

EFFECT OF SODIUM VALPROATE ON SELECTED REPRODUCTIVE HORMONES, LIPID PROFILES AND OVARIAN HISTOLOGY IN FEMALE RATS

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ABSTRACT

This study was conducted to evaluate the effect of sodium valproate (VPA) on reproductive hormones (follicle-stimulating hormone (FSH), luteinizing hormone (LH) & total testosterone), lipid profiles, total cholesterol (TC), triglyceride (TG), high density lipoprotein (HDL), low density lipoprotein (LDL) & very low density lipoprotein (VLDL), ovarian weight & histology in non epileptic female rats. Twenty-four sexually mature female Sprague-Dawley rats were included in this study, divided randomly into 3 groups, each one included 8 rats. Blood samples were collected from one group (8 rats), then dissected before starting the treatment & experimental parameters were measured. The other 2 groups, group I received distilled water & considered as control group, group II received VPA for 56 days. After treatment, blood samples were collected from animals then killed for measurement of previously mentioned parameters. VPA caused significant reduction ($P < 0.05$) in serum FSH & insignificant changes in serum LH & serum total testosterone. VPA treatment significantly reduced weights of ovaries ($P < 0.05$) but insignificantly affect lipid profiles. Ovaries of VPA treated rats did not show features of polycystic ovaries & their histology appeared similar to normal tissue. Numbers of corpus luteum & numbers of follicular cysts did not change significantly in these ovaries. It was concluded that sodium valproate did not produce changes in reproductive hormones (except FSH), lipid profiles & ovarian histology which were characteristic of polycystic ovarian syndrome (PCOS) in non epileptic female rats.

INTRODUCTION

PCOS is one of the more common endocrine disorders among reproductive age women.^[1] It is an important health concern & may represent a major health issue affecting young women today.^[2] PCOS was first described as a single disease by Stein & Leventhal in 1935, but now has been separated into several distinct entities comprising symptoms complex.^[3] It is extremely common disorder affecting 4-12% of women in reproductive age.^[4,5] Although this varies depending on diagnostic criteria used.^[6] PCOS can be diagnosed if 2 of the following 3 criteria were present, after the exclusion of other etiologies: 1) *Oligomenorrhea &/or anovulation.* 2) *Clinical &/or biochemical signs of hyperandrogenemia.* 3) *Polycystic ovaries by ultrasonography.*^[7] Actually it is a syndrome involving various organs & different symptoms & clinically presents as menstrual disturbances, infertility, excessive body weight & hirsutism.^[8] The etiology of PCOS remains obscure & there is probably more than one cause^[9] but several lines of evidence suggest that it is familial disorder.^[10] PCOS an important cause for long-term problems & women with this syndrome have an increased prevalence of hyperlipidaemia,^[11] hypertension,^[12] cerebrovascular disease^[13], cardiovascular morbidity,^[14] type 2 diabetes,^[15] endometrial

hyperplasia^[16] & endometrial carcinoma.^[17] Women with epilepsy have an apparent increased finding of polycystic ovaries and to a lesser degree of PCOS.^[18] Several studies have described an association between epilepsy and PCOS in women under treatment with various anti epileptic drugs (AEDs). One possibility explaining this association is that the seizure disorder itself increases the risk of PCOS; This hypothesis is supported by observations of an increased incidence of polycystic ovaries in un-medicated epileptic women or in treated women with epilepsy regardless of the antiepileptic therapy that is used.^[18] Another possibility is that, AEDs use may be a causal or contributory factor in increasing the prevalence of PCOS in epileptic women. These drugs can alter reproductive hormone levels & promote the development of reproductive endocrine disorders, especially PCOS. Among these AEDs, VPA has been associated with the development of characteristic PCOS features. The risk appears to be particularly high when valproate use is started in childhood or adolescence.^[19] The first association between VPA & polycystic ovaries was reported in 1993. It was found that 43% of epileptic women treated with VPA monotherapy had polycystic ovaries, compared with 22% of women treated with CBZ alone & 18% in normal controls.^[20] A

second supportive report for an association between VPA & polycystic ovaries was demonstrated in 1996. It was found that 50% of 22 obese women with epilepsy, treated with VPA, had insulin resistance & elevated androgen levels^[25], which are features of PCOS. VPA produced ovarian cysts in non epileptic Wistar rats at very high, supra therapeutic doses.^[21] while reproductive hormonal changes occurred only one month after the utilization of VPA as therapy for epilepsy in either gender.^[22]

