 hours they received daily oral administration of GTE (300mg/kg) for 30 days.

At the end of the experimental period, rats were weighed, suddenly decapitated. Brains were rapidly excised from skulls, blotted and chilled. The brain tissue was rapidly wiped dry with filter paper. Dissection was performed on an ice-cooled glass plate into the hypothalamus from the first half for assay of monoamines and free amino acids and the second half for assay of GSH and MDA and stored in (-80 °C). Also, the blood was immediately collected, incubated at 4 °C for 30 min and centrifuged at 3000 x g for 10 min. The obtained serum was stored at −80 °C for further biochemical assays.

Assay of Serotonin, Dopamine and Norepinephrine:

The first step in the determination of the brain (hypothalamus) monoamines by HPLC method involved weighing and homogenization of the tissue in 1/10 weight/volume of 75% aqueous HPLC grade methanol. The homogenate was centrifuged at 3000 r.p.m. for 10 min and the supernatant was used for monoamine determination immediately extracted from the trace elements and lipids by the use of solid-phase extraction CHROMABOND column NH2 phase Cat. No. 730031. The sample was then injected directly into an AQUA column 150 54.6 mm 5 µC18, purchased from Phenomenex, USA under the following conditions: mobile phase 97/3 20 Mm potassium phosphate, pH 3.0/methanol, flow rate 1.5 ml/min, UV 270 nm. Monoamines were separated after 12 minutes. The resulting chromatogram identified each monoamine position and concentration from the sample as compared to that of the standard, and finally, the determination of the content of each monoamine as µg/gram brain tissue (Pagel *et al.,* 2000).

Assay of Free Amino Acids:

Free amino acid neurotransmitters GABA, Glycine, Taurine, Glutamate, Aspartate and Serine were detected in the hypothalamus by high-performance liquid chromatography (HPLC) using the precolumn PITC derivatization technique employed by Heinrikson and Meredith (1984).

Endocrinological Analysis:

Determination of blood Testosterone, FSH and LH by enzyme-linked immunosorbent assay (ELISA) kits according to Chen *et al.,* (1991), Rose, (1998) and Rebar *et al.* (1982) respectively.

Assay of Malondialdehyde and Glutathione:

Lipid peroxidation (MDA) in brain tissue was determined according to the method of Ohkawa *et al.* (1979). Similarly, Glutathione (GSH) in the brain tissue was determined by the methods of Ellman (1959).

Statistical Analysis:

The experiment was set up with a completely randomized design. Data were presented as means ± S.E for the indicated number of independently performed experiments using the Statistical Package for the Social Sciences (SPSS 17.0 for Windows). The statistical significances within parameters were evaluated by one-way and multiple analysis of variation (ANOVA), where significant differences at $P < 0.05$.

RESULTS

The present study was conducted to evaluate the possible therapeutic effects of green tea extract (GTE) 300mg/kg on the hypothalamus-pituitary-testicular axis in a rat epileptic model induced by PTZ.

As shown in Table 1, the exposure to a single i.p injection of PTZ (60mg/kg) resulted in a significant decrease in 5-HT, DA and NE in the hypothalamus as compared to the control group ($P<0.05$). On the other hand, the oral administration of green tea extract in epileptic rats resulted in a significant increase in serotonin as compared to

epileptic rat models. Similarly, the content of DA and NE was significantly increased in hypothalamus as compared to the epileptic model after GTE treatment recording 2.03±0.15 and 1.57±0.08 respectively. The induction of epilepsy resulted in a significant increase in excitatory amino acids (Glutamate, Aspartate and Glycine) in hypothalamus as compared to control groups recording 2.84±0.3, 4.60±1.01 and 2.56±0.22 respectively at $p<0.05$. The treatment with GTE (300mg/kg) for 30 days after induction of epilepsy resulted in a significant reduction in glutamate content in hypothalamus as compared to the epileptic group and no significant changes were observed in the content of Aspartate and Glycine in the studied area as compared to the epileptic group at $p<0.05$ (Table 2).

Table 1: The effect of oral administration of green tea extract (GTE) (300mg/kg) for 30 days on the content of serotonin (5-HT), Dopamine (DA) and Norepinephrine (NE) in hypothalamus of epileptic rat models.

