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EFFECT OF TOPIRAMATE DRUG ON NEUROTRANSMITTERS LEVELS (ACETYLCHOLINE AND DOPAMINE) IN PREGNANT ALBINO RATS

تأثير عقار التوبيراميت على مستويات النواقل العصبية (الاسيتايل كولين والدوبامين) في الجرذان البيض الحوامل

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*** البحث مستل من رسالة ماجستير للباحث الثاني .**

ABSTRACT

The present study aimed to elucidate the effect of Topiramate drug on neurotransmitters levels (Acetylcholine and Dopamine) in pregnant albino rats for different periods (7,9,12,14 and 21 days) of gestation.The present study was conducted on female albino rats (n = 40). Their weights arranged between (195 – 285 gm). The pregnant albino rats were divided into 5 groups, each group includes 8 pregnant rats and each group subdivided into control and treated (control =4 , treatment= 4).The control groups administrated orally distilled water (1 ml), while the treated group administrated Topiramate drug (100 mg/kg b.wt) by gavage tube for 7, 9, 12, 14 and 21 days of pregnancy. The acetylcholine level represented significant decrease ($P \leq 0.05$) in treated animals in comparison with control groups, while the dopamine level recorded significant increase ($P \leq 0.05$) when compare with control groups.

Keywords : Topiramate , Acetylcholine , Dopamine , albino rat .

الخلاصة

هدفت الدراسة الحالية الـــى معرفة تأثير عـــقار التوبيراميت علـــى مستويات الـــنواقل العصـــبية (الأسيتايل كولين والدوبامين) في اناث الجرذان البيض الحوامل.

اجريت الدراسة على (40) انثى جرذ ابيض . تراوحت اوزانها بين (195-285)غرام . قسمت الاناث الحوامل الى خمسة مجاميع (8 حيوانات) ثم قسمت هذه المجاميع الى مجاميع ثانوية (4 سيطرة و4 معاملة). جرعت جميع اناث السيطرة بماء مقطر (1) مل بينما جرعت اناث المعاملة في المجاميع الخمسة بعقار التوبيراميت بجرعة(100 ملغم/ كغم) من وزن الجسم بواسطة انبوبة تجريع خاصة وللأيام 7و9و12و14و21 من الحمل . اظهر مستوى الأسيتايل كولين انخفاضا معنويا لحيوانات المعاملة مقارنة مع مجاميع السيطرة في حين سجل مستوى الدوبامين زيادة معنوية لحيوانات المعاملة بالعقار مقارنة مع مجاميع السيطرة .

** الكلمات المفتاحية :التوبيراميت ، الأسيتايل كولين ، الدوبامين ، الجرذان البيض .

INTRODUCTION

Topiramate (TPM) drug is a novel antiepileptic drug derived from the naturally occurring monosaccharide D-fructose. TPM drug is effective as initial monotherapy for generalized and partial seizures [17]. It is quite effective and safer for the pregnant than other antiepileptic drugs such as Valporic acid[19]. TPM drug has used to treat epilepsy in children and adults and it was originally used as anticonvulsant and also for the prevention of migraines [17].

Neurotransmitters are chemical secretion that secreted from vesicles located in the end of the nerve cell axis by the synapses, where it works on the transmission of nerve impulse by depolarizing the nerve cell membrane after tangles, then crushed and analyzed by special enzymes in order not to accumulate around neurons to allow field to return to the state of polarization [9].Neurotransmitters are manufactured in synaptic vesicles with a bond terminal and the fact that free, but in the end the movement and are stored in vesicles. The neurotransmitters are known as acetylcholine and catecholamine (which include dopamine, norepinephrine, epinephrine and Serotonin),and amino acids (which include Glutamate, glycine, GABA and aspartate) and gases (which include nitric oxide NO and carbon monoxide CO) [8].Neurotransmitters are manufactured in synaptic vesicles with a bond terminal and the fact that free, but in the end the movement and are stored in vesicles [3].

Neurotransmitter is released by the next action potential of the axis, as the leading wave of depolarization associated with the effort act to activate the calcium channels in the membrane by tangles, which causes calcium union interrelation vesicles with the membrane before entering the tangles and explode neurotransmitter towards synaptic cleft by process called output [20]. Clinical and experimental studies investigated the role of the major neuro-modulatory systems in epilepsy [12]. Acetylcholine [21] and dopamine [4] are all known to regulate seizure activity.

