ORIGINAL ARTICLE

Effects of long-term use Topiramate on fertility and growth parameter in adult Male Rats

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Abstract

PURPOSE: A case control study was conducted to assess the effects of long-term ingestion of Topiramate on fertility, body and reproductive organ weight and level of sex hormones in Sprague-Dawley male rats.

METHODS: Ten adult male rats were exposed to Topiramate diets at a concentration of 100 mg /kg for 60 days. Another ten control male rats received vehicle (distilled water). After 24 hours of the last dose, animals were autopsied under light anesthesia. Several parameters including, body and reproductive organ weight, sperm count and motility, serum testosterone, FSH, levels of cholesterol, triglyceride, SGOT and SGPT were all measured. Assessment of pregnancies in females mixed with tested males was also measured. The results of histological, histometrical and biochemical profiles were compared to that of the control group, and the significance of these results was measured using student's „t" and Chi- square tests.

RESULTS: Long-term ingestion of Topiramate for 60 days caused a significant decrease in spermatogenesis in seminiferous tubules of the testes. Sperm motility and density were also significantly reduced in cauda epididymides and testes of the treated group.The body weights and weight of reproductive organs (testes, epididymides, ventral prostrate and seminal vesicle) were decreased considerably. Hormonal assay also showed significant decrease in testosterone levels. Testicular cell population dynamics also demonstrated a decrease in the number of both primary and secondary spermatocytes and spermatids in the treatment group.The number of female rats impregnated by male rats on long-term Topiramate diet had decreased. The number of implantations and the number of viable fetuses were also notably decreased in female rats impregnated by male rats ingested Topiramate. Histometry of reproductive organs confirmed these results.

CONCLUSION: these results confirm that the long-term Topiramate ingestion produces adverse effects on fertility and reproductive system in adult male rat.

Introduction

Topiramate (TPM); 2,3:4,5-bis-O- (1 methylethylidene)-b-Dfructopyranose sulfamate; C12H21NO8S) is one of the new antiepileptic drugs with multiple mechanisms of action. Besides its clinical efficacy as an anticonvulsant agent, TPM may have activity to assuage or eliminate withdrawal from substances like ethanol and other addictive substances [1–5]. Topiramate (TPM) is a novel neurotherapeutic agent currently indicated mainly for the treatment of epilepsy and undergoing development for other central nervous system problems such as neuropathic pain, bipolar disorder, and migraine prophylaxis. TPM is synthesized from D-fructose and contains a sulfamate moiety that is essential for its pharmacologic activity [6]

The exact mechanism of TPM testicular toxicity is unknown. One mechanism could be due to decreased testosterone levels. Rats received Topiramate displayed slightly reduced basal serum testosterone levels; this reduction appears to be central nervous system-mediated [1–2]. However, it is unlikely that hormone changes can explain the testicular atrophy, since it has been shown that spermatogenesis can be maintained in the presence of significantly reduced intratesticular testosterone [3–4]. Thus, these data suggested that, other possible mechanisms should be considered and may raises the issue of the reproductive toxicity to high-level Topiramate exposure. The previous studies left a need for an adequate evaluation of the effect of TPM ON male fertility.

In this study, the effects of long-term ingestion of Topiramate on fertility, reproductive and growth parameter were investigated in adult male rats fed by 100 mg /kg Topiramate for a period of 60 days.

Material and method

Animals and treatment

Adult male albino rats of Sprague Dawley strain, weighing about 270 gm were raised in the Animal House Unit at Jordan University of Science and Technology, School of Medicine under controlled temperature of 21±1°C and 12:12 hr light/dark cycles. Food and water were available *ad libitum*. The control group (n=10) received vehicle. The treated group (n=10) received tablets of Topiramate (Topamax) in a dose of 100 mg /kg dissolved in distal water and given by intragastric administration (Gavages tube) for 60 days.

