ISSN: 0975 -8542

Journal of Global Pharma Technology

Available Online at: www.jgpt.co.in

RESEARCH ARTICLE

Effect of Carbonic Anhydrase Inhibitor Combination on Fertility Male Rats

Anmar Shukr Mahmood[1*], Mohammed A.S. Kamil[2], Ahmed Jasim Nawfal[3]

1. *Department of Pharmacology / University of Fallujah / Iraq.*
2. *Department of Medicine / University of Fallujah / Iraq.*
3. *Department of Physiology / University of Fallujah / Iraq.*

***Corresponding Author: Anmar Shukr Mahmood**

Abstract

Carbonic anhydrase inhibitors are many drugs, the main usage in epilepsy and raised intracranial pressure and the most commonly used are acetazolamide and topiramate. Twenty healthy adult males Wistar rats After acclimatization three weeks and divided to two groups randomly (10 rats/group) and treatment for 60 days as follows: high dose ($\mathbf{H_d}$) group administrated 30 mg/kg/B.W of Acetazolamide and 9mg/kg/B.W of Topiramate and low dose ($\mathbf{L_d}$) group received 5mg/kg B.W of Topiramate and 30 mg/kg B.W of Acetazolamide orally/day. Serum Hormones concentration tested are testosterone (ng/ml) and follicular stimulation hormones (FSH), Serum luteal hormones (LH) mlU/ml. The histological section showed the pathological changes in the $\mathbf{H_d}$ group while in the $\mathbf{L_d}$ have semi normal changes, the results confirm that the rats received of low dose more safety than the high dose of these drugs combination.

Introduction

Idiopathic intracranial hypertension (IIH) is a nervous disease characterized by increased intracranial pressure around the brain. It is resulted by increased production or decreased reuptake of cerebrospinal fluid (CSF). The standard treatment of IIH includes medicines to reduce CSF production. Acetazolamide is the most commonly used drug in treating IIH and is also considered as the first-line treatment of it. Topiramate is used to treat IIH and used to treat obesity which is a risk factor of IIH. We investigated the effect of topiramate as an adjunct drug along with acetazolamide in patients with IIH who presented to Shahid Sadoughi Hospital in Yazd, central Iran [1].

The standard treatments of IIH include carbonic anhydrase inhibitor drugs that reduce CSF production. Acetazolamide is a carbonic anhydrase inhibitor used to treat glaucoma and sometimes tonic-colonic, myoclonic, and atonic seizures, especially in women who develop seizure at menopause or whose seizure aggravates at the time of menstrual cycle [2]. Topiramate also a carbonic anhydrase inhibitor belongs to the second generation of antiepileptic drugs and is more effective in treating epilepsy and headaches. It is also used to treat psychotic disorders and obesity. Carbonic anhydrases (CAs) that participate in the regulation of ion, water, and acid-base balance by catalyzing the reversible hydration of carbon dioxide in a reaction: CO2 + H2O HCO3 - + H+ [3], their function is linked to sperm motility, fertilization, and embryonic development through acidification of epididymal fluid, alkalinization of seminal plasma and uterine fluid, and regulation of acid-base balance in amniotic fluid and the developing foetus [4].

Carbonic anhydrase is found in many portions of the female and male reproductive system, there have been no reports of infertility or reduced fertility associated with CA inhibitor therapy.

Materials and Methods

Twenty healthy adult males Wistar rats, weighed 200-250 gm were used and housed in plastic cages 70×50×15 cm and adaptation in an animal house/ Department of Pharmacology/College of Medicine/ Fallujah

University for three weeks. The animals were kept at 22 -25˚C, with 12h light/dark cycle. Animals were allowed freely access to water and pellets along the experimental period**.** After acclimatization rats were randomly divided into two groups (10 rats in each group) and treatment for 60 days as follows: **Hd** group administrated 9mg/kg/B.W of Topiramate and 30 mg/kg/B.W of Acetazolamide and **Ld** group administrated 5mg/kg B.W of Topiramate and 30 mg/kg B.W of Acetazolamide orally/day. Blood samples were collected at 0 and 60 days of the experiment. Blood was drawn by cardiac puncture technique from rats anesthetized by intramuscular injection of (Ketamine 90mg/Kg B.W. and Xylazine 40mg/kg B.W.).