Acetylcholine (ACh) was the first neurotransmitter to be identified. It was discovered by Henry Dale in 1914. It was isolated in 1920 by a German biologist called Otto Loewi. Acetylcholine is one of the most abundant neurotransmitters in the human body. It is located in both the central nervous system(CNS) and the peripheral nervous system (PNS). Acetylcholine a neurotransmitter active both in the brain, where it regulates memory and in PNS, where it controls the actions of skeletal and smooth muscle, acetylcholine is synthesized in nerve terminals from its precursor choline which is not formed in the CNS but transported there in free form in the blood. It is found in many foods such as egg yolk, liver and vegetables although it is also produced in the liver and its brain concentration rises after meals [23].

Dopamine (DA) a neurotransmitter is discovered by a Swedish biologist called Arvid Carlsson in 1950. It is an inhibitory neurotransmitter meaning that when it finds it's way to it's receptor sites, it blocks the tendency of that neurons to fire. DA is considered to be both excitatory and inhibitory. In the brain, dopamine roles as a neurotransmitter released by neurons to send signals to other nerve cells, the brain

contains several different dopamine pathways, one of which plays a major role in reward-motivated behavior and others pathways are involved in motor control and in controlling the release of various hormones, these pathways and cell groups form a dopamine system which is neuromodulators [16].

The present study amid to evaluate the effect of Topiramate drug on the neurotransmitters levels (Acetylcholine and Dopamine) in pregnant albino rats.

MATERIALS AND METHODS

This study was conducted on pregnant albino rats to demonstrate the effect of Topiramate drug on neurotransmitters (Acetylcholine and Dopamine). This study included the following steps:

1.Experimental Animals

In this study, the animals were obtained from the animal house of the Biology Department / College of Science at University of Babylon. Animals were put inside special cages for breeding which has a length of 25 cm , 18 cm width and 19.5 cm height and were stayed about 30 days. The cages were covered with sawdust, which replaced three times in a week with the care of hygiene and sterilization. The animals were provided with food and water *ad libitum*. The animals were housed in special rooms with controlled conditions of temperature (24± 1oC^{o}) and natural light periods (12 hours light/dark) [6]. Each two females were put with one male in special plastic cages with strung metal caps with dimensions of 40 cm length, 25 cm width and 19.5 cm height.

2.Mating

The animals were selected with an average weight of 195- 280 gm and with an average age of 2.5-3 months, all animals were with good health. The mature females albino rats were put with males (one male rat for each two female rats). After ensuring the pregnancy by observing vaginal plug and vaginal smear[6]. Pregnant rats were isolated from males in separated cages with writing the mating date, the mating day was considered the 0th day of gestation (GD=0) and the next day was the first day of gestation (GD =1) [13].

3.Drug Dose Preparation

Topiramate drug was used in this study, which manufactured by Mylan Company, Australia. TPM has been obtained from the local pharmacy in Hilla-Iraq, each tablet contains 100 mg from the active substance. The tablets were yellow, round, biconvex, film-coated debossed with "G" on one side and "T" over "100" on the other. Each tablet of TPM were dissolved in 10 ml of distilled water. Determination of drug doses were depended on the animals body weight [18].

4.Experimental Design

40 female albino rats were used in this study (we were used 20 males in this study for mating only). Pregnant albino rats were divided into five groups (n=8), each group was subdivided into control and treated groups. Pregnant rats were arranged into groups according to the period of treatment for 7, 9, 12, 14 and 21 days of pregnancy:

