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Research Article

Effects of valproic acid on the reproductive functions of adult male Wistar rats and consequences for offspring

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Abstract

Introduction: Valproic acid can affect the nervous system as well as other systems such as the endocrine and reproductive systems. Exposure to valproic acid is not without harmful side effects. These undesirable effects can lead to infertility. **Objective:** To assess the effects of valproic acid on reproductive functions in adult male Wistar rats and consequences for offspring. **Materials and methods:** Twenty-four adult male Wistar rats, weighing between 100 and 300 g, were used. Treatment was administered orally for sixty days. For the study, three batches, each containing eight adult male Wistar rats, were formed and treated as follows: (1) a control batch given distilled water at 10 ml/kg; (2) and (3) batches treated with valproic acid at doses of 200 and 400 mg/kg respectively. The variables studied included physiological measurements, pedigree observations, sexual behavior, fertility, prolificacy and mortality rates, spermogram and hormone levels. **Results:** Exposure of adult male Wistar rats to valproic acid at doses of 200 mg/kg and 400 mg/kg led to an alteration in body weight, a significant decrease in the weight of the prostate ($p < 0.05$), testes ($p < 0.05$) and epididymis ($p < 0.05$). Decreases in fertility rate (25%), prolificacy rate (66.67 and 68.75%), and fecundity rate (250 and 275%), as well as an increase in mortality rate (50 and 45.45%), were observed respectively in batches 2 and 3 treated with valproic acid. This exposure also led to an alteration in sperm parameters. This was illustrated by asthenospermia ($p < 0.0001$), necrospermia ($p < 0.05$), polyspermia ($p < 0.05$) for the 200 mg/kg dose and oligospermia ($p < 0.05$) for the 400 mg/kg dose. Batches treated with valproic acid showed a significant decrease in testosterone levels ($p < 0.0001$) as well as abnormalities such as hemorrhagic syndrome in neonates born to untreated females and alopecia in the pedigree. **Conclusion:** Prolonged exposure to valproic acid in adult male Wistar rats at doses of 200 mg/kg and 400 mg/kg led to a reduction in prostate, testicular and epididymal weights. This exposure resulted in a decrease in fertility, prolificacy and fecundity and an increase in mortality. Alterations in sperm parameters and depletion of testosterone levels were noted, as well as abnormalities in ascendants and descendants.

Keywords: valproic acid, sperm, hormone, infertility, brain, rat

INTRODUCTION

Valproic acid is an anti-epileptic drug. It exerts its pharmacological effects on the central nervous system ¹. Its therapeutic effect is primarily anticonvulsant ². It acts in brain tissue to stop seizures ^{1,2}. It is also used to treat the manic phase of bipolar disorder and migraines ^{3,4}. These properties are used against a wide variety of types of seizures, including convulsive seizures in animals and epilepsy in humans ^{5,6}. Exposure to valproic acid is not without harmful side effects ^{7,8,9}. Valproic acid can affect the central nervous system as well as other systems such as the endocrine and reproductive systems ^{4,9,10}.

Infertility is a public health problem and around 30% of infertilities may be due to a male factor ¹¹. Exposure to factors such as drug treatments, toxins, inadequate nutrient intake and pollution can have adverse effects on spermatogenesis and normal sperm production ¹². In addition to these harmful effects on the brain ^{9,10}, Depakine ® can alter sperm quality and hormone levels. These adverse effects can lead to infertility, highlighting the link between the brain and fertility or reproductive function ^{4,9,13}. Reproduction is controlled by hormones, which requires normal functioning of the hypothalamus and pituitary gland ^{13,14}. In this study, we evaluated the

effects of valproic acid on reproductive functions in adult male Wistar rats and consequences for offspring.

MATERIALS AND METHODS

Animals and treatments

Twenty-four adult male Wistar rats, weighing between 100 and 300 g, from the Faculty of Health Sciences animal house, were used. They were kept in their natural habitat, with free access to water and food. The treatment consisted of prolonged oral administration of the various products at different doses for sixty days. For the study, three batches, each containing eight male Wistar rats, were formed and treated as follows:

- the first batch served as a control and received distilled water at 10 ml/kg;
- the second batch was treated with valproic acid at a dose of 200 mg/kg;
- the third batch was treated with valproic acid at a dose of 400 mg/kg.