Groups	5-HT (µg/g tissue)	DA (µg/g tissue)	NE (µg/g tissue)
Control	1.34±0.07	3.01±0.90	2.45±0.81
GTE	1.53±0.03	3.32±0.68	2.19±0.64
PTZ	0.83±0.02[a]	1.93±0.07[a]	0.98±0.03[a]
PTZ +GTE	1.09±0.11[a]	2.03±0.15[ab]	1.57±0.08[ab]

Data are expressed as means ± standard error (SE) for 10 animals/ group.
a: Significance change at P < 0.05 in comparison with the control group.
b: Significance change at P < 0.05 in comparison with the epileptic group.

Table 2: The effect of oral administration of green tea extract (GTE) (300mg/kg) for 30 days on the content of excitatory amino acids (Glutamate, Aspartate and Glycine) in hypothalamus of epileptic rat models.

Groups	Glutamate (µg/g tissue)	Aspartate (µg/g tissue)	Glycine (µg/g tissue)
Control	0.76±0.03	2.78±0.23	0.73±0.01
GTE	0.89±0.05	2.39±0.14	0.81±0.05
PTZ	2.84±0.3[a]	4.60±1.01[a]	2.56±0.22[a]
PTZ +GTE	1.15±0.44[ab]	3.99±0.32[a]	2.03±0.37[a]

Data are expressed as means ± standard error (SE) for 10 animals/ group.
a: Significance change at P < 0.05 in comparison with the control group.
b: Significance change at P < 0.05 in comparison with the epileptic group.

The data represented in table (3) recorded a significant decrease in inhibitory amino acids (GABA, Taurine and serene) in animals exposed to PTZ as compared to the control group. The treatment with GTE resulted in a significant elevation in the inhibitory amino acids in hypothalamus as compared to the epileptic group and still significantly decreased as compared to the control group.

Table 3: The effect of oral administration of green tea extract (GTE) (300mg/kg) for 30 days on the content of inhibitory amino acids (GABA, Taurine and serene) in hypothalamus of epileptic rat models.

Groups	GABA (µg/g tissue)	Taurine (µg/g tissue)	Serene (µg/g tissue)
Control	4.46±0.34	1.87±0.08	1.06±0.12
GTE	4.92±0.92	1.49±0.15	1.40±0.09
PTZ	1.91±0.18[a]	0.63±0.03[a]	0.36±0.01[a]
PTZ +GTE	4.90±0.04[ab]	1.96±0.05[ab]	1.62±0.05[ab]

Data are expressed as means ± standard error (SE) for 10 animals/ group.
a: Significance change at P < 0.05 in comparison with the control group.
b: Significance change at P < 0.05 in comparison with the epileptic group.

The results recorded in table (4) showed that the induction of epilepsy resulted in a significant decrease in serum testosterone level as compared to the control group. However, daily oral administration of GTE (300mg/kg) for 30 days in epileptic rats resulted in a significant elevation in serum testosterone level as compared to PTZ group at P<0.05. on the other hand, The results recorded in table (5**)** showed that the induction of epilepsy resulted in a significant increase in serum FSH and LH levels as compared to the control group. However, daily oral administration of GTE (300mg/kg) for 30 days in epileptic rats resulted in a significant decrease in LH levels as compared to PTZ group at P<0.05.

Table 4: The effect of oral administration of green tea extract (GTE) (300mg/kg) for 30 days in the serum testosterone level of epileptic rat models.

Groups	Testosterone (mmol/g)
Control	34.74±1.87
GTE	36.98±1.45
PTZ	20.82±1.92[a]
PTZ +GTE	30.46±2.07[b]

Data are expressed as means ± standard error (SE) for 10 animals/ group.
a: Significance change at P < 0.05 in comparison with the control group.
b: Significance change at P < 0.05 in comparison with the epileptic group.

Table 5: The effect of oral administration of green tea extract (GTE) (300mg/kg) for 30 days in serum FSH and LH levels of epileptic rat models.

Groups	FSH (mmol/g)	LH (mmol/g)
Control	5.14±1.87	3.98±0.54
GTE	4.98±1.05	3.21±0.98
PTZ	9.87±0.92[a]	7.12±0.14 [a]
PTZ +GTE	7.46±0.17[a]	4.46±0.73 [b]

Data are expressed as means ± standard error (SE) for 10 animals/ group.
a: Significance change at P < 0.05 in comparison with the control group.
b: Significance change at P < 0.05 in comparison with the epileptic group.

