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Neurophysiological Effect of Pentoxifyllin on Male Albino Rats

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Abstract

The objective of the present work is to study the possibility of involved neurotransmitters in the CNS side effects due to the administration of Pentoxifyllin in male albino rats. The frontal cortex of Pentoxifyllin treated groups revealed decrease of glutamate, GABA and elevation of aspartate, asparagines, glycin, serine levels and AChE activities in a time related effect. In the hippocampus area there are elevation of asparaginet, GABA, glycine, serine level but reductions of glutamate, aspartate level and AChE activities in a time related effect. Overall, the data suggest that there is a shift in the balance between neurotranamitters towards increased production of excitatory potency in groups subjected to Pentoxifyllin administration.

Keywords: Pentoxifyllin, Neurotransmitters, Male albino rats, Acetylcholinesterase, Asparaginet, GABA, Glycine, Serine glutamate, Aspartate level.

Contents

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1. Introduction

The nervous system regulates all aspects of bodily function. It is staggering in its complexity. Despite of the complexity of the nervous system we known, for instance, those electrical impulses are produced along the length of every nerve cell. A neurotransmical is defined as a chemical that is selectively released from a nerve terminal by an action potential, interacts with a specific receptor on an adjacent structure and elicits a specific physiological response [1]. Neurotransmitters are chemicals that are released from nerve endings into the synapse, Where they produce effects by binding to receptors on postsynaptic.The receptors may be on other neurons of effectors ′organs such as smooth muscle [2].

Neurotransmitters are classified into groups of substances within chemical categories: amino acids [3] catecholamine [4] and neuropeptides [5]. Other substances that may participate in central synaptic transmission include purines such as adenosine and ATP [6].

Fonnum [7] cited that the majority of the synapses in the brain, and perhaps as many as 90% use amino acids as transmitters. Now, amino acids transmitters involved in most brain functions, and amino acids are the prime mediators of fast synaptic signaling. Glutamate is the most common neurotransmitter in the brain [8], Asparate is an excitatory neuro transmitter [9], Asparagine is a non essential amino acid which is made from aspartic acids plus ATP (adenosine tri-phosphate) [10].

GABA (Y-amino butyric acid) was identified as unque chemical constituent of brain in 1950, but its potency as a CNS depressant was not immediately recognized [11]. Glycine acts as a post-synaptic inhibitory transmitter in the spinal cord and brain stem [12]. Serine has been proposed to act as a neurotransmitter(inhibitory amino acids) [13].

Antibiotics are among the most frequently prescribed medications in modern medicine. Antibiotics cure disease by killing or injuring bacteria.The present study aimed to evaluate the impact of oral administration of Pentoxifyllin on the levels of amino acids neurotransmitter (Glutamate, Asparate, Asparagine, GABA, Glycine, Glycine Glycine) and acetylecholinesterase activities in the frontal cortex and the hippocampus of brain areas of adult male albino rats (Rattusnorvegicus) through 1,3,7 and 14 days post Pentoxifyllin administration.

2. Material and Methods

2.1. Drug and Doses

Pentoxifylline (PTX) (Trental ®, Aventis Pharma, Cairo, Egypt), was orally administered 4 wks post infection (PI) 5 days/wk at dose of 400 mg/Kg body weight. The treatment was continued until the date of sacrifice.

2.2. Experimental Animals

This study was carried out on adult male albino rats (Ratusnorvegicus). One hundred eight animals with average weight ranged 100g ± 20g. They were obtained from the Egyptian Institution of Serum and Vaccine (Helwan).

The male albino rats were housed in iron mesh cages with seven rats each. Clean sawdust was used to keep the animals dry and clean throughout the experimental periods. The experimental animals were allowed to acclimate under the laboratory conditions two weeks before the beginning of the experiments. The animals were kept under controlled temperature of 21oC and 12 hours light 12/hours dark cycle throughout the course of experiment. A commercial pelleted diet and fresh vegetables were used before the experiment. The food debris, feces and urine were removed daily to prevent food and water contamination.

