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ORIGINAL ARTICLE

The effect of Vigabatrin, Lamotrigine and Gabapentin on the fertility, weights, sex hormones and biochemical profiles of male rats

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Abstract

PURPOSE: A case control study was conducted to assess the effect of Sabril (Vigabatrin), Lamictal (Lamotrigine) and Neurontin (Gabapentin) on fertility in male rats. Their effect on the body and organs weight and certain biochemical profiles including total serum protein, cholesterol, triglycerides, serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), serum testosterone, and FSH levels were also measured.

METHODS: several parameters, concerning fertility were measured in 40 albino male rats of Sprague Dawley strain, they were divided into 4 groups, group one received vehicle (distilled water), group two received Vigabatrin in a dose of 200 mg/kg body weight, group three received Lamotrigine in a dose of 30 mg/kg body weight, and group four received Gabapentin 100mg/kg body weight. All the male rats in these groups received the different medications for a complete reproductive cycle (60 days). After 24 hours of the last dose, the animals were weighed and autopsied under light ether anesthesia. Parameter of fertility that has been measured in this study includes: sperm count and motility, weight of different reproductive organs, germ cell and interstitial cell population, serum testosterone and FSH levels and assessment of pregnancies in females mixed with tested males. Biochemical profiles such as serum cholesterol, serum triglycerides, serum bilirubin, SGOT, SGPT level are all measured. The results of the histological, histometerical studies and biochemical profiles were compared to that of the control group, and the significance of these results was measured using student's "t" test.

RESULTS: There was significant reduction in the body weight and the weight of the testes, epididymis, seminal vesicles, ventral prostate, and vas deferens in the antiepileptic fed male rats in comparison to the control group ($p>0.001$). There was significant reduction in testicular cells population dynamics including both germinal cell types and interstitial cell types in the antiepileptic fed male rats in comparison to the control group. There was also significant reduction in histometrical parameters and sperm dynamics in the antiepileptic fed male rats his-

tologies in comparison to the control group. There was significant reduction in both testosterone and FSH levels ($p<0.001$) in the antiepileptics fed male rats in comparison to the control group. There was also significant reduction in pregnancy rate observed in female rats exposed to the tested male rats among antiepileptic fed male rats compared to controls. The results of biochemical profiles assessment showed significant reduction in serum glucose, serum cholesterol, serum triglycerides levels and significant increase in serum bilirubin, SGOT, and SGPT levels in antiepileptics fed male rats in comparison to the control group.

CONCLUSIONS:

Fertility rate and other parameters concerned with fertility, sex hormones and certain biochemical profiles were significantly disturbed in male rats fed with three of the second-generation antiepileptic drugs Vigabatrin, Lamotrigine, and Gabapentin, indicating a possible toxic effect of these three medications on sexual organs, liver, and lipid metabolism.

Introduction

Pharmacotherapy with antiepileptic drugs is the mainstay of treatment of epilepsy and the reduction of epileptic fits in epileptic patients. An expansion of the use of these drugs in the treatment of other central and peripheral nervous system disorders has been observed lately.

In recent years several new antiepileptic drugs (AEDs) have been licensed forward to be in use in general and neurological practice and they are designs as second-generation AEDs (1). These drugs have proven efficacy as add-on therapy in resistant cases of partial and generalized epilepsy, as 20–50% of patients with add-on trials experienced a seizer reduction of >50% (1). Monotherapy trials have been conducted, the newer drugs were often as efficacious as conventional drugs, and their tolerability is even better. However the methodology of the trials can be criticized (2). These drugs include: Felbamate, Vigabatrin, Lamotrigine, Levetiracetam, Oxcarbazepine, Topiramat, Vigabatrin and Zonisamide.

The effects of AEDs on serum sex hormones have mainly been studied in male patients; however, several studies have been published recently evaluating female patients. Patients with epilepsy have reduced fertility and suffer from hyposexuality more frequently compared to average population (3,4).

Vegabatrin (gamma-venyl GABA) is an inhibitor of GABA transaminase enzyme, was found to produce dose related reduction of food intake in rats after both single (125–1000 mg/kg IP or 500 mg/kg PO) and repeated (250 mg/kg/dayIP) administration, this suggest a role of Vigabatrin in weight reduction through its anorexic effect (5,6)

Lamotrigine does not show an effect on weight in human epileptic patients in comparison to Valproat-treated patients in another study (7). This controlled study was conducted to assess the effect of selected second-generation antiepileptic drugs Vigabatrin, Lamotrigine, and Gabapentin on male rats regarding, fertility, weight of body and organs, sex hormones and certain biochemical profiles.