Pedigree observation

Adult male Wistar rats were observed to monitor daily treatment for signs of toxicity that might be associated with changes in plasma/serum concentrations. The second generation was observed after mating and parturition. A battery of functional observations was based on a complete description of the subject's appearance, behavior and functional integrity. All animals were carefully examined for this purpose, ignoring the treatment received by the animal to limit observer-related variability. The frequency of occurrence of an abnormality in a population was measured by calculating the proportion of abnormalities or events present in the population at a given time.

Mating behavior of Wistar rats

The behavior of untreated female Wistar rats relative to adult male Wistar rats exposed to valproic acid was observed as well as that of the control lot after the treatment phase. The first few minutes of mating were taken into account as well as variables such as aggression, rejection, isolation, mating after one hour, grooming and sniffing between the two sexes, while taking into account the lordosis posture of the untreated females⁹.

Reproduction data

When the rodents gave birth, a second generation was obtained. It was obtained from mating between untreated female Wistar rats and adult male Wistar rats treated with valproic acid as well as those from the

control batch. Observation of the offspring enabled anomalies to be taken into account and reproductive parameters such as fertility, fecundity and prolificacy rates to be assessed, as well as the mortality rate and the presence of macroscopic anomalies (visible external abnormalities).

Blood and organ sampling

Blood was collected from the retroorbital sinus on dry tubes following the end of treatment and behavioral observations. The serum was decanted after centrifugation and used for biomedical analysis. The animals in each batch were anesthetized by inhalation of ethyl ether for dissection. The testes, epididymis, spermiducts, seminal vesicles and prostate were removed, weighed and directed for analysis.

Semen analysis

The spermogram is the basic examination carried out in men in the event of infertility within the couple. The epididymis and spermiduct were excised, dilated and then ground in 1 ml of physiological water. A fixed volume of sperm suspension was obtained and used to determine the sperm parameters of the adult male Wistar rat.

Hormone levels

The ELISA test with the Cypress ® kit was used for the determination of FSH, LH and testosterone.

Ethics

The study was conducted in compliance with the 3R rule for animal experimentation. Directive 2010/6106/EU on the protection of laboratory animals was considered¹⁵.

Statistical analysis

Results were expressed as mean $\pm$ standard error. Batches of treated animals were compared with the control group for each variable. Comparisons of non-evolving parameters were made using Student's t-test and one-factor ANOVA, followed by a Tukey test for comparison between several experimental batches. Data were entered into Excel 2019. Tests were performed using Statistical Package for Social Science, version 20. The significance threshold corresponded to a risk of error of $p < 0.05$.

RESULTS

Body weight

Prolonged administration of different substances for sixty days resulted in weight variations in adult male Wistar rats. These variations are represented in the following figure.