Experimental Design

Male rats were divided into two groups.Control Group: Rats of this group received vehicle for 60 days.Treatment Group: Rats of this group received powdered of Topamax (100 mg / kg) dissolve in distal water for reproductive cycle, 60 days. After 24 hours of the last dose, the animals were weighed and autopsied under light ether anesthesia. The blood was collected through cardiac puncture using a dry clean syringe for serum studies.

Fertility Test. Fertility tests was estimated in adult male rats received Topiramate and in the control male counterparts. Each male rat was placed in an individual cage with two virgin untreated females of the same strain; they were left together for ten days, during which two estrous cycles should have elapsed [5]. One week after the removal of the exposed males, females were killed by cervical dislocation under light ether anesthesia and the number of pregnant females, number of implantation sites, number of viable fetuses and number of resorptions were recorded.

Sperm Motility and Count. To determine sperm motility and sperm counts, 100 mg of cauda epididymides was minced in 2 ml of physiological saline. One drop of an evenly mixed sample was applied to a Neubauer's counting chamber under a cover slip.Quantitative motility expressed as a percentage was determined by counting both motile and immotile spermatozoa per unit area. Cauda epididymal and testicular sperm counts were made by routine procedure and expressed as million/ml of suspension [6].

Body and Organ Weights. Initial and final body weights of the animals were recorded. The reproductive tract was taken out trimmed free of fat and each organ was weighed separately on an electronic balance. The male reproductive organs used for the studyincluded testes, epididymides, ventral prostrate, seminal vesicle and vas deferens. Some vital organs such as liver, kidney, adrenal, heart and thyroid were also taken out and weighed. Reproductive organs along with a small piece of liver, heart and kidney were fixed in Bouin's fixative for histological studies.

Histological Studies. The Bouin's fixed reproductive organs (testes, epididymides, seminal vesicle, ventral prostate and vas deferens) along with liver, kidney and heart muscles were cut into small pieces and processed. The paraffin embedding was followed by section cutting (5 μm) and staining (Harris haematoxyline and eosin).

Histometry. With the help of Camera Lucida hundred circular appearing seminiferous tubules were traced at x80 and the diameter of each tubule was measured separately. The measurement was expressed as the mean of all the traced tubules. Similarly, Leydig cell nuclei were traced at x800. The epithelial cell height of cauda epididymides, caput epididymides and seminal vesicle were also traced at x360.

Testicular Cell Population Counting. Spermatogenic elements i.e. spermatogonia, spermatocytes and spermatids were counted in 5 μm thick cross sections of 10 seminiferous tubules in 10 animals of each group.All raw counts were transformed to true counts by an adaptation of Abercrombie formula [7] from the germ cell diameter measurement.

Interstitial cell types such as fibroblast, immature and mature Leydig cells and degenerating cells were estimated, applying a differential count of over 200 cell population and statistically verified by the binomial distribution [8].

Serum Biochemistry. Total protein, cholesterol, triglycerides, serum aspartate aminotranferase (AST),

and serum alanine aminotranferase (ALT) were obtained using commercial kits.

Hormonal Assays. Plasma FSH and testosterone concentrations were measured by Radioimmunoassay using commercial kits.

Statistical analysis. Data were expressed as mean ± standard deviation (SD). The differences between Topiramate exposed groups and control were analyzed using either Chi-square test or Student "t" test. P values less than 0.05 were considered significant [9].

Results

Effect of Topiramate on the body and organs weight. Table 1, shows that Topiramate caused a slight decrease in body weight, when initial and final body weights were compared in the experimental group.On the contrary, an increase in the body weight was observed in the control group.However, the weight of the testes, epididymides, seminal vesicle, ventral prostate and vas deferens were significantly decreased in the treated male rats when compared to the control group.

Effect of Topiramate on Sperm Dynamics and Histometrical Parameters. While sperm motility in cauda epididymides was significantly decreased in treated animals in comparison to the control, sperm density, seminiferous tubule diameter and Leydig cell nuclear diameter in treated male rats were significantly increased. Epithelial cell height in epididymides (cauda, caput and seminal vesicle) was also considerably increased (Table 2).