Material and methods

Animals and treatment. Adult male and female albino rats of Sprague Dawley strain, weighing about 300 gm. were raised in the Animal House Unit in Faculty of Medicine at Jordan University of Science and Technology under controlled temperature of 21 ±1C and 12 hours light and 12 hours darkness schedule (lights on 06.00 –18.00 hr). Food and Water were available *ad libitum*.

Drugs material and Doses. Drugs material was obtained from a local market (pharmacy) in Irbid (Jordan). This material was dissolved in distilled water and administered orally to rats using animal feeding intubations needles (Popper and Sons, New York). Doses were selected by doubling the maximum dose recommended for human and was given to rats once daily.

Experimental Design. Male rats were divided into following groups:

Group 1 – Intact (Control): The rats of this group received vehicle (distilled water) for 60 days.

Group 2 – Intact + Vigabatrin [200 mg/kg body weight for reproductive cycle, 60 days]

Group 3 – Intact + Lamotrigine [30 mg/kg body weight for reproductive cycle, 60 days].

Group 4 – Intact + Gabapentin [100 mg/kg body weight 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 and clean syringe, for serum studies.

Fertility Test. Fertility was estimated in adult male rats treated with solution of different medicinal drugs groups and in control male's counterparts. Each male 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 (8). 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 resorption were recorded.

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

Body and Organ Weights. The initial and final body weight of the animal was recorded. The reproductive tract was taken out trimmed free of fat and each organ was weighed separately on electronic balance. The reproductive organs taken into account for study in male include testes, epididymis, 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, epididymis, seminal vesicle, ventral prostate, 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 um) 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 in terms of mean of all the traced tubules. Similarly, Leydig cell nuclei were traced at x800. The epithelial cell height of cauda epididymis, caput epididymis and seminal vesicle were also traced at x360.

Testicular Cell Population Counting. Spermatogenic elements i.e. spermatogonia, spermatocytes and spermatids were counted in 5 um 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 (9) from germ cell diameter measurement.

Interstitial cell types (such as fibroblast, immature and mature Leydig cells and degenerating cells) were estimated, applying a differential count over 200 cells population and statistically verified by the binomial distribution (10).

Serum Biochemistry. Total serum protein, cholesterol, triglycerides, serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT) were measured using commercial kits.

Hormonal Assays. Plasma FSH and testosterone concentrations was measured by Radio immunoassay using two commercial kits.

Statistical Calculation. All the values of body/organ weight biochemical estimation and histometry were expressed in terms of mean value ± S.D. The different treatment groups were compared with control group using chi-square test and Student's "t" test (11).

Results

The results of our study are detailed in the followings tables:

Table 1, designed to show the body and organ weights of different medicine fed male rats, it showed significant reduction in the testicular, epididymis, seminal vesicles, ventral prostate and vas deferens weights in the Vigabatrin, Lamotrigine, and Gabapentin fed male rats in comparison to the normal controls ($p<0.05$, $p<0$, 01, and $p<0.001$) the results were also expressed as mean standard deviation.

Table 2, was designed to show the histometrical parameters and sperm dynamics of different medicine fed male rats, it showed significant reduction in sperm motility (%), sperm density (million/ml), seminiferous tubules diameter, leydig cell nuclear diameter, and epithelial cell heights in the Vigabatrin, Lamotrigine and Gabapentin fed male rats in comparison to the control group ($p<0.05$, $p<0.01$, and $p<0.001$). the results were also expressed as mean standard deviation. Minor differences are observed in the analysis of the results obtained from these three drugs.

Table 3, shows the results of testicular cell population dynamics of different medicine fed male rats, it showed significant reduction in all germinal cell types (spermatogonia, primary and secondary spermatocytes), and in all interstitial cell types (spermatid, fibroblast, immature leydig cell, mature leydig cell) and increase in the number of degenerating cells in Vigabatrin, Lamotrigine, and Gabapentin fed male rats in comparison to the control group ($p<0.05$, $p<0.01$

Table.1: Body and organ weights of **Different medicine** fed male rats.