The aim of this study was to evaluate the effect of sodium valproate on reproductive hormones (FSH, LH & total testosterone), lipid profiles & ovarian histology in non epileptic female rats.

MATERIALS & METHODS

Animals: 24 sexually mature female Sprague-Dawley rats used in this study which were supplied by & housed in the animal house of Kufa Medical College. All rats age ranged from 16-18 weeks & their weights ranged from 190-210 grams. They were housed in controlled conditions, temperature ranged from 22-25°C & 12:12 hours light-dark cycles. They had free access to drink tap water & fed standard chow diet. Each 5 rats were housed together in a standard cage for rats. Before starting the treatment, blood samples were taken from 8 rats (*in estrus stage of estrus cycle as determined by vaginal smears*) for measuring serum FSH, LH, total testosterone & lipid profiles; then killed, dissected & their ovaries were taken for determination of ovarian weight. These values were considered as baseline parameters. The remaining 16 rats, were randomly divided into two groups, each group included 8 rats, one group received distilled water & considered as control group. The second group received sodium valproate. The treatment was continued for 52 to 56 days.

Sodium valproate: "Depakine syrup" was used in this study, manufactured by Sanofi Winthrop Industries (France), Batch no. 285. Each 1.75 ml of syrup contains 100 mg of sodium valproate. A dose of 600 mg/kg had been used.^[21] The dose was calculated according to body weight of the rat & given orally once daily at same time each day through gastric tube.

Vaginal Smears: Hormone levels vary in female rats depending on estrus cycle stage^[23], so microscopic examination of vaginal smears

was considered as routine method to determine the stage of the estrus cycle of female rats in reproductive researches.^[24] Before starting the experiment (for 8 rats) & after 52 days of treatment (for all rats used in the experiment), vaginal smears were done in the morning at about 8.00 A.M, to determine which animal was in the estrus stage of estrus cycle in order to kill them,^[25] while animals in other stages of cycle (proestrus, metestrus or diestrus) received treatment according to their groups & left for another vaginal smears in the next day. This was done for 4 successive days. So all female rats were killed in estrus stage of estrus cycle. Vaginal smears were done as follow^[23]: A sterile cotton-tipped swab was moistened with sterile saline then inserted in the vaginal opening, rotated gently against the dorsal vaginal wall & withdrawn. Then the swab was immediately rolled onto a glass slide & allowed to air dry. All slides were then stained with Methylene blue & left to dry, then washed by running tap water, left to dry again then examined under a microscope.

Animal Sacrification: Before starting the experiment (for 8 rats) & after 52 days of treatment for all groups, female rats in estrus stage of estrus cycle (as proved by vaginal cytology) which were also fasting over night, were anaesthetized by using chloroform. Then 5 ml of blood were drawn from each animal by cardiac puncture. These blood samples were put in serum tubes, and then serum was separated by centrifugation (3000 xg for 15 min).

The anaesthetized rat was dissected in order to get ovaries. Both ovaries were removed from each rat, cleaned from fat & connective tissue which were attached to them, dehydrated by filter paper then weighed by using balance, and then the ovaries were kept in 10% formalin solution for fixation & preparation of histological sections.

Immunoassay: Serum FSH & LH were determined by immunoradiometric assay (IRMA) according to procedures & kits specific for each hormone supplied by Immunotech company (USA)^[26,27] using gamma counter (Hettich, Germany). Serum total testosterone was determined by radioimmunoassay (RIA) according to procedure & kit specific for this hormone supplied by Immunotech company

(USA)^[28] using gamma counter (Hettich, Germany).