2.3. Experimental Design

In order to study the physiological effect of Pentoxifyllin on the levels of amino acids and monoamines neurotransmitters and activities of acetylcholinesteras in frontal cortex and hippocampus brain areas. The rats divided into three main groups. The rats of the first group (n=12 rats) were administered 2 ml of distilled water daily, whereas those of the second group(n=48 rats) were administered 400 mg/Kg body weight Pentoxifyllin dissolved in 2 ml water.At the end of the experimental periods 1, 3, 7 and 14 days, twelve animals sacrificed after 12 hours from the last administration by rapid decapitation. The brains were dissected out quickly weighed and cleaned. Four brains from each treated group served for biochemical analysis. For biochemical analysis and in case of control group three animals were selected at each experimental period to achieve a single control group representing the control of the study. The frontal cortex and hippocampus brain areas were separated from each whole brain. Each brain area divided into two halves the first half served for acetylcholinesterase activity assay and the second half was homogenized in 75% high performance liquid chromatography (HPLC) methanol (1/10) weight/volume) using a homogenizer surrounded with an ice jacket and the homogenates and used for the determination of the brain contents of amino acids and monoamines neurotransmitters.

2.4. Estimation of the Biochemical Parameters

2.4.1. Estimation of the Free Amino Acids

Free amino neurotransmitters were estimated by aid of high performance liquid chromatography (HPLC), using the precolumn PTC derivatization technique according to method of Heinrikson and Meredith [14].

The amino acids (glutamic acids, aspsrtic acids, asparagines, GABA (y-amino-butyric acids), glycine and serine,) were determined in the brain cortex and hippocampus.

Derivatized amino acids standard and derivatized sample were injected, the injected volume is 20µl into the column for separation by HPLC. The resulting chromatogram identified each amino acids position and concentration from the sample as compared to that of the amino acids standard and finally the determination of the µ mole content of each amino acids per gram brain tissue was achieved. The chromatogram was integrated by Turbochrome software program [Perkin-Elmer, USA]. The brain content of each amino acid was expressed as µmol/g tissue and was calculated using the following formula:

2.4.2. Estimation of Acetylcholinesterase (AChE) Activity

Acetylcholinesterase activity in the brain cortex and hippocampus were determinate according to the modified of Ellman, et al. [15] method as described by Gorum, et al. [16]. The principle of the method is based on measurement of the rate of thiocholine as a result of Acetylthiocholinehydrolysis.The reagents (analytical grade) were phosphate buffer, pH 7.6 (20 mmol), acetylthiocholine iodide (5mmol). DTNB-phosphate- ethanol reagent (12.4 mg 5,5-dithiobis-2-nitrobenzoic acid dissolved in 120 ml of 96% ethanol, 80 ml of distilled water and 50 ml of 0.1 M phosphate buffer, pH 7.6). the solution can be kept at room temperature for at least six months. Glutathione (2.5mmol) was used as a standard for cholinesterase activity.

The brain cortex and hippocampus tissue samples were weighed and homogenized in a 20 mmol- phosphate buffer, pH 7.6 (4%w.v). 0.14 ml phosphate buffer 20 mmpl pH 7.6), 0.05 ml 5-mmol-acetylthiocholine iodide and 0.01 ml of tissue homogenate were pipettes in a cuvette that incubated for 10 min at 38oC, then the reaction was stopped by 1.8 ml of DTNB-phosphate ethanol reagent. The color produced was read immediately at 412 nm on Shimadzu apectrophotometer uv-1601. The cholinesterase was determined as µmolsh from a standard curve.

3. Result

3.1. Biochemical Studies

3.1.1. Effect of Pentoxifyllinon Glutamic Acid Concentration in Frontal Cortex and Hippocampus Tissues

3.1.1.1. Frontal Cortex

Data presented in Table (1) recorded the effect of Pentoxifyllin on glutamic acid concentration in the frontal cortex of male albino rats. The data showed a significant decrease throughout the experimental periods which is inversely proportional to the duration as the maximum effect achieved after the 1st injection of and exhibited 55.4, 43.5, 34.2 and 16.83% Percentage difference from control value after experimental duration 1, 2, 7, 14 days, respectively.