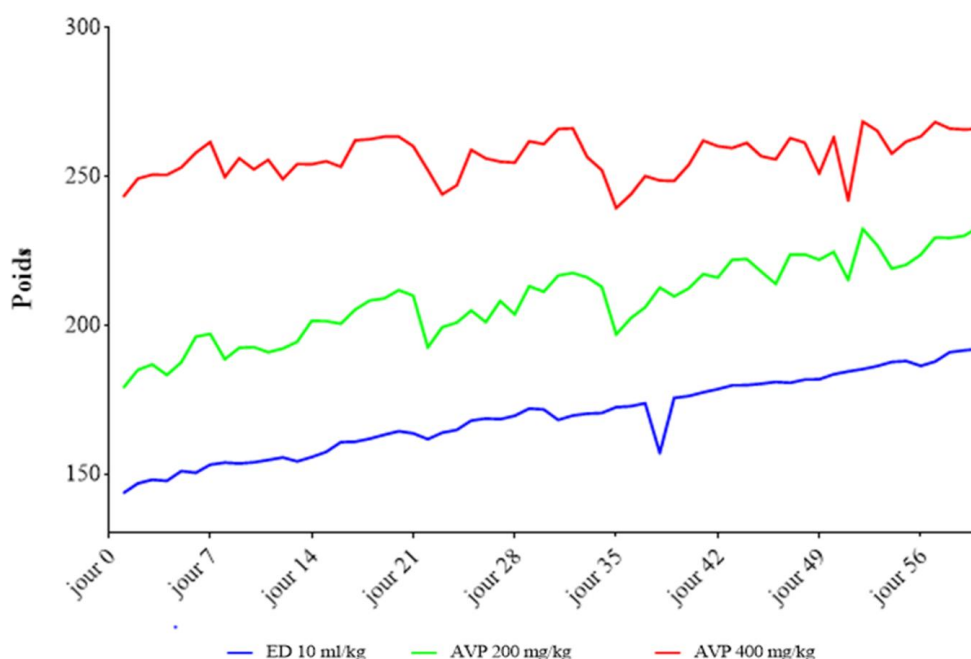


Figure 1. Effects of valproic acid on body weight in adult male Wistar rats

Observation of adult male Wistar rats treated with valproic acid

Prolonged exposure to valproic acid may cause abnormalities in adult male Wistar rats. The table below

shows the various alterations observed during the treatment phase and their frequency of occurrence.

Table I. Abnormalities in adult male Wistar rats treated with valproic acid

Parameters	ED 10 ml /kg	VPA 200 mg/kg	VPA 400 mg/kg
Alopecia	0	37.5	37.5
Stress	0	75	75
Blackening of the tongue	0	37.5	25

ED: Distilled water; VPA: Valproic acid; Frequency: %.

Sexual behavior

Batches (2 and 3) of adult male Wistar rats exposed to valproic acid at doses of 200 mg/kg and 400 mg/kg,

respectively, were subject to rejection, isolation, and the aggressiveness of untreated females. The table below shows the various observations made during the mating period and their frequencies.

Table II. Behavior of untreated female Wistar rats towards treated males

Parameters	ED 10 ml /kg	VPA 200 mg/kg	VPA 400 mg/kg
Aggressivity	0	75	75
Rejection of the male	0	50	50
Isolation	0	75	50
Grooming/sniffing between males and females	100	0	25
Action of mating after 1 hour	100	0	0

ED: Distilled water; VPA: Valproic acid; Frequency: %.

Reproduction data

The mating of the rodents resulted in a second generation. Observation of the offspring made it possible

to take into account reproductive parameters such as fertility, fecundity, and prolificacy rates, as well as the mortality rate.

Table III. Effects of valproic acid on reproductive parameters

Parameters	ED 10 ml /kg	VPA 200 mg/kg	VPA 400 mg/kg
Number of rat pups	16.5±0.70	7.5±9.19 *	8±9.89 *
Fertility rate	100	75	75
Prolificity rate	96.96	66.67	68.75
Fertility rate	800	250	275
Mortality rate	3.12	50	45.45

Results were expressed as mean ± standard error. (*): $p < 0.05$; (**): $p < 0.001$; (***): $p < 0.0001$; NS (not significant) by student's t-test; Rate: Percentage (%); ED: Distilled water; VPA: Valproic acid.

Testicular macroscopy

Animals exposed to valproic acid showed testicular atrophy, the effect of which may be dose-dependent.

Figure (2) shows the condition of testes from the control group (A) and those treated with valproic acid at doses of 200 mg/kg (B) and 400 mg/kg (C).

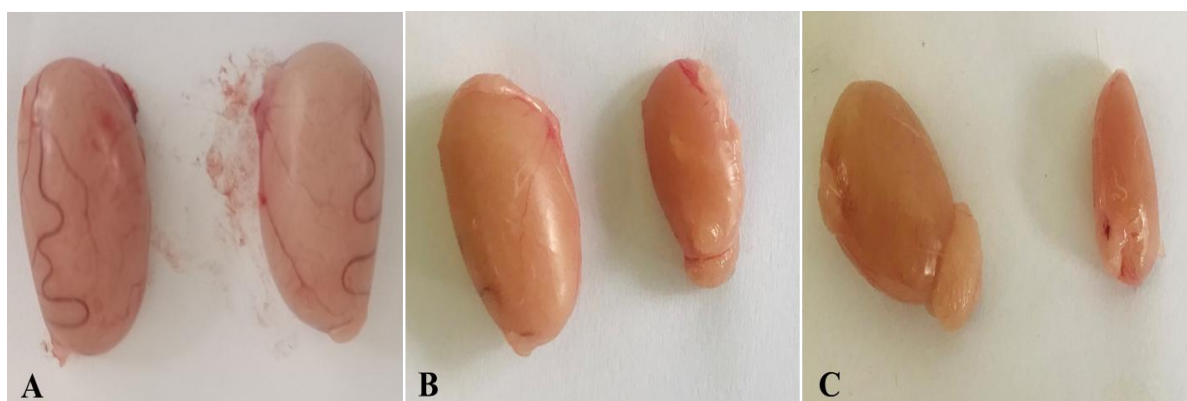


Figure 2. Photographs of the testes of adult male Wistar rats from the control batch (A), the second batch (B) treated with valproic acid at 200 mg/kg and the third batch (C) at 400 mg/kg