Blood sample were kept in tube followed by centrifugation for 15 minutes at 3000 rpm. Serum were isolated and frozen at -20˚C until analysis. At the end of the experimental period, the animals were sacrificed using high-dose of (Ketamine and Xylazine) and their scrotum were opened and the testes were taken and fixed in 10% formalin solution, dehydrated in a graded alcohol series, embedded in paraffin wax, and serially sectioned using a microtome at 5 µm and stained using H&E (Hematoxylin and Eosin) staining and photographs were taken using a light microscope with a camera attachment has been carried out with image (Java-based image processing program developed at the National Institutes of Health).

Statistical Analysis

Values in tables are given as mean ± S.E. Data were analyzed using SPSS version 22. Differences between groups were analyzed by a one-way analysis of variance (ANOVA). *p*-value ≤0.001 were considered significant.

Hormones Test

Serum Hormones concentration tested are testosterone (ng/ml), and (follicular stimulation hormones (FSH), Serum luteal hormones (LH) ml U/ml.

Result

Table (1) showed significant differences (P<0.001) (P= 0.009) in the serum testosterone concentration in **Hd** (1.09 ± 0.07) and **Ld** (1.69 ± 0.13) of Topiramate and Acetazolamide after 60 days of experiment period when compared between to each other that clarified fall the level in **Hd** more than **Ld** group. However, it was lack of significant differences (P<0.001) between **Hd** (1.38 ± 0.22) and **Ld** (1.99 ± 0.18) of Topiramate and Acetazolamide in LH and FSH was detected after 60 days of experiment when compared to each other (LH: P= 0.082) (FSH: P= 0.111). Data in Table (2) revealed a significant (P<0.05) increase in serum Testosterone concentration in **Ld** group after 60 days of treatment with Topiramate and Acetazolamide (1.69 ± 0.14) compared with the pretreated period (1.18 ± 0.17) and **Hd** treated groups (1.09 ± 0.08).

However, rats treated with **Hd** showed a significant (P<0.05) decrease in serum FSH concentration at the end of experiment (1.69 ± 0.39) compared to **Ld** (2.67 ± 0.35) and pretreated period (2.23 ± 0.21). After 60 days of treatment the result also showed a significant decrease (P<0.05) in Serum concentration of LH in rats subjected to **Hd** (1.38 ± 0.23) and **Ld** after 60 days of the experiment as compared to other experimental groups. Histological examination results also supported the above findings. As histological changes were observed in **L d** and **Hd** groups (Figure: 1, 3) after 60 days of combination.

Discussion

There are no significant differences were found in rats administrated orally L_d and H_d of Topiramate and Acetazolamide combination in male reproductive hormones in both pretreated and after 60 days of treated period (Table:1, 2). A significant decline in serum testosterone concentration in H_d and no effect in L_d of Topiramate and acetazolamide groups (Table. 2) as compared with pretreated period (Table.1), these result compatible with [5, 7].

That is the antiepileptic drugs (AEDs) affect reproductive hormones differentially in male rats, and confirms that the long-term Topiramate and acetazolamide administration produces harmful effects on fertility and genital tract in adult male rats. Enzyme-inducing antiepileptic drugs result in decreased estrogen and androgens including testosterone. Level of sex hormone binding globulin (SHBG) is elevated. The combination of decreased reproductive sex hormones and elevated SHBG leads to reduced free reproductive hormones [8, 10].

Table 1: Clarified the treated values after 60 days of serum Testosterone (ng/ml), FSH (mlU/ml) and LH (mlU/ml) concentrations of two high and low dose groups of Topiramate and Acetazolamide combination

low dose	Day	Mean ± SE	P value
Testosterone (ng/ml)	zero day	1.18 ± 0.17	0.057
	60 day	1.69 ± 0.14	
LH (mlU/ml)	zero day	2.24 ± 0.28	0.49
	60 day	1.99 ± 0.19	
FSH (mlU/ml)	zero day	2.15 ± 0.31	0.308
	60 day	2.67 ± 0.35	
High dose	**Day**	**Mean ± SE**	**P value**
Testosterone (ng/ml)	zero day	1.2 ± 0.11	0.448
	60 day	1.09 ± 0.08	
LH (mlU/ml)	zero day	1.57 ± 0.29	0.627
	60 day	1.38 ± 0.23	
FSH (mlU/ml)	zero day	2.23 ± 0.21	0.274
	60 day	1.69 ± 0.39	

Values are expressed as mean ± SE, n = 8 animals/ each group.