3.1.1.2.Hippocampus

Data presented in Table (1) recorded the effect of Pentoxifyllin On glutamic acid concentration in the hippocampus tissue of male albino rats. The data showed significant decrease in the 1st,3th, 7th and 14th days of the experimental periods as compared with the control. Administration of Pentoxifyllin and exhibited 10.4, 7.3, 5.2 and 1.04% percentage difference from control value after experimental durations 1, 3, 7 and 14 days, repectively.

Table-1. Effect Pentoxifyllin of the levels of glutamic (µmole/g) in frontal and hippocampus of the cortex of male albino rats

Tissue	Group	Experimental periods							
		1 day	% Change	3 days	% Change	7days	% Change	14 days	% Change
Frontal cortex	Control	10.34±0.6		10.34±0.6		10.34±0.6		10.34±0.6	
	Pentoxifyllin	4.61±0.3**	55.4%	5.84±0.4**	43.5%	6.8±0.6**	34.2%	8.6±0.7*	16.83%
Hippocampus	Control	9.6±0.62		9.6±0.62		9.6±0.62		9.6±0.62	
	Pentoxifyllin	8.6±0.5*	10.4%	8.9±0.23	7.3%	9.1±0.36	5.2%	9.5±0.34	1.04%

Table-2. Effect Pentoxifyllin of the levels of aspartic acid (µmole/g) in frontal and hippocampus of the cortex of male albino rats

Tissue	Group	Experimental periods							
		1 day	% Change	3 days	% Change	7days	% Change	14 days	% Change
Frontal cortex	Control	3.14±0.23		3.14±0.23		3.14±0.23		3.14±0.23	
	Pentoxifyllin	3.84±0.45	22.29	3.94±0.41	25.48	4.5±0.33	43.31	4.87±0.65	55.1
Hippocampus	Control	2.23±0.33		2.13±0.33		2.13±0.33		2.13±0.33	
	Pentoxifyllin	1.64±0.08	-26.46	1.86±0.12	-12.68	1.96±0.77	-7.98	2.1±0.51	-1.41

Table-3. Effect Pentoxifyllin of the levels of asparagine (µmole/g) in frontal and hippocampus of the cortex of male albino rats

Tissue	Group	Experimental periods							
		1 day	% Change	3 days	% Change	7days	% Change	14 days	% Change
Frontal cortex	Control	0.56±0.22		0.56±0.22		0.56±0.22		0.56±0.22	
	Pentoxifyllin	0.54±0.52	-3.7%	0.62±0.3	10.71	0.67±0.8	19.64	0.74±0.4	32.14
Hippocampus	Control	0.24±0.2		0.24±0.2		0.24±0.2		0.24±0.2	
	Pentoxifyllin	0.32±0.3	33.3	0.38±0.6	58.3%	0.41±0.6	70.83%	0.42±0.8	75

Table-4. Effect Pentoxifyllin of the levels of serine (µmole/g) in frontal and hippocampus of the cortex of male albino rats

Tissue	Group	Experimental periods							
		1 day	% Change	3 days	% Change	7days	% Change	14 days	% Change
Frontal cortex	Control	0.32±0.26		0.32±0.26		0.32±0.26		0.32±0.26	
	Pentoxifyllin	0.53±0.1	65.63	0.37±0.12	58.83	0.34±0.18	6.25	0.39±0.22	21.88
Hippocampus	Control	0.13±0.24		0.13±0.24		0.13±0.24		0.13±0.24	
	Pentoxifyllin	0.21±0.8	61.54	0.18±0.82	38.46	0.15±0.66	15.38	0.20±0.21	53.85

Table-5. Effect Pentoxifyllin of the levels of GABA (µmole/g) in frontal and hippocampus of the cortex of male albino rats