There was a significant rise in malondialdehyde (MDA) and a significant reduction in glutathione (GSH) levels in brain tissue homogenate of epileptic animal models as compared to the control group (P<0.05). On the other hand, the treatment with GTE postnatally for 30 days resulted in a significant decrease in MDA content and a significant increase in GSH content as compared to PTZ group as shown in table (6).

Table 6: The effect of oral administration of green tea extract (GTE) (300mg/kg) for 30 days on the brain Malondialdehyde (MDA) and Glutathione (GSH) of epileptic rat models.

Groups	MDA (mmol/g tissue)	GSH (mmol/g tissue)
Control	0.05±0.006	60.2±3.05
GTE	0.05±0.01	63.76±6.03
PTZ	0.19±0.05[a]	39.87±4.23[a]
PTZ+GTE	0.06±0.02[b]	57.76±3.95[b]

Data are expressed as means ± standard error (SE) for 10 animals/ group.
a: Significance change at P < 0.05 in comparison with the control group.
b: Significance change at P < 0.05 in comparison with the epileptic group.

DISCUSSION

This study was designed to investigate the effect of *Camellia sinensis* extract treatment on the hypothalamic-pituitary-testicular axis in male epileptic model rats. The prospective and retrospective studies demonstrate that PTZ is widely used as a convulsing drug in experimental studies (Morimoto *et al.,* 2004). PTZ interacts competitively with the picrotoxin-binding site of the $GABA^A$ receptor, thereby decreasing the chloride flux throughout the membrane and producing generalized tonic-clonic seizures (Seo *et al.,* 2020). In addition, PTZ resulted in an increase of oxidative stress, affecting mainly the brain in comparison to other organs (Yang *et al.,* 2020).

Our results recorded a significant decrease in 5-HT, DA and NE content in hypothalamus of PTZ induced epileptic seizures. Christensen *et al.* (2019) reported a decrease in the brain serotonin system of epileptic patients of post-mortem brain tissue taken from epileptic donors compared to healthy controls. Similarly, the decrease recorded in DA content in the present study was also consistent with the study of Akyuz *et al.* (2021), which recorded an elevation in dopamine hydroxylase and homovanillic acid in epileptic patients and reported the involvement of DA dysfunction in the production of epileptic symptoms. In addition, the observed reduction in NE is consistent with the recorded decrease in DA, as vital neurotransmitters for the normal function of the brain and serves as a precursor of NE production, this may explain our present results.

On the other hand, it is known that glutamate and GABA are two neurotransmitters substances that are linked to widespread synaptic communication in the CNS. Glutamate is the principal excitatory neurotransmitters substance in the brain and spinal cord whereas GABA is responsible for most of the inhibitory communication in the brain, and glycine is the principal inhibitory transmitter in the spinal cord and in the lower brain (Kandel *et al.,* 1995)

Most theories regarding these amino acids neurotransmitters in epilepsy suggest that the GABAergic system is suppressed resulting in an elevation in the glutamate system. The overactivity of glutamate could result in excitotoxicity which could cause aberrant neuronal development (Bittigau and Ikonomidou 1997). If this system is hyperfunction, it is possible that neuronal growth and connectivity are damaged during critical periods of development. Excessive glutamatergic stimulation is also associated with seizures, which are common among individuals with epilepsy.

Similarly, Mäkelä *et al.* (2018), was examined the brain level of glutamate decarboxylase in epileptic and control subjects. They found that this enzyme was reduced 48-61 % in parietal and cerebellar areas of brains of the epileptic group when compared to control. These results may explain our present results in the elevated level of glutamate and reduced level of GABA in epileptic animal models.

Epilepsy is associated with abnormalities of reproductive physiology, but the mechanisms of hormonal dysregulation are not clear (Luef, 2010). Hyposexuality is the most frequent abnormality in men and women with epilepsy. In men with epilepsy, hypoandrogenimia, hypogonadism and sperm abnormalities are common. Testicular atrophy was also infrequently reported (Hamed, 2016).

The present results recorded a marked increase in LH and FSH and a significant decrease in testosterone in serum of epileptic rat model. Furthermore, Stoffel-Wagner *et al.* (1998) stated that LH and FSH levels in the blood of epileptic men were significantly increased than in controls. They suggested that an there is impairment in Leydig cells function might explain these observations. Despite an increased release of LH from the

pituitary gland, the impaired Leydig cells are not able further to increase their testosterone synthesis, causing a decreased testosterone level. From our results and results of other investigators, we can conclude that patients with epilepsy are exposed to hypergonadotropic hypogonadism if replaced mechanisms in the hypothalamo-pituitary–gonadal-axis are inadequate to normalizing serum testosterone.