Table 2: Showed the mean values of serum Testosterone (ng/ml), FSH (mlU/ml) and LH (mlU/ml) concentrations of two high and low dose groups of Topiramate and Acetazolamide combination before and after treated

60 day	group	Mean ± Std. Error	P value
Testosterone (ng/ml)	High dose	1.09 ± 0.07	0.009
	Low dose	1.69 ± 0.13	
LH (mlU/ml)	High dose	1.38 ± 0.22	0.082
	Low dose	1.99 ± 0.18	
FSH (mlU/ml)	High dose	1.69 ± 0.39	0.111
	Low dose	2.67 ± 0.34	

Values are expressed as mean ± SE, n = 8 animals/ each group.

As the AED combination dose was increased, consecutive semen analyses were completed and averaged for each dose, the results showed a noticeable decline in the serum FSH and LH measurements in H_d group when compared with L_d and pretreated groups, in the population of epileptic male patients, dysregulation of the gonadotropic hormones, sexual dysfunctions and a decrease in fertility capacity have been notified, one probable cause of the decline in fertility capacity, which was reported in some previous studies, is the effect of antiepileptic drugs [11, 12].

The role of the hypothalamic-pituitary axis (HPA), including the production of luteinizing, follicle-stimulating, gonadotropin-releasing, and prolactin hormones, the concentrations and metabolism of its end products, such as estrogen, testosterone, and dehydroepiandrosterone, appear to be change in many people with epilepsy. Effects of the disturbance itself and effects of antiepileptic drugs (AEDs) both show to participate to these hormonal changes, which may be associated with sexual dysfunction [13]. Hormones may increase susceptibility to develop seizures. The meaning of the interrelation between epilepsy, hormones, and hormonal therapy is currently emerging. Although, epilepsy and its medications are associated with hormonal disorders lead to altered endocrine reproduction and sexual functions [14]. The histological findings in rats testis of the present study reveal that the H_d of Topiramate and acetazolamide combination for 60 days induced histological alterations in testes (Figure: 1, 2). The sections of testis H_d group showed mononuclear cells aggregation in the lumen of seminiferous ducts and interstitial edema. A significant positive correlation was found between the carbonic anhydrase activity of the testes and carbonic anhydrase inhibitor drugs which is agreement with [15, 16].

That demonstrated carbonic anhydrase inhibitor significantly reduced the carbonic anhydrase activity in the testes, epididymides and ductus deferens. As the dose was increased, the carbonic anhydrase activity of the reproductive tract tissues decreased. The reduction in carbonic anhydrase activity was associated with significantly lower volumes of semen, spermatozoa and seminal plasma per collection. Section in the testis of animal treatment with L_d of Topiramate and acetozolamide combination shows normal arrangement of seminiferous tubules with complete spermatogenesis and no lesion (Figure: 3, 4), these result indicate negligible effect of low dose in combination two drugs on reproductive male system.