Serum lipid profile assay: Total cholesterol, triglycerides, high density lipoproteins were measured according to procedures supplied by BioMerieux Company using Shimadzu UV-visible 1650PC spectrophotometer^[29]. Serum LDL & VLDL were measured according to the Friedewald equation^[30]:

$$\text{VLDL} = \text{TG}/5.$$

$$\text{LDL} = \text{TC} - \text{HDL} - \text{VLDL}.$$

Histological Sections: were prepared according to a method reported by Drury & Wallington.^[31] Four sections were made & examined for each ovary (right & left ovaries were studied for each rat). These sections were made at maximum transverse diameter of ovary. The sections were examined microscopically under magnification power $\times 4$, $\times 10$, $\times 40$ for features of PCO. The numbers of corpus luteum & numbers of

follicular cysts were also calculated in each histological section to determine variations between groups if present.

Statistical methods: The data expressed as mean \pm SEM unless otherwise stated. The statistical analysis had been done by using paired t-test to compare between baseline & after treatment parameters of each group. Chi-square test was used to compare between the proportions of histopathological changes. Values of $P < 0.05$ were considered statistically significant.

RESULTS

Effect of the treatment on hormones: Serum FSH level significantly decreased ($P < 0.05$) in VPA group, but not in control group. Serum LH & total testosterone did not significantly changed in both VPA & control group, (**Table-1**).

Table 1. Mean values of serum FSH, LH, total testosterone in control & VPA groups, before & after 56 days of treatment with VPA (600 mg / kg) (no.= 8 in each group).

Parameters	Control group			VPA group		
	Before treatment	After treatment	P-value	Before treatment	After treatment	P-value
FSH (IU/L)	8.154 \pm 1.79	8.334 \pm 1.79	NS	8.154 \pm 1.79	4.725 \pm 1.52	$P < 0.05$
LH (IU/L)	0.304 \pm 0.02	0.30 \pm 0.02	NS	0.304 \pm 0.02	0.292 \pm 0.03	NS
Total testosterone (ng/l)	0.204 \pm 0.10	0.217 \pm 0.11	NS	0.204 \pm 0.10	0.192 \pm 0.07	NS

Effect of treatment on lipid profiles: serum TC, TG, HDL, LDL & VLDL were not changed in both VPA & control group, (**Table-2**).

Table 2. Mean values of serum TC, TG, HDL, LDL & VLDL in control & VPA group, before & after 56 days of treatment with VPA (600 mg / kg) (no. = 8 in each group).

Parameters	Control group			VPA group		
	Before treatment	After treatment	P-value	Before treatment	After treatment	P-value
TC (mg / dl)	52.95 \pm 3.47	52.85 \pm 3.51	NS	52.95 \pm 3.47	53.25 \pm 2.03	NS
TG (mg /dl)	48.07 \pm 5.44	47.92 \pm 5.47	NS	48.07 \pm 5.44	45.73 \pm 2.95	NS
HDL (mg / dl)	32.13 \pm 2.62	31.80 \pm 2.62	NS	32.13 \pm 2.62	34.95 \pm 2.17	NS
LDL(mg / dl)	11.11 \pm 1.98	11.47 \pm 2.07	NS	11.11 \pm 1.98	9.15 \pm 0.86	NS
VLDL(mg / dl)	9.78 \pm 1.09	9.58 \pm 1.09	NS	9.78 \pm 1.09	9.15 \pm 0.58	NS

Effect of treatment on weights of ovaries: weights of ovaries significantly decreased ($P < 0.05$) in VPA group, but not in control group, (Table-3).