Effect of Topiramate on Testicular Cell Population Dynamics. Table 3, demonstrates that the administration of Topiramate caused a considerable decrease in the germinal cell population, spermatocytes (primary and secondary) and spermatids were also decreased to a significant level. Similarly the immature and mature Leydig cells numbers were also considerably decreased. However the degenerating cells number was greatly increased. The numbers of Fibroblast and spermatogonia were slightly decreased.

Effect of Topiramate on Serum Biochemical Markers. Results presented in Table 4, show that glucose, bilirubin, total cholesterol and triglycerides levels were within the normal range. Serum SGOT and SGPT levels were significantly increased in the treated group when compared to the control. On the contrary, levels of plasma testosterone were extensively decreased in the treated group when compared to the control group.However, FSH levels were significantly decreased.

Effect of Topiramate on Male Rat Fertility. Table 5, shows that the fertility of adult male rats ingested Topiramate was significantly reduced ($P<0.001$). This was reflected by a decrease in the number of pregnant females impregnated by Topiramate exposed males. However, the number of implantations and the number of viable fetuses were not statistically different (although decreased) from control group.The total number of resorptions was significantly increased ($P<0.001$) in females impregnated by male rats ingested Topiramate.

Discussion

In this study the effects of long-term exposure of Topiramate at a dose of 100 mg /kg/day on fertility and reproductive system in adult male rats were investigated.

Up to date, to our knowledge there are no published data showing the effects of long-term ingestion of Topiramate on various parameters of biological and reproductive capacity in adult male rats. The animal model used in this work has been previously used to assess the adverse effects of metal salts ingestion on behavior and fertility in small laboratory animals [10–11] without compromising the health of the experimental animals.

The dose of 100 mg /kg (ten times of the dose recommended in human) of Topiramate used in this study was selected because of the reported toxicity potentials of higher doses of this compound including decreased body weight, water consumption and clinical signs of toxicity such as dehydration, lethargy and hunched posture [12]. This dose was also selected to obtain broader range of information on the effects of Topiramate on growth parameters and reproduction.

In rats, the whole spermatogenic process requires 53 days out of which spermatozoa spend the last 6 to 7 days in the final transit through epididymides [13]. Topiramate was administrated for one complete spermatogenic cycle. The present study shows that, administration of Topiramate resulted in a decrease of all fertility parameter in male albino rats. The weight of all reproductive organs was markedly reduced when compared to that in the control group (Table 1). The weight, size and secretary function of testes, epididymes, seminal vesicles, ventral, prostate, vas deferens are regulated by androgens [14–15]. The drug may act

Table.1: Effect of Topiramate (100mg/kg) for 60days on body and organ weights male rats.

Treatment	Body weight (gm)		Testes	Epididymides	Seminal vesicle	Ventral Prostate	Vas deferens
	Initial	*Final*	*(mg/100 gm body weight*				
Control	267±12.21	296±11.76	925± 9.83	395± 8.62	404.58± 5.5	215± 3.01	87± 1.78
Topiramate	268±8.35	243±7.66	787***±8.66	321.21***±3.81	347.3***±9.37	163***±5.38	71*± 0.66

Results are expressed as mean ±S.D.
Ten rats were included per group.
*p < 0.05, **p < 0.01, ***p < 0.001 significantly different from control group (Student's "t" test).

Table 2: Effect of Topiramate (100mg/kg) on Histometrical parameters and sperm dynamics in male rats

Treatment	Sperm motility	Sperm densitymillion/ml		Seminiferous tubule	Leydig cell nuclear	Epithelial cell height		
	Cauda	Testes	Cauda	diameter	diameter	Caput	Cauda	Seminal vesicle
				μm				
Control	74.1±1.94	4.75±0.47	56.0±1.94	290.6±3.2	6.45±0.96	38.8±0.4	26.08±0.32	17.32±0.17
Topiramate	18.66***±1.07	1.07***±0.17	5.66***±1.33	73.6***±3.28	3.14***±0.66	12.36***±1.17	12.69***±1.88	9.85***±0..36

Results are expressed as mean ±S.D.
n rats were included per group.
*p < 0.05, **p < 0.01 ***p < 0.001 significantly different from control group (Student's "t" test).