Treatment	Body weight (gm)		Testes	Epididymis	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
Vigabatrin	296±12.35	170.3±9.66	787***±8.66	263.16***±3.81	343.6***±9.37	134***±5.38	68*± 0.66
Lamotrigine	273±5.77	243±4.71	874***±6.73	311***±5.36	358***±7.66	168.6***±3.66	77*±1.03
Gabapentin	287.5±15.77	277±4.71	937 ***±16.73	306.87***±3.87	363**±3.88	162.33**±4.71	71.23*± 1.22

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: Histometerical parameters and sperm dynamics of **Different medicine** fed male rats

Treatment	Sperm motility %	Sperm density million/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
Vigabatrin	7.272***±0.17	0.107***±0.02	0.278***±0.08	69.0***±1.35	2.96***±0.423	9.66***±0.876	6.33***±1.14	5.46***±0.115
Lamotrigine	17.34***±1.08	1.55***±0.14	6.185***±1.08	91.27***±21.35	3.79***±0.762	14.68***±2.66	13.4***±2.68	8.45***±0.27
Gabapentin	19. 96***±0.66	1.83***±0.14	11.33***±1.75	104.59***±15.36	4.03***±1.06	17.29***±1.87	17.03***±4.22	12.56***±2.33

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 .3: Testicular cell population dynamics of **Different medicine** fed male rats.

Treatment Groups	Germinal cell types			Interstitial cell type				
	Spermatogonia	Spermatocyte (Primary)	Spermatocyte (Secondary)	Spermatids	Fibroblast	Immature Leydig cell	Mature Leydig cell	Degenerating Cell
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
Vigabatrin	9.87***±1.84	4.66***±1.41	5.31***±2.84	21.33***±1.34	28.70***±0.95	32.40***±1.24	34.10***±1.18	107.0***±1.19
Lamotrigine	17.05***±4.44	12.96***±2.36	17.97***±3.73	53.37***±1.18	38.66***±0.95	41.66***±1.24	46.66***.±0.78	48.33***± 2.78
Gabapentin	14.11*±2.36	11.33**±2.41	13.77***±4.33	39.32***±1.82	34.18**±1.33	36.17****±1.65	43.97. ***±0.78	52.73***±0.76

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 4: Effect of fed **Different medicine** intact on male rats fertility.

Treatment	No. of male	No. of female	No. of pregnant females	No. of implantationsites	No. of viable Fetuses	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%)
Vigabatrin	10	20	9/20† (45%)	7.36***±2.83	7.25 ***± 1.67	9/66† (13.6%)
Lamotrigine	10	20	13/20† (65%)	8.49***± 3.31	6.63 ***±1.54	6/110(5.45%)
Gabapentin	10	20	14/20† (75%)	8.82***± 3.31	6.93***± 1.54	5/123† (4.06%)

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). † P < 0.05 (chi -square test)

Table 5: Serum biochemistry of **Different medicine** fed male rats.

Treatment	Glucose Mmol	Cholesterol Mmol	Triglycerides Mmol	Bilirubin µmol	SGOT U/L	SGPT U/L	Testosterone nmol/l	FSH u/L
Control	7.3± 0.212	1.4±0.147	0.8±0.07	3.1750.142±	36.7±1.66	77.7±2.12	14.4±2.53±	21.870.47±
Vigabatrin	6.16***±0.301	0.89***±0.33	.66***0±6.89	4.13***±0.45	104***±2.77	54.33***±3.11	8.87***1.07±	14.83***± 0.23
Lamotrigine	5.76***±0.45	1.25***±0.165	0.74***±4.83	3.37***±0.901	76***±1.88	103***±1.66	10.48***±0.02	17.28***±0.23
Gabapentin	5.33***±0.45	1.06***±0.66	0.73***±2.19	3.86***±1.04	82***±2.33	115***±1.66	9.36***±2.09	16.37***±0.48

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).

and p<0.001) the results were also expressed as mean standard deviation.

Table 4, shows the effect of different medicine fed male rats fertility, it showed significant reduction of number of pregnant female, number of implantation sites, and number of viable fetuses in Vegabatrin, Lamotrigine, and Gabapentin fed male rats copulated with virgin females rats in comparison to the control group (p<0.001).