Table 3. Mean values of ovarian weight (summation weights of both right & left ovaries in each rat) (mg) of control & VPA group, before & after 56 days of treatment with VPA (600 mg/kg) (no = 8 in each group)

Groups	Weights of ovaries (mg)		P-value
	Before treatment	After treatment	
Control	63.45±4.22	63.24±4.26	NS
VPA	63.45±4.22	50.19±3.50	$P < 0.05$

Ovarian Histology:

Macroscopical Features:

Control group: Ovaries that were removed from rats of this group at estrus stage of estrus cycle, all had normal color, consistency & appearance of rat ovary, their size ranged from 3×4×5 mm to 4×5×6 mm, as shown in (Figure-1).

VPA group: All ovaries that were removed from rats in this group at same stage of estrus cycle, had no difference in their macroscopical features from that of control group, their sizes also within size range of control group ovaries, as shown in (Figure-2).



Fig 1. Macroscopical appearance of normal rat ovaries.

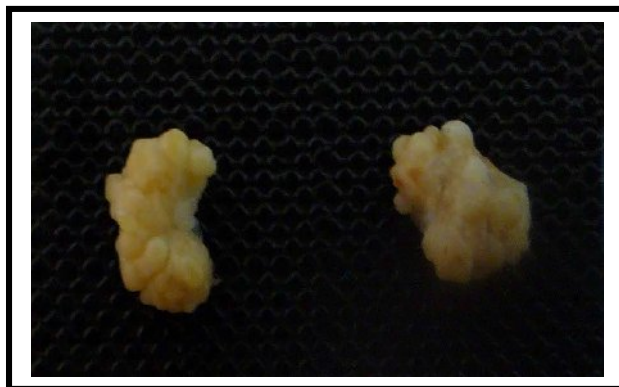


Fig 2. Macroscopical appearance of VPA treated rat ovaries. No morphological change compared with normal ovaries.

Microscopical Features:

Control group:

By using light microscope, all ovaries in this group had normal rat ovarian histology. Ovarian follicles in different stages of development were found (primordial, primary, secondary, tertiary & mature Graffian follicles) with normal stromal cells, as shown in (Figure-3).

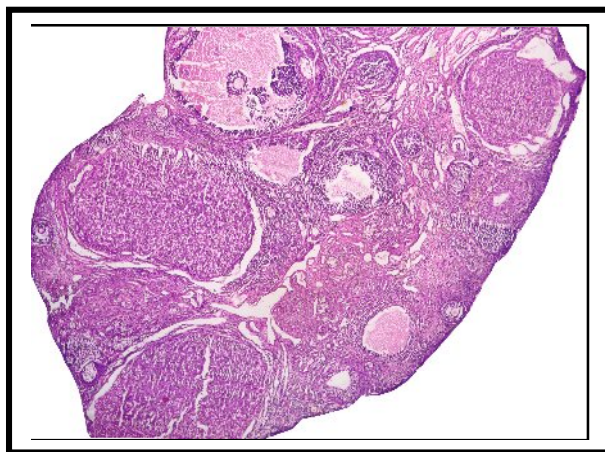
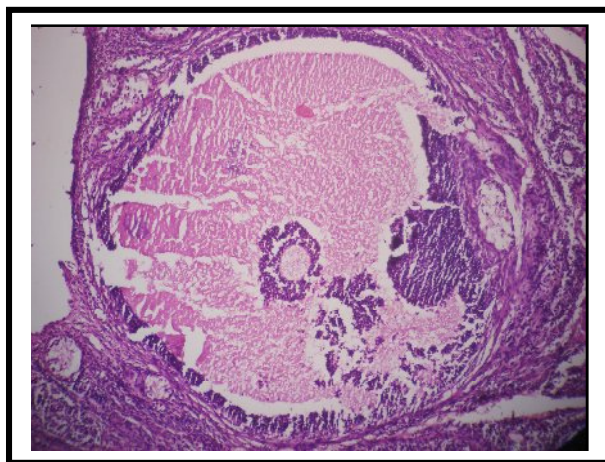


Fig 3. Histological section of normal rat ovary (x 4, x10).