Tissue	Group	Experimental periods							
		1 day	% Change	3 days	% Change	7days	% Change	14 days	% Change
Frontal cortex	Control	2.2±0.12		2.2±0.12		2.2±0.12		2.2±0.12	
	Pentoxifyllin	1.61.6±0.38	-26.82	1.68.6±0.32	-23.64	1.63.6±0.8	-25.91	1.78.6±0.92	-19.1
Hippocampus	Control	1.6±0.65		1.6±0.65		1.6±0.65		1.6±0.65	
	Pentoxifyllin	1.85.6±0.66	15.63	1.94.6±0.45	21.25	2.2±0.58	37.5	2.6.6±0.84	62.5

Table-6. Effect Pentoxifyllin of the levels of glycine (µmole/g) in frontal and hippocampus of the cortex of male albino rats

Tissue	Group	Experimental periods							
		1 day	% Change	3 days	% Change	7days	% Change	14 days	% Change
Frontal cortex	Control	1.5±0.72		1.5±0.72		1.5±0.72		1.5±0.72	
	Pentoxifyllin	1.9±0.14	26.66	2.4±0.42	60	2.6±0.5	73.3	2.84±0.55	86.6
Hippocampus	Control	1.14±0.5		1.14±0.5		1.14±0.5		1.14±0.5	
	Pentoxifyllin	1.3±0.45	14	1.6±0.2	40.35	1.84±0.6	61.4	2.1±0.34	84.2

Table-7. Effect Pentoxifyllin of the levels of AchE (µmole/g) in frontal and hippocampus of the cortex of male albino rats

Tissue	Group	Experimental periods							
		1 day	% Change	3 days	% Change	7days	% Change	14 days	% Change
Frontal cortex	Control	15±0.93		15±0.93		15±0.93		15±0.93	
	Pentoxifyllin	17.5±0.33	16.6	20.7±0.85	38	23±0.7	73.3	25±0.44	66.7
Hippocampus	Control	18±0.87		18±0.87		18±0.87		18±0.87	
	Pentoxifyllin	16±1.17	-11.1	15.4±0.27	-14.4	13.7±0.5	-23.9	11.2±0.66	-37.8

3.1.2. Effect of Pentoxifyllin on Aspartic Acid Concentration in Frontal Cortex and Hippocampus Tissues

Collection data of aspartic acid concentrations in the frontal cortex and hippocampus tissues of male albino rats affected by Pentoxifyllin administration are given in Table (2).

3.1.2.1. Frontal Cortex

The data in Table (2) showed a significant increase ($P<0.05$) throughout the experimental durations and the maximum effect achieved after 14 days post Pentoxifyllin Treatment and exhibited 22.29, 25.48, 43.31, and 55.1 percentage difference from control value after experimental durations 1, 2, 7 and 14 days, respectively.

3.1.2.2. Hippocampus

Data presented in table (2) recorded the effect of Pentoxifyllin on aspartic acid concentration in the hippocampus tissue of male albino rats. The data showed significant decrease in the 1st,3rd and 7th days in Pentoxifyllin Administered groups.But after the 14th day of drug administration aspirate elevated restoring to some extent it´s control value. Administration of Pentoxifyllin exhibited -26.46, -12.68, -7.98 and -1.41 Percentage difference from control value after experimental duration 1, 2, 7 and 14 days respectively.

3.1.3. Effect of Pentoxifyllinon Asparagine Concentration in Frontal Cortex and Hippocampus Tissues

The general pattern about the data of asparagines concentrations in the frontal cortex and hippocampus tissues of male albino rats affected by Pentoxifyllin administration are given in Table (3)

3.1.3.1. Frontal Cortex

The data in Table (3) showed asparagines level increased significantly from the 3rd days of Pentoxifyllin administration till the last 14th day as compared to the control value in the frontal cortex recording the percentage differences -3.7%,10.71, -19.64and-32.14 at the increase ($P<0.05$) the 1st,3rd and 7th days, respectively,

3.1.3.2. Hippocampus

Data recorded in Table (3) represented the effect of Pentoxifyllin on asparagines concentration in the hippocampus tissue of male albino rats. The data showed significant increase from the 1st to the 14th day of Pentoxifyllin administration. Administration of Pentoxifyllin exhibited 33.3, 58.3%, 70.83% and 75 percentage difference from control value after experimental duration 1,2,7 and 14 days respectively.