Observation of young Wistar rats

The figures below illustrate cases of hemorrhagic syndrome in neonates born to untreated female Wistar rats, and alopecia in young rats. These observations were made in the offspring of adult male Wistar rats exposed

to valproic acid. They were born from mating with untreated female Wistar rats. Figure (3), highlighted the hemorrhagic syndrome in neonates born to untreated female Wistar rats and adult male Wistar rats treated with valproic acid at doses of 200 and 400 mg/kg.

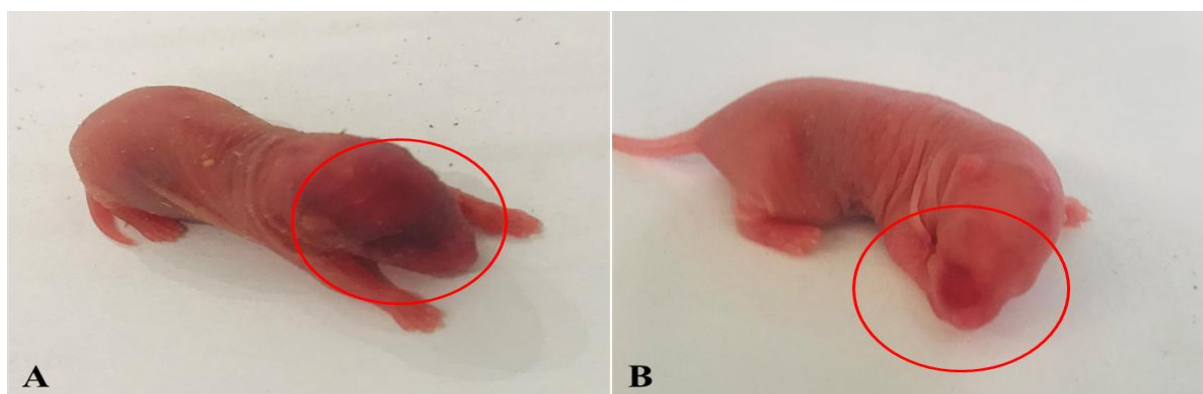


Figure 3. Photographs (A and B) of hemorrhagic syndrome in neonates from untreated female Wistar rats mated to adult male Wistar rats treated with valproic acid

Figure (4), highlighted the alopecia observed in young rats mated with batches exposed to valproic acid (B, C, E and F) at doses of 200 and 400 mg/kg compared with the control batch (A and D), which received distilled water.

Microphotographs A, B, and C show young rodents at postnatal day 8 (PN 8). Microphotographs D, E and F showed young rodents on postnatal day 15 (PN 15).