Table 5, shows serum biochemistry profile of different medicines fed male rats including serum glucose, serum cholesterol, serum triglycerides, serum bilirubin, SGOT, SGPT, and the level of sex hormones including testosterone, and FSH. There was significant reduction in serum glucose, cholesterol, triglycerides levels in comparison to the control group, and significant increase in liver enzymes in comparison to the control group. There was also significant reduction in sex hormones level in comparison to the control group.

Discussion

The autopsy of the Vigabatrin, Lamotrigine, and Gabapentin fed male rats and the control group showed significant reduction ($p<0.05$, $p<0.01$ and $p<0.001$) in testicular, epididymides, seminal vesicles, ventral prostate and vas deference weight in comparison to the control group (Table 1).

In our study the sperm motility and sperm density (million/ml) showed significant reduction in all second-generation antiepileptic drugs fed male rats ($p<0.001$) in comparison to the control group (Table 2). The same was also noticed regarding seminiferous tubes diameter, leydig cells diameter and epithelial cell height in the caput, cauda, and seminal vesicles showing more significant reduction in these parameters in the histological examination of the testes of the antiepileptic fed male rats in comparison to the histological findings observed in the control group.

Regarding germinal cells population; our study showed highly significant reduction in the germinal cell population in Vegabatrin, Lamotrigine and Gabapentin fed male rats ($p<0.05$, <0.01 and <0.001) in comparison to the control group. Histological examination of the testicle of different medicine fed male rats and the control group also showed highly significant reduction in the population of all interstitial cell types ($p>0.001$) in the 3 antiepileptic fed male rats in comparison to the control group (Table 3).

We think that there is a possible toxic effect of these three antiepileptic drugs on sex organs and spermatogenesis but unfortunately we could not find similar studies to compare these results to, neither in male rats nor in humans.

Testosterone and FSH levels were found to be significantly reduced in antiepileptic fed male rats in comparison to the control group (Table 5), this may suggest another possible role of reduction in fertility and libido among the antiepileptics fed male rats.

Several studies on humans targeting the level of sex hormone in both males and females showed no significant affection of sex hormones or adrenal and gonadal steroids in patients using Gabapentin or Lamotrigine (12). Schwartz (13) have reported the same findings postulating that this may due to the fact that, these drugs do not affect the P450 liver enzyme in humans thus they don't alter the metabolism of endogenous steroids. Isogarvi et al, (14) founded that Lamotrigine decreases the level of testosterone in female patients with polycystic disease of the ovaries. The effect of these drugs found in our study may be attributed to the higher dosages of these antiepileptic drugs used in testing these animals in comparison to the conventional treatment of the same drugs in human epileptic patients. We are unable to find any study on the effect of vigabatrin and sex hormones or gonadal steroids in rats or humans.

This study also shows a significant reduction in the number of pregnancies, fertilization and implantation sites ($p<0.001$) occurs after exposing these second generation antiepileptcs fed male rats to virgin females of the same species, in comparison to the control group (Table 4).

There was a significant reduction in body weight of rats (Table 1) observed in all the three antiepileptics fed male rats in comparison to the control group. This finding was in consistence to previous reports on the effect of Vigabatrin on rats (5,6). In contrary to studies on humans on the Gabapentin and Vigabatrin-treated epileptic patients using conventional doses had revealed risk of weight gain in those patients (2). Biton et al. (7) reported that Lamotrigine-treated patients do not show any change in their weight in comparison to Valporate-treated patients. This controversy of the effect of second-generation antiepileptic drugs on body weight may be due to the different doses used or due to the differences between humans and rats.

The results of biochemical profiles assessment showed significant reduction ($p<0.001$) in serum glucose, serum cholesterol, serum triglycerides levels and significant increase in serum bilirubin, SGOT, and SGPT levels (Table 5) in the antiepileptic fed male rats in comparison to the control group, This may be explained by the induction of liver enzymes in the antiepileptics fed male rats, a finding similar to other studies in human epileptic patients, however there is less induction of liver enzymes in the second-generation antiepileptic drugs in comparison to the first generation antiepileptic drugs (15,16).

Conclusions

Fertility rate and other parameters concerned with fertility, sex hormones and certain biochemical profiles were significantly affected in male rats fed with three of the second-generation antiepileptic drugs Vigabatrin, Lamotrigine, and Gabapentin, compared to controls, indicating a possible toxic effect of these three medications on sexual organs, liver, and lipid metabolism.

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