3.1.4. Effect of Pentoxifyllinon Serine Concentration in Frontal Cortex and Hippocampus Tissues

3.1.4.1. Frontal Cortex

The general pattern about the data of serine concentrations in the frontal cortex of male albino rats affected by Pentoxifyllin administration are given in Table 4. Pentoxifyllin administration exhibited significant increase after the 1st dose by 65.63 % percentage change from control value. Serine frontal cortex level increased then decreased insignificantly, at the, 3rd and 7th duration representage 58.83 and 6.25 as percentage difference from control level. At the last duration serine level increased significantly by 21.88 Percentage changes from control value (Table 4).

3.1.4.2. Hippocampus

Data presented in Table 4. Recorded the effect of Pentoxifyllin on serine concentrations in the hippocampus tissue of male albino rats. The data showed significant increase after the 1st day Pentoxifyllin administered group as compared to control level. But after the 7th day of drug administration serine decreased significantly as compared to its control value. Then serine level increased significantly after the 14th day of Pentoxifyllin administration compared to control (<0.05). Administration of Pentoxifyllin exhibited 61.54 , 38.46, 15.38 and 53.85 percentage difference from value after experimental duration 1,2,7.and 12 days, respectively.

3.1.5. Effect of Pentoxifyllinon GABA Concentration in Frontal Cortex and Hippocampus Tissues

The general pattern about the dat of GABA concentrations in the frontal cortex and hippocampus tissues of male albino rats affected by Pentoxifyllin administration are giPentoxifyllinven in Table 5.

3.1.5.1. Frontal Cortex

GABA concentration decreased significantly (P<0.05) as compared to the control level from the 1st day of Pentoxifyllin administration till the 14 th day post administration. The data revealed -26.82, -23.64, -25.91 and -19.1 percentage difference from control value after experimental duration 1,2,3,7 and 14 days,respectively(Table 5).

3.1.5.2. Hippocampus

The data presented in Table 5. Recorded the effect of Pentoxifyllin concentration in the hippocampus tissue of male albino rats. The data showed significant increase throughout of the experimental periods post Pentoxifyllin administration in the GABA levels as compared to the control value. The data revealed 15.63, 21.25, 37.5 and 62.5 percentage difference from control value after experimental duration 1,2,7 and 14 days, respectively

3.1.6. Effect of Pentoxifyllinon Glycine Concentration in Frontal Cortex and hippocampus Tissues

The general pattern about the data of glycine concentrations in the frontal cortex and hippocampus tissues of male albino rats affected by Pentoxifyllin administration are given in Table 6.

3.1.6.1. Frontal Cortex

Data presented in Table 6 showed that glycine concentration increased significantly (P<0.05) as compared to the control level from the 1th day of Pentoxifyllinadminstration till the end of the expermintal periods. The data revealed 26.66, 60, 73.3 and86.6 percentage difference from control value after experimental duration 1,2,7, and 14 days. Respectively.

3.1.6.2. Hippocampus

Data presented in Table (6) recorded the effect of Pentoxifyllin on glycine concentration in the Hippocampus tissue of male albino rats. The data showed significant increase from the 1st day in Pentoxifyllin administrated group as compared to control level till the 14th day of drug administration. Administration Pentoxifyllin exhibited 14, 40.35, 61.4 and 84.2 percentage difference from control value after expermintal duration 1,2,5 and 14 dayss,respectively.

3.1.7. Effect of Pentoxifyllinon Acetylcholinesterase (AchE) Concentration in Frontal Cortex and Hippocampus Tissues

The general pattern the data of AchE activity in the frontal cortex and Hippocampus tissues of male albino rats affected by Pentoxifyllin administration are givein in Table (7).