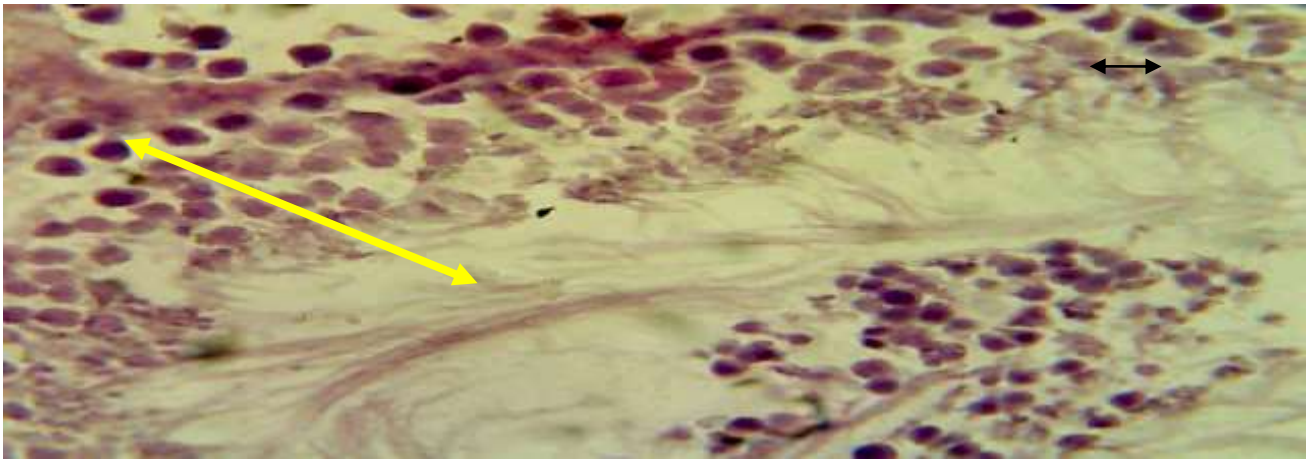

Fig. 1: Section in the testis of animal treatment with high dose (H_d) of Topiramate and acetozolamide combination shows mononuclear cells aggregation in the lumen of seminiferous ducts ⟷ (H and E stain 400x)

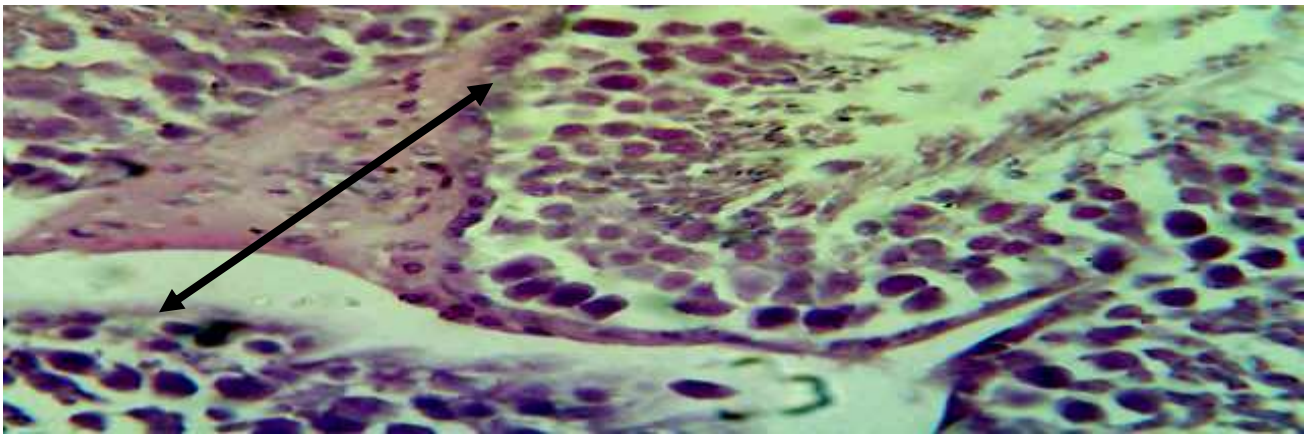

Fig.2: Section in testis of animal treatment with high dose of Topiramate and acetozolamide combination shows interstitial edema ⟷ (H and E stain 400x)