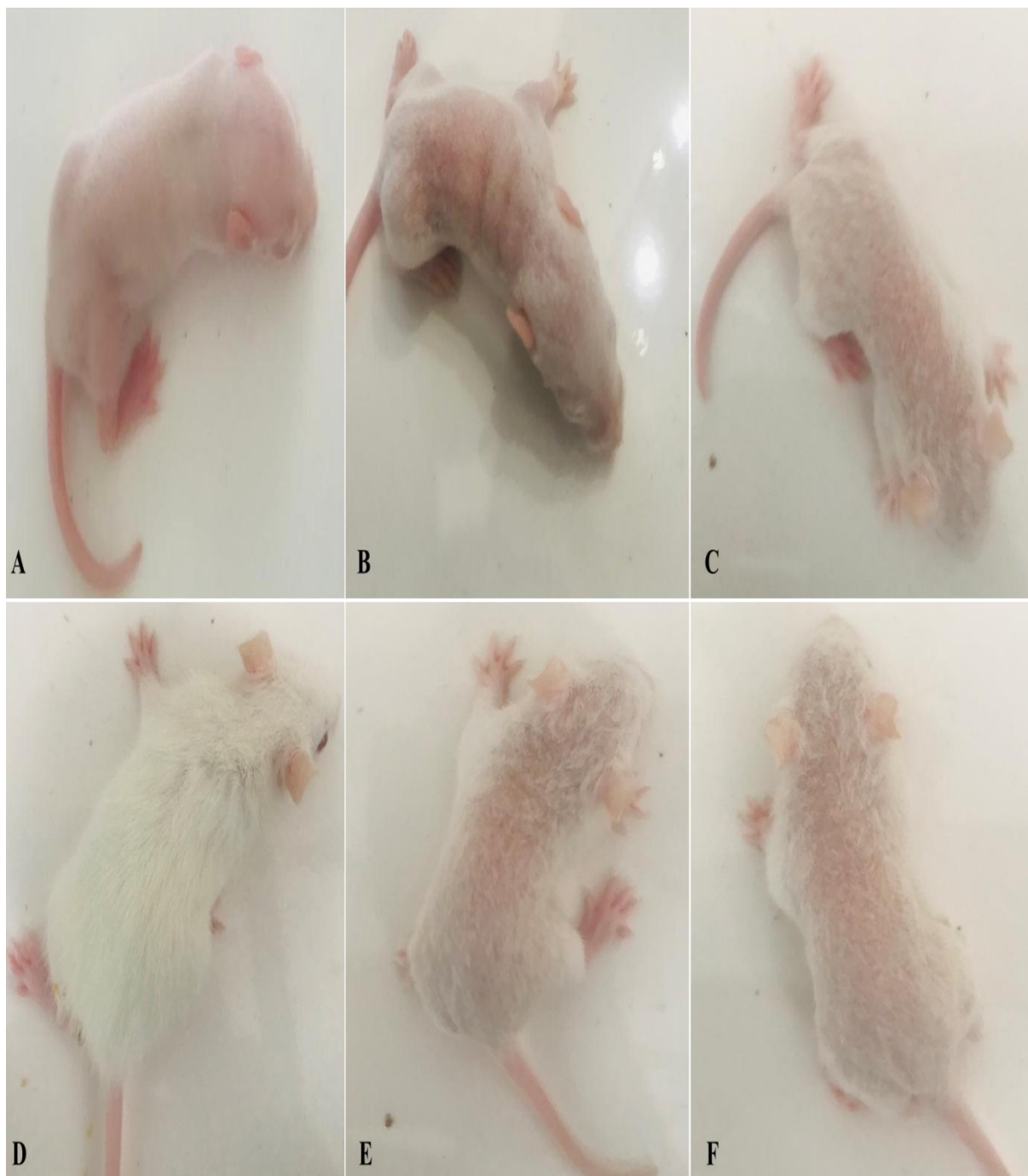


Figure 4. Photographs at PN 8 (A, B and C) and PN 15 (D, E and F) of pups from untreated female Wistar rats mated to adult male Wistar rats treated with valproic acid (B, C, E and F) at doses of 200 and 400 mg/kg, as well as the control lot (A and D)

Organ weight

Valproic acid was administered orally for sixty days at doses of 200 and 400 mg/Kg to adult male Wistar rats in

batches 2 and 3 respectively. The control lot received 10 ml/kg of distilled water. Organ weights were recorded after dissection.

Table IV. Organ weight index

Organs	ED 10 ml/kg	VPA 200 mg/kg	VPA 400 mg/kg
Prostate	0.91±0.27	0.65±0.17 ^{NS}	0.55±0.02 [*]
Left testicle	1.96±0.03	0.65±0.39 [*]	0.64±0.55 [*]
Right testicle	1.98±0.14	1.12±0.03 ^{NS}	1.18±0.07 ^{NS}
Left epididymis	0.33±0.03	0.20±0.08 [*]	0.31±0.20 ^{NS}
Right epididymis	0.31±0.01	0.26±0.08 ^{NS}	0.30±0.18 ^{NS}
Left spermiduct	0.06±0.01	0.10±0.02 [*]	0.22±0.19 ^{**}
Right spermiduct	0.08±0.01	0.13±0.02 [*]	0.23±0.18 ^{**}
Left seminal vesicle	0.30±0.07	0.38±0.08 ^{NS}	0.44±0.12 ^{NS}
Right seminal vesicle	0.33±0.06	0.46±0.13 ^{NS}	0.42±0.09 ^{NS}

Results were expressed as mean ± standard error. (*): p<0.05; (**): p<0.001; (***): p<0.0001; NS (not significant) by student's t-test. ED: Distilled water; VPA: Valproic acid.