Table 3: Effect of Topiramate (100mg/kg) on Testicular cell population dynamics of in male rats

Treatment Groups	Germinal cell types				Interstitial cell type			
	Spermatogonia	Spermatocyte(primary)	Spermatocyte(secondary)	Spermatids	Fibroblast	Immature Leydig cell	Mature Leydig cell	Degeneratingcell
Control	23.99±0.93	18.85±0.80	64.126±3.51	147.71±4.87	63.83±1.64	65.195±3.47	70.64±1.03	18.34±1.67
Topiramate	15.36**±1.53	10.46***±1.66	14.33***±2.84	49.73***±1.26	39.34***±1.14	40.35***±1.36	45.33***±1.32	63.13***±2.04

Results are expressed as mean ± D.
Ten rats were included per group. *p < 0.05, **p < 0.01 significantly different from control group (Student's "t" test).

Table 4: Effect of Topiramate (100mg/kg) on Serum biochemistry of male rats

Treatment	GlucoseMmol	CholesterolMmol	TriglyceridesMmol	Bilirubinμmol	SGOTU/L	SGPTU/L	Testosteronenmol/l	FSHIu/L
Control	7.3± 0.212	1.4±0.147	0.8±0.07	3.175± 0.142	36.7±1.66	77.7±2.12	14.4±2.53	21.87±0.47
Topiramate	7.12*±0.103	0.93*±0.04	0.75*±0.21	2.47*±0.66	91***±2.33	113 ***±1.75	9.33***±2.04	14.17**± 0.45

Results are expressed as mean ± D.
Ten rats were included per group. *p < 0.05, **p < 0.01 significantly different from control group (Student's "t" test).

Table 5: Effect of Topiramate (100mg/kg) on fertility in male rats.

Treatment	No.of male	No.of females	No.of pregnant females	No.of implantationsites	No.of viablefetuses	No.of resorption / total No.of implantation sites
Control group	10	20	18/20 (90%)	9.63± 2.66	9.37±1.16	4/173(2.31%)
Topiramate	10	20	11/20† (55%)	7.48***±1.63	7.32 ***± 1.44	8/71† (11%)

Results are expressed as mean ± D.
Ten rats were included per group. *p < 0.05, **p < 0.01 significantly different from control group (Student's "t" test). † p < 0.05 (chi -square test)

on pituitary gland and decreases the main hormone of spermatogenesis. The process of spermatogenesis and accessory reproductive organs function are androgen dependent. Decreased androgen production will cause a decrease in the number of mature Leydig cells and their functional status. In the present study the number of degenerating Leydig cells were significantly decreased, this reflects the decrease of androgen level (Table 2, 3). Previous report had confirmed this, by showing that a decrease in androgen caused a decreased in number of spermatocytes (both primary and secondary) and spermatids [16]. Significant decrease in the sperm motility was also observed in the treated group in this study compared to controls. This may be due to the effects of Topiramate on the enzymes of oxidative phosphorylation.

Table 4, show that serum SGOT and SGPT levels were significantly increased in the treated group when compared to the control. On the contrary, levels of plasma testosterone and FSH were significantly decreased in the treated group when compared to the control group.

The results presented in this paper show that ingestion of Topiramate by adult male rats decreased the number of females impregnated by the exposed males (Table 5). Also, the number of implantations and the number of viable fetuses were notably decreased. This effect may be due to a decrease in sperm motility and sperm density. It is important to mention that the effect of Topiramate on fertility, reproductive and growth parameter on adult male rats were not different from that of Lamotrigine, Gabapentin and Vegabatrin reported previously by our laboratory [17].

In conclusion, our results confirm that the long-term Topiramate ingestion produce adverse effects on fertility and reproductive system in adult male rat.

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