Moreover, it is known that epilepsy caused a marked increase in corticosterone levels in plasma. The higher plasma corticosterone levels suggested that epileptic seizures activate the hypothalamic-pituitary-gonadal axis which may induce alterations in plasma levels of male reproductive hormones (Mejías-Aponte *et al*., 2002).

The decrease in Testosterone leads to failure in neuromodulator system, through changes in the synthesis, release, reuptake, or catabolism of neurotransmitters (Barth *et al.*, 2015). The testosterone acts on gene expression in serotonin neurons in a manner that could increase 5-HT neurotransmission (Bethea *et al.*, 2014). So, the decrease in testosterone level may cause a decline in 5-HT content in hypothalamus.

Camellia sinensis (green tea) has been consumed as the most popular drink all over the world and is believed to have a wide range of health benefits. L-Theanine, the major component of the free amino acids in green tea, has been reported to display neuronal protection and tumour inhibition (Yang *et al.*, 2013). L-Theanine is absorbed in the small intestine after oral injection into the bloodstream and easily crosses the blood-brain barrier (Vuong *et al.*, 2011). Theanine is structurally similar to the excitatory neurotransmitters glutamate and in accordance binds to glutamate receptors. Regarding the possible mechanisms, theanine showed an antagonistic effect on AMPA/kinate type glutamate receptors (Kawada *et al*., 2010). In addition, it inhibits glutamate/glutamine cycle and thus blocks the reuptake of glutamate (Sugiyama *et al.*, 2004). On the other hand, some neurochemical studies reported that L-theanine caused increased brain DA, 5-HT and GABA levels (Liu *et al*., 2009). Similarly, Yokogoshi *et al.* (1995) reported that administration of green tea extract caused a significant increase in DA concentration in the brain especially in striatum, hypothalamus and cerebral cortex. This may explain the amelioration in neurotransmitters contents in the present study in the group of epilepsy after treatment with green tea extract (GTE).

Our present results recorded a significant decrease in gonadotropic hormones (LH and FSH) and significant increase in testosterone level in serum. The results obtained by Figueiroa *et al.* (2009), recorded that the green tea extract coused a decrease in secreation of testosterone from seroli cells *in vitro,* due to the presence of catechins. These data are also in agreement with Das and Karmakar (2015), who reported the decrease in serum testosterone level after exposure of catechin in animlas. These results are contradict with our present results. The elevation in testosterone level in our experiment may be due to the effect of antioxidant constituents (polyphenol) in the watery extract of *Camellia Sinensis* on testicular tissues which may cause improve in the hormonal production of Sertoli cells (Kaplanoglu *et al.*, 2013). The increase in testosterone level in circulation resulted in feedback decrease in gonadotropin hormones from pituitary gland. Moreover, green tea diet can alter hypothalamic function and decrease the level of LH and FSH by its anitioxidant and free radicle scavengers effect (Schreihofer *et al.*, 1993).

The production of reactive oxygen species (ROS) has been involved in numerous pathological alterations of CNS including neurodegeneration and in particular epilepsy (Waldbaum and Patel, 2010). The cytotoxic activity of PTZ is due to the generation of hydrogen peroxide, hydroxyl radicle as well as reducing the levels of the enzymatic antioxidant system (Mao *et al.*, 2019). The antioxidant decrease may be due to lower production or greater consumption rates, implying greater vulnerability of the epileptic

brain to oxidant. These findings are also in line with a recent study on brain tissue that reports a significant increase in lipid peroxide level and a significant decrease in glutathione level in epileptic model rats.

In the present study, the treatments of epileptic rats with daily oral administration of green tea extract (300mg/kg), resulted in a significant decrease in MDA, a significant increase in GSH. The green tea extract (GTE) serves as a free radicle scavenger as it diminishes the generation of lipid peroxides. The neuronal protective properties of GTE could be correlated to the bioactive constituents known as Catechins which are superior in antioxidant action compared to vitamin C and E (Rice-Evans *et al*., 1995). Babu *et al.* (2006) reported that the GTE by scavenging the free radicles directly, may reduce the utilization of GSH and thereby exhibiting an increase in its content. As polyphenols are capable of crossing the blood-brain barrier, they could avert the effects of PTZ evoking neuronal protection (Weinreb *et al*., 2004).

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