Group I:
Control group (n=4) administrated (1ml) distilled water orally by gavage tube for 7 days of pregnancy.
Treated group (n=4) administrated orally 100 mg/kg b.wt. of TPM drug by gavage tube for 7 days of pregnancy.
Group II:
Control group (n=4) administrated (1ml) distilled water orally by gavage tube for 9 days of pregnancy.
Treated group (n=4) administrated orally 100 mg/kg b.wt. of TPM drug by gavage tube for 9 days of pregnancy.
Group III:
Control group (n=4) administrated (1ml) distilled water orally by gavage tube for 12 days of pregnancy.
Treated group (n=4) administrated orally100 mg/kg b.wt. of TPM drug by gavage tube for 12 days of pregnancy.
Group IV:
Control group (n=4) administrated (1ml) distilled water orally by gavage tube for 14 days of pregnancy.
Treated group (n=4) administrated orally 100 mg/kg b.wt. of TPM drug by gavage tube for 14 days of pregnancy.
GroupV:
Control group (n=4) administrated (1ml) distilled water orally by gavage tube for 21 days of pregnancy.
Treated group (n=4) administrated orally 100 mg/kg b.wt. of TPM drug by gavage tube for 21 days of pregnancy.

5.Neurotransmitters Procedures by Elisa Assay

Neurotransmitters were measured in serumby using Elisa kit (Elabscience, co, China) for both control and treatment animals according to the different dosing periods in pathological analysis laboratory in Biology Department - College of Science – University of Babylon.

5.1. Estimation of Acetylcholine

1.Add 100 µL of Standard, Blank, or Sample per well. Incubate for 90 minutes at 37 oCº.
2.Immediately add 100 µL of Biotinylated Detection Ab working solution to each well . Incubate for 1 hour at 37oCº.
3.Aspirate and wash 3 times.
4.Add 100µL of HRP Conjugate working solution to each well. Incubate for 30 minutes at 37°Cº.
5.Aspirate and wash 5 times.
6.Add 90µL of Substrate Solution to each well. Incubate for about 15 minutes at 37°Cº.
7.Add 50µL of Stop Solution to each well. Read at 450nm immediately.
8.Calculation of results. (Elabscience , co).

5.2.Dopamine Assay Procedures

1. Add 50µL standard or sample to each well.
2. Immediately add 50µLBiotinylated Detection Ab to each well.
3. Incubate for 45 minutes at 37°Cº.
4. Aspirate and wash 3 times.
5. Add 100µL HRP Conjugate to each well. Incubate for 30 minutes at 37°Cº.
6. Aspirate and wash 5 times.
7. Add 90µL Substrate Reagent. Incubate 15 minutes at 37°Cº.
8. Add 50µL Stop Solution. Read at 450nm immediately.
9. Calculation of results. (Elabscience , co).

RESULTS

1.Effect of Topiramate Drug on Acetylcholine level in Pregnant Albino Rats for 7, 9, 12, 14 and 21 days of Pregnancy.

The results of this study showed that acetylcholine means decrease significantly (P≤0.05) in treated groups (1, 2, 3 and 4) (7, 9, 12 and 14) days of pregnancy with TPM drug (100 mg/kg b.wt) (30.903±5.756, 43.7±5.36, 39.61±14.83 and 28.02±21.36) respectively, as compared with the control groups (52.84±8.07, 52.03±4.38, 50.43±5.03 and 52.62±2.98) respectively. While 21 days of pregnancy showed non-significant changes (P>0.05) in treated and control groups (Table 1).

Table(1):Effect of Topiramate drug on Acetylcholine level in pregnant albino rats for 7, 9, 12, 14 and 21 days of pregnancy.

Period (days) / Groups	Acetylcholine (Pg/ml)		Significance level
	Control Mean±S.D.	Treatment Mean±S.D.	
7	52.84±8.07	* 30.903±5.756	P≤0.05
9	52.03±4.38	* 43.7±5.36	
12	50.43±5.03	* 39.61±14.83	
14	52.62±2.98	* 28.02±21.36	
21	52.13±3.52	51.33±1.62	

*means significant decrease

2.Effect of Topiramate Drug on Dopamine level in Pregnant Albino Rats for 7, 9, 12, 14 and 21 days of Pregnancy.

Results of this study showed that dopamine means changed in treated groups with TPM drug (100 mg/kg b.wt), as compared with the control groups (Table 2). All groups (except 9 days of pregnancy) showed significantly increase ($P \leq 0.05$) for treated groups (197.66±13.650, 240.666±19.008, 116.066±15.772 and 291.20±6) as compared with control groups (140.826±7.944, 120.833±9.200, 112.666±15.534 and 145.40±5) respectively.