VPA group:

All ovaries in this group were reviewed on microscopical examination (four sections were examined for each ovary). All ovaries demonstrated normal rat ovarian histology. Ovarian follicles in different stages of development were found with normal stromal cells. Features of PCO in human, multiple superficial cortical cysts, region of subcortical stromal fibrosis^[18], hyperplasia of theca stromal cells surrounding arrested follicles & presence of luteinized theca cells^[32], were not identified in any of these ovaries, as shown in (**Figures-4**).

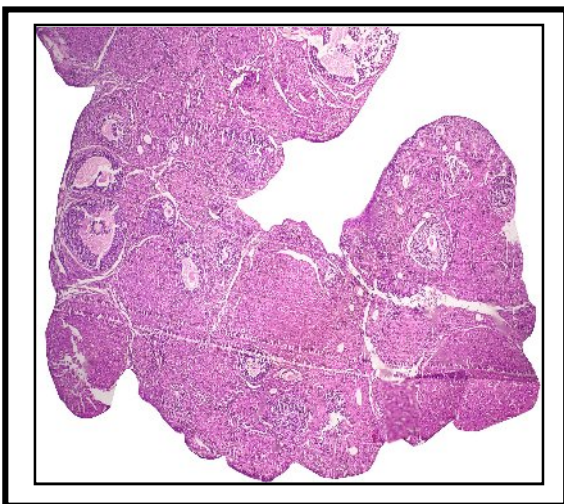
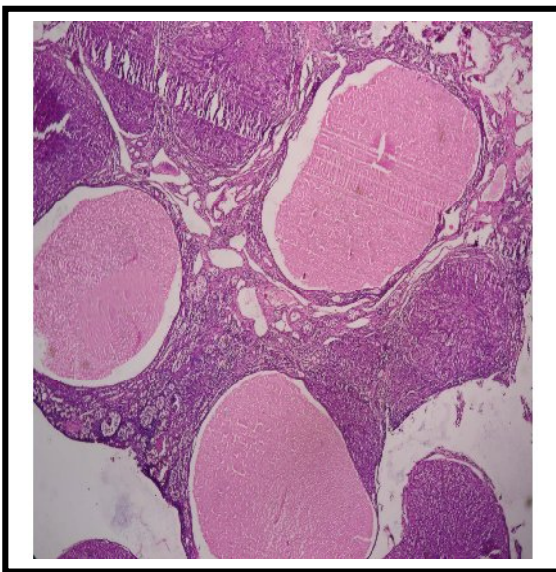


Fig 4. Histological section of rat ovary treated with VPA showed absence of specific features of polycystic ovaries, except appearance of follicular cysts (x10, x4).

Counting the numbers of corpus leutum & follicular cysts:

By using light microscope, the number of corpus leutum was counted for each ovary (for control & VPA groups) & summation of these numbers for right & left ovaries of each rat where obtained. The same method was applied for counting the number of follicular cysts, the results were shown in (**Table-4**).

Table 4. Number of corpus leutum & follicular cysts in control & VPA groups after 56 days of treatment with VPA (600 mg / kg).

Groups	Numbers of corpus leutum	Numbers of follicular cysts
Control	25.0 ± 8.12	2.60 ± 0.89
VPA	11.83 ± 3.31	5.20 ± 1.92

All data expressed as mean ± SD

The number of corpus leutum & the number of follicular cysts changed insignificantly in VPA group as compared to control group.

DISCUSSION

This study reported a significant reduction in serum FSH of VPA treated group. This result was in agreement with several others.^[33-35] While Stephen et al.^[36], found that FSH did not change significantly in epileptic women treated with VPA. The possible explanation for the reduction in FSH was that, VPA can lead to increase levels of γ -aminobutyric acid (GABA), so density of GABA input to medial preoptic area of hypothalamus was altered. By this way changing the release of gonadotropin releasing hormone (GnRH) which could in turn affect the secretion of gonadotropins (causes reduction of FSH & inappropriate secretion of LH). These facts are based on the finding that GABA ergic neurons modulate noradrenergic inputs to GnRH neurons & that valproate seems to modify GABAergic neurotransmitters.^[37]