Sperm parameters

Valproic acid was administered orally for sixty days at doses of 200 and 400 mg/Kg in adult male Wistar rats.

The effects observed on sperm parameters are presented in Table V.

Table V. Effects of valproic acid on sperm parameters

Parameters	ED 10 ml/kg	VPA 200 mg/kg	VPA 400 mg/kg
pH	6.62 ±0.47	6.12±0.25 ^{NS}	6.12±0.25 ^{NS}
Motility	81.25±8.53	12.5±6.45 ^{***}	7.5± 2.88 ^{***}
Vitality	94.25±0.5	77.5±8.66 [*]	78.75±10.30 [*]
Sperm count	22.5±1.91	27.25±2.21 [*]	17.25±2.21 [*]

Results were expressed as mean ± standard error. (*): p<0.05; (**): p<0.001; (***): p<0.0001; NS (not significant) by student's t-test. ED: Distilled water; VPA: Valproic acid.

Hormone levels

Valproic acid was administered orally for sixty days at doses of 200 and 400 mg/Kg in adult male Wistar rats.

The effects observed on hormonal parameters are presented in Table VI.

Table VI. Effects of valproic acid on hormonal parameters

Parameters	ED 10 ml/kg	VPA 200 mg/kg	VPA 400 mg/kg
FSH	1.75±0.44	1.94±0.19 ^{NS}	2.80±1.66 ^{NS}
LH	1.05 ±0.06	0.99±0.06 ^{NS}	1.01±0.10 ^{NS}
Testosterone	0.42±0.07	0.26±0.13 [*]	0.15± 0.08 ^{***}

Results were expressed as mean ± standard error. (*): p<0.05; (**): p<0.001; (***): p<0.0001; NS (not significant) by student's t-test. ED: Distilled water; VPA: Valproic acid.

DISCUSSION

Exposure to valproic acid is not without harmful side effects ^{7,8,9}. Valproic acid can affect the central nervous system, as well as other systems such as the endocrine and reproductive systems ^{4,9,10}. Its influence can be felt in the neuroendocrine axis, which includes hypothalamic GnRH neurons and pituitary cells that secrete FSH and LH in response to GnRH ^{13,14,16}. Valproic acid use can reduce male sex hormone levels and alter sperm parameters. This exposure may be responsible for impaired fertility in adult male Wistar rats. The aim of our study was to evaluate the effects of valproic acid on reproductive functions in adult male Wistar rats and consequences for offspring. Valproic acid was administered orally for sixty days at doses of 200 and 400 mg/Kg to male Wistar rats in batches 2 and 3 respectively. The control lot received 10 ml/kg of distilled water.

Exposure to valproic acid at doses of 200 and 400 mg/Kg respectively in rats for sixty days resulted in weight variation in Wistar rats. Figure (1) shows the effects of valproic acid on body weight in male Wistar rats. The control batch showed a constant growth curve, whereas the valproic acid-treated batches showed altered growth. Impaired growth can have various effects on fertility, management and growth in animals ^{9,17}.

Prolonged exposure to valproic acid resulted in abnormalities in adult male Wistar rats. Table (I) above shows the anomalies observed and their frequency of occurrence in adult male Wistar rats treated with valproic acid. The study reported alopecia (37.5%), stress (75%) and blackening (37.5% and 25%) of the tongue in rodents after prolonged exposure to valproic acid at doses of 200 and 400 mg/Kg respectively ^{18,19,20}. Alopecia may be androgenetic and may be associated with significant. It may be induced by hormonal imbalance.