3.1.7.1. Frontal Cortex

The data in Table 7 showed dose response significant increase (P<0.05) in AchE activity from 1st achieving the maximum activity in the 14th doses of Pentoxifyllin treatment as compared to the control enzyme activity representing percentage change from control 16.6, 38, 73.3 and 66.7 after experimental duration 1,3,7 and 14 days post Pentoxifyllintreatment,respectively.

3.1.7.2. Hippocampus

Data presented in Table 7 recorded the effect of Pentoxifyllin on AchE activity in the Hippocampus tissue of male albino rats. The data showed significant decrease in AchE activity from the 1st till the 14th day´ Pentoxifyllinadministration groups as compared to the control enzyme activity. The data revealed -11.1, -14.4, -23.9 and -37.8 percentage difference from control value after expermintal duration 1,3,5 and 14 dayss, respectively.

4. Discussion

In the present study the response of cortex and hippocampus areas to Pentoxifyllin at different duration are clearly investigated through the determined amino acids monoamines neurotransmitters and the recorded activities of the acetylcholinesterase enzyme and the excitatory potencies of Pentoxifyllin recorded throw the elevation ofaspartic and asparagines level and the increase of the activities of AchE in spite of decrease glutamic level I the cortical area as a result of Pentoxifyllin administrations in a time related effect. In addition there is decrease GABA level in a manner as aforementioned effect.

The excitatory potencies of the antibiotics under investigation varied as regard to the hippocampus area where Pentoxifyllin in the hippocampus area recorded elevation of asparagines, GABA, serine, glycine levels but decrease of glutamate level in a time related effect. In addition there is decrease of AchE activities and aspartate, glutamate levels in a dose related effect.

The previous finding, which recorded in brain cortical areas from groups subjected to the antibiotics, is in line with alterations in extrahippocampal regions in epileptic rat models. This alterations support the extrahippocampal with GABAergic inhibition [17].

The alterations in the hippocampus area evidence provided links early sezures with the later development of epilepsy and selective hippocampus neuronal loss [18].

The anexiogenic effects due to hippocampus may be supported through the recorded changes in the concentrations of frontal cortex amino acid. There were decreases in GABA and glutamate levels with a concomitant increase in aspartate, asparagines, glycine and serine contents which similar to those recorded in the frontal cortex of epileptic rat models [19, 20].

The regional differences in GABA level and acetylcholinesterase activity between decrease of GABA level and increase of AChE activity in the cortical area and increase of GABA level and decrease of AChE activity in the hippocampal area in antibiotic treated groups in a dose related effect which mimics that predicted in rat epileptic

models [21] and support the proconvulsant effect of the quino;ones previously discussed [22]. Biochemical studies have proposed a role for AChE in brain mechanisms responsible by development to status epilepticus through decrease in the n the hippocampus [23].

The decreased glutamate level in the cortical area of Pentoxifyllin treated groups and treated group hippocampus area, may be explained as fluoroquinolones did not bind to the glutamate or glycine-binding site of the NMDA receptor. It has been shown that fluoroquinolonesand MK-801 binding to the NMDA receptor [24]. The decrease of glutamate and increase of aspartate may be explained through the glucose homeostasis abnormalities (dysglycemia) associated with the use of Pentoxifyllin [25].

The elevated level of glycine in cortical and hippocampus areas of rats subjected to Pentoxifyllin may be declared the effect of the inhibitory neurotransmitter glycine on slow destructive processes in brain cortex slices under anoxic conditions as glycine, the simplest of the amino acids, is an essential component of important biological molecules, a key substance in many metabolic reactions,the major inhibitory neurotransmitter in the spinal cord and brain stem, and an anti-inflammatory,cytoprotective [26, 27]. In hippocampus area the experimental model of epilepsy during kianic acid induced epilepsy the results indicated that the levels of glutamate,aspartate, glycine and GABA were ststistically increased in rat′s hippocampus [28].

As regard to the recorded data about the amino acid serine Kartvelishvily, et al. [29] study suggestion may declare the mechanism through which the serine concentration elevated in the frontal cortex of the antibiotics treated as D-serine is a co-agonist of N-methyl-D-aspartate (NMDA) RECEPTORS that occurs at high levels in the brain.