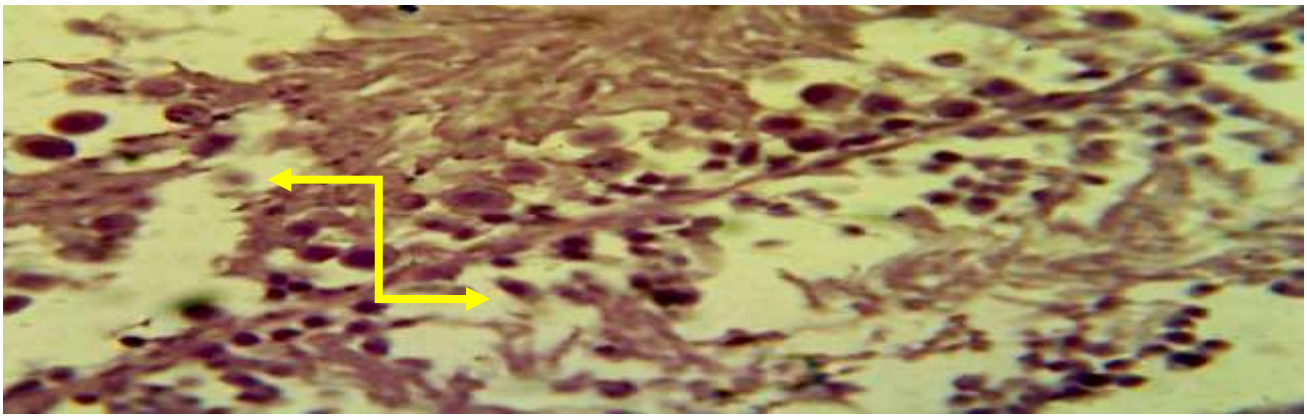

Fig.3: Section in the testis of animal treatment with low dose of Topiramate and acetozolamide combination shows normal arrangement of seminiferous tubules with complete spermatogenesis ⟷ (H and E stain 400X)

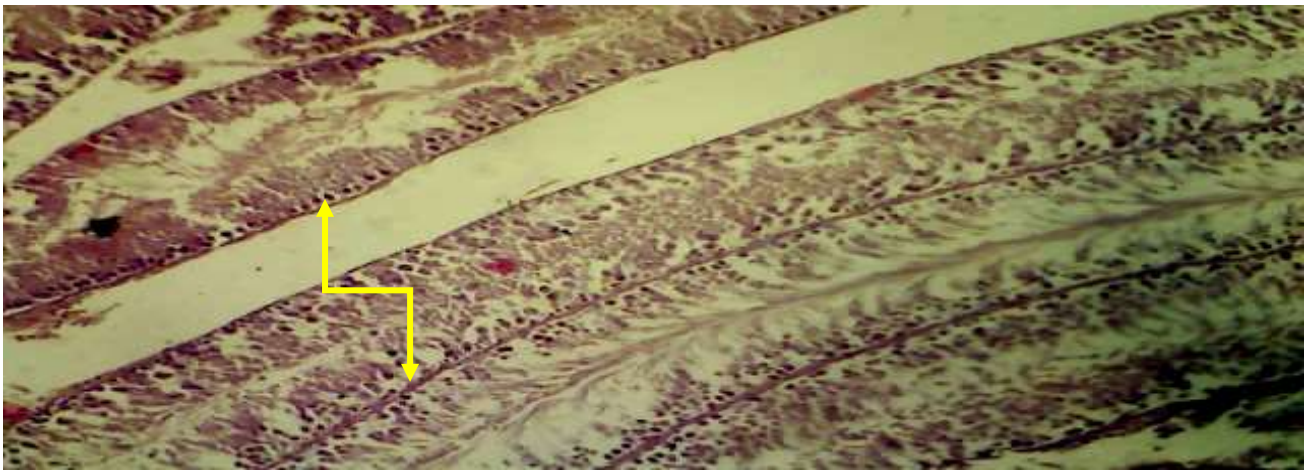

Fig.4: Section in the testis of animal treatment with low dose of Topiramate and acetozolamide combination shows normal arrangement of seminiferous tubules with complete spermatogenesis ⟷ (H and E stain 100X)

Conclusion

The aim of the present study was to ascertain the drugs of acetazolamide and topiramate had the greatest effect on lowering ICP in healthy rats, and so provide preclinical evidence to support their use in the pharmacological treatment of IIH. It is possible that if higher doses were used (although these would be less clinically relevant), a greater effect could have been seen as noted in other papers.

In addition, if a repeated dosing regimen was used, a higher steady state concentration of acetazolamide would likely have been reached, compared to our model where only a single dose was used. This is obviously also true of the other drugs, however, including topiramate [17, 18]. Reproductive endocrine dysfunction in men with epilepsy is an important issue, and in recent years there is growing evidence to support the effect on sex hormones of both epilepsy per se and various antiepileptic drugs (AEDs).