In this study, serum LH nonsignificantly changed in VPA treated group, this is similar to results obtained by Ferin et al^[18] (who found the same result in non epileptic Rhesus monkeys treated with VPA for 12-15 months), Isojarvi et al^[38] and Stephen et al^[36] (who reported similar results in epileptic women treated with VPA). According to previously mentioned explanation that VPA affects GABA neurotransmitter, LH is supposed to increase in VPA treated group, but our study & other studies reported that LH was not changed. This might be due to the short duration of treatment because in one study^[39] serum LH increased from second year & onward in epileptic women treated with VPA & evaluated for 3 years. Serum total testosterone, in the present study, was reduced insignificantly in VPA group. Ferin et al^[18] reported non significant changes in total testosterone in non epileptic monkeys treated with VPA. While a significant reduction in total testosterone of non epileptic VPA treated rats had been also reported.^[40] In contrast, Isojarvi et al^[41] & Hamed et al^[42], found elevated total testosterone levels in epileptic women treated with VPA. The possible pathogenic mechanisms leading to the development of hyperandrogenism during VPA therapy were unknown. It was possible that VPA had a direct effect on ovarian androgen production^[43]. VPA also inhibits cytochrome P450 2C19 & to lesser extent P450 2C9, which catalyzes the reversible 17-position oxidation of testosterone to form androstenedione. So the inhibition of this pathway by VPA could lead to increase level of testosterone.^[44] The results of our study & other studies on non epileptic animals differ from results of clinical studies on epileptic women. This discrepancy might suggest that the effects of VPA in women with epilepsy could be due to in the fact that VPA exacerbating an already disturbed system (from epilepsy related effects) which is unable to compensate for its androgen promoting actions.^[20] This study reported, non significant changes in serum lipid profiles of VPA treated group: These results were similar to that obtained by Ferin et al^[18] Karikas et al^[45] also reported non significant changes in serum lipid profiles (except LDL-C) after VPA treatment. In addition, Tekgul et al^[46], found that serum lipids did not significantly change (except TG) in VPA treated epileptic women.

While, Nikolaos et al^[47] and Mc Intyre et al^[48] reported significant changes in serum lipids of VPA treated women. This controversy may be due to an impact of treatment duration because the effect of VPA on serum lipids represents a long term result.^[49]

In this study, ovarian weight is significantly reduced in VPA group. This result is consistent with Roste et al.^[21] While Taublii et al^[40] reported significant increase in ovarian weight of non epileptic female rats treated with VPA. This reduction in ovarian weight might be due to reduction in corpus luteum numbers of these ovaries (as reported by this study). Histopathological examination for rat ovaries of VPA group, revealed non significant reduction in numbers of corpus luteum. Roste et al.^[21] found significant reduction in numbers of corpus luteum in ovaries of non epileptic rats after treatment with VPA for 90 days. This result may be explained by the significant reduction in serum FSH (as reported by this study), which decreases the number of developing follicles in each cycle & decreases frequency of ovulation. Thus, it decreases number of corpus luteum. The duration of treatment may be the possible cause for non significant changes in corpus luteum numbers which have been reported by this study. The number of follicular cysts in these ovaries, was non significantly increased. However, a significant increase in number of ovarian follicular cysts in non epileptic female rats after 90 days of VPA treatment had been reported.^[40] Ovarian morphology in PCOS was characterized by multiple superficial cortical cysts, region of subcortical stromal fibrosis,^[18] hyperplasia of theca stromal cells surrounding arrest follicles & presence of luteinized theca cells.^[32] In this study, ovaries that had been removed from rats at the end of 56 days VPA treatment did not have any of these features & in fact all ovaries demonstrated normal ovarian histology. This result was similar to that obtained by Ferin et al.^[18] who reported the same result after 12-15 months of VPA treatment in monkeys.

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