Exposure of adult male Wistar rats to doses of 200 and 400 mg/Kg valproic acid respectively induced disturbances during mating. Impaired olfactory preference was observed. Table (II) shows the sexual behavior of untreated females compared with adult male Wistar rats exposed to valproic acid. The lordosis behavior of untreated females in the presence of male Wistar rats treated at doses of 200 and 400 mg/Kg was significantly affected compared with male Wistar rats from the control batch. We observed that untreated females mated more aggressively (75%), isolated (75% and 50%) and rejected (50%) the batches treated with valproic acid than the control batch. A lack of grooming and sniffing (0 and 25%) between the two sexes, as well as mating (0%) after one hour, were observed. In rodents, during the pre-copulatory phase, both partners emit pheromones and sniff each other ^{9,21,22}. This olfactory stimulation is very important for the introduction of reproductive behaviors, such as the male's emission of the ultrasonic vocalizations needed to attract the female and keep her close ^{9,21,22}. The copulatory phase begins with mounts by the male, to which the female responds with a lordosis posture. In the female, male pheromones stimulate the neurons of the olfactory bulb, which transmit this chemosensory

information to those of the medial amygdala, then to those of the nucleus of the stria terminalis ²².

Mating of the rodents resulted in a second generation. This is the result of mating between untreated female Wistar rats and adult male Wistar rats treated with valproic acid, as well as the control lot, which received distilled water. Table (III), presents the reproductive data after valproic acid exposure of first-generation adult male Wistar rats. Reproductive parameters such as fertility rate, fecundity and prolificacy, as well as mortality, were taken into account when observing the offspring ⁹. Our results showed a significant reduction in the number of offspring produced by mating untreated female rats and batches treated with valproic acid at doses of 200 and 400 mg/kg respectively, compared with the control batch at parturition ⁹. We observed a 25% decrease in fertility rate in untreated females mated to adult male Wistar rats treated with valproic acid compared with 100% of the control batch ⁹. We also observed a 66.67% and 68.75% decrease in prolificacy in untreated females mated to adult male Wistar rats treated with valproic acid at 200 and 400 mg/kg respectively, compared with 96.96% in the control lot ⁹. Mating of untreated females to adult male Wistar rats treated with valproic acid at doses of 200 and 400 mg/kg respectively revealed a 250% and 275% decrease in fecundity compared with 800% in the control lot. There was also a 50% and 45.45% increase in the mortality rate for the 200 and 400 mg/kg valproic acid doses respectively, compared with 3.12% for the control batch ⁹. This analysis highlighted the alterations in young Wistar rats resulting from the mating of untreated female rats and adult male Wistar rats exposed to valproic acid at doses of 200 mg/kg and 400 mg/kg respectively.

Testicular macroscopy plays an important role in the study of male infertility ²³. Evaluation of testicular morphology supports the hypothesis of a testicular mechanism of male infertility ²³. Figure (2), illustrated testicular atrophy in adult male Wistar rats treated with valproic acid at doses of 200 and 400 mg/Kg respectively. Our results showed that valproic acid can induce testicular atrophy ^{24,25,26}.

Figures (3) and (4) above illustrate cases of hemorrhagic syndrome in neonates born to untreated female Wistar rats and alopecia in young rats ^{27,28,29}. Figure (3), highlighted the hemorrhagic syndrome in neonates born to untreated female Wistar rats and adult male Wistar rats treated with valproic acid at doses of 200 and 400 mg/kg ^{27,28}. Hemorrhagic syndrome in neonates has often been associated with maternal use of drugs that inhibit vitamin K activity, such as antiepileptic drugs ²⁸. Figure (4), highlighted cases of alopecia observed in young rats resulting from mating with batches exposed to valproic acid (B, C, E, F) at doses of 200 and 400 mg/kg compared with the control batch (A, D), which received distilled water ^{20,28,29}. Microphotographs A, B, and C showed young rodents at postnatal day 8 (PN 8). Microphotographs D, E, and F showed young rodents on postnatal day 15 (PN 15).

Evaluation of organ morphology may support the hypothesis of a testicular mechanism of infertility ²³.

Table (IV), presented the effects of valproic acid on organ weights. Adult male Wistar rats treated with valproic acid showed a non-significant and significant decrease in prostate weight at 200 and 400 mg/Kg respectively, compared with the control lot. Our results revealed a decrease in testicular weight in adult male Wistar rats treated with valproic acid at doses of 200 and 400 mg/Kg respectively, compared with the control batch. This decrease was significant for left testicular weight in batches exposed to valproic acid. Our results also showed a decrease in epididymides weight in rats treated with valproic acid. This decrease in weight was significant for the left epididymides of the batch treated with 200 mg/kg valproic acid. Adult male Wistar rats treated with valproic acid showed a significant increase in spermiductal weight compared with the control lot. Our results showed a non-significant increase in seminal vesicle weight in valproic acid-treated rats compared with the control batch. These alterations may be correlated with infertility^{24,25,26}.