References

[1] A. G. Gilman, L. S. Goodman, and A. Gilman, *The pharmacological basis of therapeutics*, 10th ed. London: MacMillian Comp., 2001.

[2] R. J. Miller, "Presynaptic receptors," *Ann. Rw. Pharmaco Toxical*, vol. 38, pp. 201-227, 1998.

[3] R. H. Duvilanski, L. Garcia, M. Bonache, and A. I. Esqufino, "Daily variation of amino acid concentration in mediobasal hypothalamus, in rats injected with freund′ adjuvant. Effect of cyclosporine," *J.Neuroimmunol*, vol. 87, pp. 189-196, 1998.

[4] A. A. Grace, "Phasic. Jversus dopamine release and the modulation of dopamine system responsitvity: A hypothesis for the etiology of schizophrenia," *Neuroscience*, vol. 41, pp. 1-24, 1991.

[5] S. Ghose, B. Wroblewska, L. Corsi, D. R. Grayson, A. L. De-Blas, A. Vicini, and J. H. Neale, "N- acetylaspartylglutamate stimulates metabotropic glutamate receptor 3 to regulate expression of the GABA(A) alpha 6 subunit in cerebellar granule cell," *J. Neurochem.*, vol. 69, pp. 2326-2335, 1997.

[6] M. Williams. Purinoceptors in central nervous system function: Targets for therapeutic intervention. In: Psychopharmalcology: The fourth generation of progress. (Bloom FE and Kupfer DJ.Eds). New York: Raven Press. pp: 643-655, 1994.

[7] F. Fonnum, "Glutamate A. neurotransmitter in mammalian brain," *J. Neurochem.*, vol. 42, pp. 1-11, 1984.

[8] C. Goudet, V. Magnaghi, M. Landry, F. Nagy, R. W. Gereau, and I. P. Pin, "Metabotropic receptors for glutamate and GABA in pain," *Brain Res, Rev.*, vol. 60, pp. 43-56, 2009.

[9] A. A. Farooqui, W. Y. Oag, and L. A. Horrocks, "Glutamate and aspartate in brain," *Neurochemical Aspects of Excitotoxici*, vol. 1, pp. 3-30, 2008.

[10] C. W. Cotman, J. S. Kahie, J. Ulas, and R. J. Bridges, *Excitatory amin acid neurotransmission. In: Psychopharmacology: The fourth generation of progress. (Bloom FE and Kupfer DJ, Eds)*. New York: Raven Pree, 1994.

[11] E. A. Kravitz, S. W. Kuffler, and D. D. Ptter, "Gamma aminobutyric acid and other blocking compounds in crustacca. Their relative concentrations in separated motor and inhibitory axons," *J. Neurophysiol*, vol. 26, pp. 739-751, 1963.

[12] W. Pei, J. Misumi, N. Kubota, M. Morikawa, and N. Kimura, "Two new reactive targets of 2,5-hexanedione in vitro-beta-alanine and glycine," *Amino Acids*, vol. 32, pp. 261-264, 2007.

[13] M. Z. Berkman, S. Palaoglu, T. Erbengi, and A. Erbengi, "Neurotransmitter and amino acid analysis and ultrastructural observations of fetai brain cortex transplantation to adult rat brain under the effect of dexametliasone," *Neurosurgery*, vol. 42, pp. 1126-1134, 1998.

[14] R. L. Heinrikson and S. C. Meredith, "Amino acids analysis by RP-HPLC: Precolumn derivatization with phenylisothiocyanate," *Anal. Biochem.*, vol. 136, pp. 65-74, 1984.

[15] G. L. Ellman, K. D. Courtney, V. Andres, and R. M. Featherstone, "A new and rapid colorimetric determination of cholinesterase activity," *Biochem. Pharmcol.*, vol. 7, pp. 88-95, 1961.

[16] V. Gorum, L. Proinov, V. Baltescu, G. Balaban, and O. Barzu, "Modified ellman procedure for assay of cholinesterase in crude enzymatic prepsrstion," *Anal.Biochwm.*, vol. 86, pp. 324-326, 1978.