References

1. Rahimdel Meibodi, Abolqasem, 1 Melat Ardekani Ali, 1 Shafiee Mohammad, 2 Mansouri, Mehrdad, 2Akbarian, Abolfazl2 (2018) The effect of topiramate and acetazolamide in patients with idiopathic intracranial hypertension compared to acetazolamide alone. Bali Medical Journal (Bali Med J.) 7 (1): 201-204 .P-ISSN.2089-1180, E-ISSN.2302-2914
2. Binder DK, Horton JC, Lawton MT, McDermott MW (2004) Idiopathic intracranial hypertension. Neurosurgery, 54 (3): 538-52.
3. Sly WS, Hu PY (1995) Human carbonic anhydrases and carbonic anhydrase deficiences. Annu. Rev. Biochem., 64: 375-401.
4. Karhumaa, Pepe (2002) Carbonic anhydrases in the reproductive system With special emphasis on isoenzymes VI, IX, XII, and a novel nuclear non classical form Department of Anatomy and Cell Biology, University of Oulu, P.O. Box 5000, FIN-90014 University of Oulu, Finland Oulu, Finland.
5. Morrell MJ, Flynn KL, Seale CG, Done S, Paulson AJ, Flaster ER, Ferin M (2001) Reproductive dysfunction in women with epilepsy: antiepileptic drug effects on sex-steroid hormones. CNS Spectr., 6: 771-772 783-86.
6. Otoom S1, Batieneh H, Hassan Z, Daoud A (2004) Effects of long-term use Topiramate on fertility and growth parameter in adult male rats. Neuro Endocrinol Lett., 25(5):351-5.
7. David Spencer MD (2017) Levetiracetam in Men With Epilepsy: Testosterone Is Left Alone But Sperm Count Is Paramount. Epilepsy Curr., 17(2): 99-100.
8. Herzog AG, Drislane FW, Schomer DL, Pennell PB, Bromfield EB, Dworetzky BA, Farina EL, Frye CA (2006) Differential effects of antiepileptic drugs on neuroactive steroids in men with epilepsy. Epilepsia 47: 1945-1948.
9. Mohammad Reza Najafi, 1 Behnaz Ansari, 1 Mohammad Zare, 1 Farzad Fatehi, 2 Ali Sonbolestan1 (2012) Effects of antiepileptic drugs on sexual function and reproductive hormones of male epileptic patients. Iran J. Neurol., 11(2): 37-41.
10. Sigrid Svalheim[a] Line Sveberg[a] Monika Mochol[b] Erik Taubøll[ac] (2015) Interactions between antiepileptic drugs and hormones. j. Seizure. 02 022
11. Roste LS, Tauboll E, Haugen TB, Bjornenak T, Saetre ER, Gjerstad L (2003) Alterations in semen parameters in men with epilepsy treated with valproate or carbamazepine monotherapy. Eur. J. Neurol., 10(5): 501-6
12. Hatice Kose-Ozlece1, Faik Ilık2, Kursat Cecen3, Nergiz Huseyınoglu1, Ataman Serım1 (2015) Alterations in semen parameters in men wıth epilepsy treated with valproate. Iran J. Neurol., 14(3): 164-167
13. Morris GL 3rd[1], Vanderkolk C (2005) Human sexuality, sex hormones, and epilepsy. Epilepsy Behav., 7(2): S22-8. Epub 20.
14. Hamed SA[1] (2008) Neuroendocrine hormonal conditions in epilepsy: relationship to reproductive and sexual functions. Neurologist, 14 (3): 157-69. doi: 10.1097/NRL.0b013e3181618ada
15. Harris GC Jr, Goto K (1984) Carbonic anhydrase activity of the reproductive tract tissues of aged male fowls and its

relationship to semen production. J. Reprod. Fertil., 70: 25-30.

16. Setchell BP (1967) Fluid secretion by the testis. J. Reprod. Fertil., 14: 347-348.
17. Karimy, JK, Zhang, J, Kurland DB (2017) Inflammation-dependent cerebrospinal fluid hypersecretion by the choroid plexus epithelium in post hemorrhagic hydrocephalus. Nat. Med., 23: 997-1003.
18. William J Scotton*, Hannah F Botfield*, Connar SJ Westgate (2018) Topiramate is more effective than acetazolamide at lowering intracranial pressure. First Published, 13 Research Article