Sperm quality can be taken as a prime indicator of natural fertility²³. The spermogram plays a key role in the assessment of male infertility. Table (V) shows the various parameters that highlight an alteration in the spermogram. These have been correlated with fertility²³. Our results showed a non-significant decrease in pH in valproic acid-treated rats compared with the control batch. This decrease in pH may be related to abnormal seminal vesicle secretion²³. Exposure of adult male Wistar rats to valproic acid at doses of 200 and 400 mg/kg respectively resulted in a highly significant decrease in motility compared with the control lot. Poor sperm motility can lead to asthenospermia. A significant decrease in vitality was observed in valproic acid-treated rats compared with the control batch. This may reflect necrospermia. Our results showed a significant increase and decrease in sperm count respectively for rats treated with valproic acid at doses of 200 and 400 mg/kg compared with the control batch. These two variations can result in polyspermia and oligospermia respectively. The former is associated with alterations in the quality of movement and is caused by abnormalities in sperm formation. It can be the cause of infertility, since such a high concentration of sperm can make their progressive motility difficult. The second can be caused by alterations in spermatogenesis (the formation of spermatozoa in the testicle), or by obstruction in the seminal tract. The decrease in pH may be linked to a deficit in seminal fluid secretion, justifying the apparent increase in sperm density. Thus, the observed increase in sperm count may be linked to hyposecretion of the seminal vesicles. Altered parameters such as pH, motility, vitality and sperm count may lead to infertility^{26,27,30,31}. Our results suggest a dose-dependent effect of valproic acid on sperm parameters and a deleterious effect on male fertility^{31,32,33}.

In men, hormonal testing can be used to diagnose hypothalamic-pituitary gonadotropic deficiency or primary testicular failure by measuring total testosterone and gonadotropins (LH and FSH). LH primarily stimulates testosterone production, while FSH stimulates sperm production^{13,14,16}. The testes must be

able to respond to this hormonal stimulus^{13,14,16}. Table (VI) shows the effects of valproic acid on the various hormones measured in the present study. Valproic acid caused a variation in FSH and LH levels, although a non-significant increase in FSH levels was observed in adult male Wistar rats treated with valproic acid compared with the control batch. An elevated plasma FSH concentration may be due to testicular damage, but it may also be due to hypogonatrophic hypogonadism. Our results showed a non-significant decrease in LH levels in adult male Wistar rats treated with valproic acid compared with the control batch. However, a similar observation was made, namely a highly significant decrease in testosterone levels in rats treated with valproic acid at doses of 200 and 400 mg/kg respectively, compared with the control batch. Decreased testosterone levels may be associated with testicular and other organ atrophy, as well as spermogram alterations^{23,24,26}. Testosterone deficiency and impaired sperm production may reflect hypogonadism. It may result from a testicular abnormality and/or a disorder of the hypothalamic-pituitary axis^{33,34,35}. Disruption or alteration of FSH, LH and testosterone levels may indicate infertility^{35,36,37}. Testosterone deficiency in adulthood may be associated with reproductive dysfunction and cognitive impairment^{9,10,29,34}.

CONCLUSION

Exposure of male Wistar rats to valproic acid at doses of 200 mg/kg and 400 mg/kg for sixty days resulted in altered body weight, and reduced prostate, testicular and epididymal weights. This exposure led to a decrease in fertility, prolificacy and fecundity, and an increase in mortality. Alterations in sperm parameters and depletion of testosterone levels were noted, as well as abnormalities in ascendants and descendants. Studies have shown that valproic acid can affect the brain. This study may confirm the close relationship between the brain and fertility. Further investigations may be necessary.

Conflicts of interest: All authors declare that they have no conflicts of interest in relation to this work.

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Authors' contributions : Participation in research design : AME Mbougou Malonga.

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Ethical approval: Ethical approval was obtained from Faculty of Health Sciences, Marien Ngouabi University, Brazzaville, Congo

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