Table(2):Effect of Topiramate drug on Dopamine level in pregnant albino rats for 7, 9, 12, 14 and 21 days of pregnancy.

Groups / Period (days)	Dopamine (Pg/ml)		Significance level
	Control Mean±S.D.	Treatment Mean±S.D.	
7	140.826±7.944	* 197.66±13.650	P≤0.05
9	127.9136±26.996	130.556 ±9.932	
12	120.833±9.200	* 240.666±19.008	
14	112.666±15.534	* 116.066±15.772	
21	145.40±5	* 291.20±6	

* means significant increase .

DISCUSSION

1.Acetylcholine

As shown in table(1) the present data show that, the oral administration of TPM drug (100mg/kg b.wt) for 7, 9, 12, 14 and 21 days of pregnancy. A significant decrease ($P \leq 0.05$) in acetylcholine levels in 7, 9, 12 and 14 days of pregnancy of rats that treated with TPM drug (100mg/kg b.wt) in comparison with control groups. The disruptions of the neurotransmitter acetylcholine was recorded after exposure to TPM drug and was parallel with the observed inhibition of AChE activity. Accordingly, the decreased GSH is obviously related to the reduction of AChE due to oxidative inactivation of the enzyme thiols and formation of disulfide bonds resulting in reduction in AChE [24].

The significant alterations of brain neurotransmitter (AChE) in treated pregnant rats may be attributed to the increased rate of formation of O2 and H2O2 in the hippocampus and may be responsible for exerting neurodegenerative diseases and Lewy bodies aggregations[5]. The results of the current study is in agreement with results of those researchers who remind that TPM drug leads to increase GABA and increase GABAergic transmission [1].

2.Dopamine

As shown in table (2) the present data show the oral administration of TPM drug (100 mg/kg b.wt) for 7, 9, 12, 14 and 21 days of pregnancy showed increased significantly ($P \leq 0.05$) in dopamine levels. The role of DA in epilepsy is most likely mediated by the neuro-modulatory effect of this molecule on structures belonging to the basal ganglia and elements of the limbic system. These structures are strongly inter connected and defective DA signaling either in the basal ganglia or in the limbic system might affect the electric properties of neurons located at distal sites through either direct interactions or through feedback mechanisms connecting the cortex to the striatum or other areas. In agreement, it has been postulated that the DAergic transmission in the basal ganglia may provide an important main inhibitory role [15].

Anticonvulsants and clozapine may share common mood stabilizing mechanism since clozapine is reported to have mood stabilizing effects and increase prefrontal dopamine by 5HT1A receptor activation [11]. Carbamazepine produces an increase in the growth hormone because it may alter brain serotonin and dopamine functions in therapeutic and mild overdoses groups [7].

Several antiepileptic drugs increase extracellular (EC) levels of dopamin (DA) and/or serotonin (5HT) in brain areas involved in epileptogenesis.Behavioural and electrocorticographic studies in rats have shown at DA controls hippocampalexcitability via opposing actions at D1 and D2receptors. Seizure enhancement is presumed to be a specific feature of D1 receptor stimulation, whereas D2 receptor stimulation is anticonvulsant [14].

The results of this study is in disagreement with the results obtained by those researchers who found that decrease of dopamine concentration in brain in high dose of aspartame. They suggest that the reason go back to phenylalanine which is one of aspartame metabolism products and it works to block the neural amino acid transporters (NAAT) and then prevent essential amino acid tyrosine that necessary to synthesis dopamine from crossing blood brain barrier [2].

It has been reported that reduced calcium levels decrease calcium/calmodulin-dependent-dopamine synthesis in the brain, increasing the susceptibility to epileptic convulsions, and that there is an increase in the numbers of D2 receptors in the neostriatum. According to these findings, it may be concluded that decreased calcium levels and the resulting decreased dopamine synthesis lead to epilepsy, while convulsions and increased D2 receptors are a secondarily-induced phenomenon[22] and this results agreed with the results of the current study which showed the role of TPM drug in increase the dopamine level and decrease the epileptic convulsions. After the exposure of dopaminergic mesencephalic neuronal cell cultures to 6-hydroxydopamine or TPM a loss of dopaminergic neurons was observed[10].

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