[17] A. V. Silva, E. R. G. Sanabria, F. A. Cavaltheiro, and R. Spreafico, "Alterations of the neocortical GAB Aergic system in the pilocarpine model of temporal lobe epilepsy: Neuronal damage and immunocytochemical changes in chronic epilepsy rats," *Brain Research Bulletin*, vol. 58, pp. 417-421, 2002.

[18] S. Koh, T. W. Storey, T. C. Santosm, A. Y. Mian, and A. J. Cole, "Early-life seizures in rats increase susceptibility to seizure induced brain injury in adulthood," *Neurology*, vol. 53, pp. 915-921, 1999.

[19] Z. Li, Y. Yamamoto, T. Morimoto, J. Ono, S. Okadam, and A. Yamatodani, "The effect of pentylenetetrazole-kindling on the extracellular glutamate and taurine levels in the frontal cortex of rats," *Neurosci. Lett.*, vol. 282, pp. 117-119, 2000.

[20] J. Szyndler, P. Maciejak, D. Turzynska, A. Sobolewska, M. Lehner, E. Taracha, J. Walkowiak, A. Skorzewska, A. Wislowskastanek, A. Hamed, and A. Bidzinski, "Change in the concentration of amino acids in the hippocampus of pentylenetetrazole-kindled rats," *Neurosci. Lett.*, vol. 439, pp. 245-249, 2006.

[21] M. E. Appleyard, A. R. Green, and A. D. Smith, "Acetylcholinesterase activity in regions of the rat brain following a convulsion," *J. Neurochem.*, vol. 46, pp. 1789-1793, 1986.

[22] I. Smolderser, C. Goussen, S. Marchand, W. Couet, G. Ebinger, and Y. Michotte, "Convulsant and subconvulsant doses of norfloxacin in the presence and absence of biphenylacetic acid alter extracellular hippocampal glutamate but not gamma-aminobutyric acid levels in conscious rats," *Antimicrobial Agents and Chemotherapy*, vol. 46, pp. 471-477, 2002.

[23] K. M. Freitas, F. C. Sousa, G. S. Viana, and M. M. Fonteies, "Acetylcholinesterase activities in hippocampus, frontal cortex and striatum of Wistar rats after pilocarpine-induced status epilepticus," *Neurosci. Lett.*, vol. 399, pp. 76-78, 2006.

[24] S. Sen, A. K. Jaiswal, S. Yanpallewar, and S. B. Acharys, "Anxiogenic potential of ciprofloxacin and norfloxacin in rats Singapore," *Med. J.*, vol. 43, pp. 1028-1032, 2007.

[25] J. Jose, B. Jimmy, and K. Saravw, "Dysglycemia associated with the use of fluoroquinolones-focus on gatifloxacin," *Jouenal of Clinical and Diagnostic Research*, vol. 3, pp. 185-187, 2007.

[26] R. Y. Gundersen, P. Vaagenes, T. Preivik, F. Fonnum, and P. K. Opstad, "Glycine- an important neurotransmitter and cytoprotective agent," *Acta Anaesthesiol Scand.*, vol. 50, pp. 261-262, 2006.

[27] A. Tonshin, N. Lobysheva, L. Yagazhinsky, E. Bezgina, D. Moshkow, and Y. A. Nartsissov, "Effect of the inhibitory neurotransmitter glycine on slow destructive processes in brain cortex slices under conditions," *Biochemistry Moscow*, vol. 72, pp. 509-517, 2007.

[28] J. Liu and J. Cheng, "Change of amino scids release in rat´s hippocampus during kainic acid induced epilepsy and acupuncture chen," *Tzu-Yen-Chiu*, vol. 20, pp. 50-54, 1995.

[29] E. Kartvelishvily, M. Shleper, L. Balan, E. Dumin, and H. Wolosker, "Neuron- derived D-serine release provides a novel means to activate N- methyl-D-aspartate receptors," *J. Biol. Chem.,* vol. 281, pp. 14151-14162, 2006.