CRITICAL ASPECTS OF MALE RAT FERTILITY IN THE ASSESSMENT OF EXPOSURE TO LAPROL-604*

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The present experiment studied the sexual behavior, biochemical profile of the testes, follicle stimulating hormone, luteinizing hormone and testosterone levels of male rats have been administered by Laprol-604 for 60 days. The surfactants are one of the potentially harmful chemicals released in the environment. The potentially toxic effects of surfactants are presently being studied with increasing intensity. Laprol-604 is a non-ionic surfactant which is widely used an ingredient of manufacturing epoxide rubber, enamels, varnishes, plastic, fiber, glues, emulsifiers, etc. Laprol-604 has been shown to produce some adverse health effects. A study was conducted to asses the effects of Laprol-604 on the reproductive system and sexual behavior of thirty sexually naive male Wistar rats. The Laprol-604 was administered to rats orally at dose levels of 1/10, 1/100 and 1/1000 LD50 – 12.5g/kg, respectively is the 1-st; 2-nd and 3-rd group for 60 days (one cycle of spermatogenesis). Sexual behavior was evaluated in a procedure consisting of an open field, it in the context of one interaction to one estrus female rat. Prolonged oral administration of Laprol-604 led to disturbance sn sexual behavior of male rats. Both the mount latency and intromission latency of male rat's 1-st, 2-nd and 3-rd groups was significantly lengthened compared to controls. In contrast, the numbers of mountings and intromissions was significantly reduced in Laprol-604 treatment groups compared to control animals. Study has been found that control male rats could ejaculate 1.3 times/hour by contrast with male rats of 1-st, 2-nd and 3-rd groups which did not ejaculate. The significant relationships between the sexual activity and testosterone levels were found. In comparison to the control rats, there were the significant reductions in the weight of testes, epididymis, seminal vesicle and ventral prostate. Testicular and epididymal sperm density was decreased in the animals, which were treated with Laprol-604. Biochemical profile of the testis revealed a significant decline in the contents of protein, testicular cholesterol and glycogen. At the same time, the activity of testicular acid phosphatase was significantly increased, but decreasing alkaline phosphatase activity was found. The level of testosterone, luteinizing hormone and follicle stimulating hormone levels were significantly suppressed by Laprol-604. Results of this study clearly show that Laprol-604 induces the toxic impact on the male rats' reproductive system. The study found the relationship between the Laprol-604 administration effects on testosterone, luteinizing hormone and follicle stimulating hormone levels and sexual behavior of adult male rats.

Key words: Laprol-604, surfactant, male rats, testis, testosterone, sperm dynamics, reproductive toxic effects, sexual behavior, mount, intromission, ejaculation.

У експерименті досліджували статеву поведінку, біохімічні показники яєчок, рівні фолікулостимулюючого, лютеїнізуючого гормонів та тестостерону в сироватці крові самців щурів, яким вводили Лапрол-604 впродовж 60 днів. Поверхнево-активні речовини є потенційно небезпечними хімічними речовинами, що потрапляють у навколишнє середовище. В даний час активно вивчається токсичний вплив поверхнево-активних речовин на живі організми. Лапрол-604 є неіонною поверхнево-активною речовиною, яка широко використовується в якості інгредієнту для виготовлення епоксидних смол, емалей, лаків, пластику, волокна, клею, емульгаторів та ін. У дослідженні оцінювали вплив Лапролу-604 на репродуктивну систему і статеву поведінку тридцяти статево незайманих самців щурів лінії Wistar. Лапрол-604 вводили щурам перорально у дозах 1/10, 1/100 і 1/1000 ЛД 50 - 12,5 г/кг, відповідно, 1-ій; 2-ій та 3-ій групі протягом 60 днів (один цикл сперматогенезу). Статеву поведінку оцінювали за допомогою модернізованого відкритого поля, в контексті взаємодії з однією самицею-еструс. Тривале пероральне введення Лапролу-604 призвело до порушення статевої поведінки самців щурів. Як тривалість ссадок, так і тривалість інтроміссій у щурів 1-ої, 2-ої та 3-ої груп були значно довшими в порівнянні з контролем. Навпаки, кількість ссадок та інтроміссій вірогідно зменшилися в даних експериментальних групах в порівнянні з контрольними тваринами. Самці контрольної групи здійснювали еякуляцію 1,3 рази /год, тоді як щури 1-ої, 2-ої та 3-ої груп не еякулювали. Виявили взаємозв'язок між статевою активністю і рівнем тестостерону у самців усіх груп. Тварини, які вживали Лапрол–604 мали вірогідно меншу вагу яєчок, придатків яєчок, сім'яних пухирців та передміхурових залоз, щільність сперми у порівнянні з контрольними щурами. При біохімічному дослідженні яєчок щурів виявили значне зниження вмісту білка, холестерину і глікогену. У той же час активність кислої фосфатази вірогідно збільшилася, але активність лужної фосфатази вірогідно зменшилася. Рівень тестостерону, лютеїнізуючого і фолікулостимулюючого гормонів у сироватці крові щурів 1-ої, 2-ої та 3-ої груп вірогідно нижчий в порівнянні з контролем. Результати дослідження свідчать, що Лапрол-604 токсично діє на репродуктивну систему самців щурів, існує зв'язок між рівнем тестостерону, лютеїнізуючого та фолікулостимулюючого гормонів та статевою поведінкою дорослих самців щурів.

Ключові слова: Лапрол-604, поверхнево-активна речовина, самці щурів, яєчка, тестостерон, сперма, репродуктивний токсичний ефект, статева поведінка, ссадка, інтромісія, еякуляція.

Introduction

A great variety of surfactants are currently used and their usage is increasing. They are used in both industry and home, so there are many occupations and home activities in which humans can be exposed to them. According to the water-soluble properties, the term «surfactants» includes two general groups: ionic surfactants and nonionic surfactants [17]. Laprol-604 is included in to numerous non-ionic surfactants [18]. Laprol-604 has been produced industrially for several decades for use primarily as ingredients of manufacturing epoxide resin, enamels, varnishes, plastic, fiber, glues, emulsifiers ets. [19]. Nonionic surfactants have been most intensively studied from a toxicological standpoint [7; 9; 10; 18]. The toxicity of Laprol-604 has been studied and the findings showed that Laprol-604 was a moderately toxic substance. Exposure to Laprol-604 throughout of gestational period showed the adverse effect on pregnant rats and their progenies, such as decreasing of body weight of rat

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newborns, diminished number of live pups and the viability of the progeny during the first ten days after birth, delayed developmental progress. Maternal toxicity of Laprol-604, indicated by deficits in weight gain and liver enlargement, is associated with biochemical disturbances in pregnant rats [1; 12].

Nowadays, the effects of nonionic surfactants on sexual hormone levels of female rats are known and impact of Laprol-604 on female reproductive system of rats has been found. Nevertheless, until quite recently, to our knowledge no one has previously described male hormonal levels and sexual behavior across studies of non-inorganic polyols and the influence of Laprol-604 on male reproductive system. The present experiment has focused mainly on the sexual behavior, fertility, testicular biochemistry and serum hormonal levels of male rats have been administered by Laprol-604 for 60 days.

Materials and methods

Laprol-604 was obtained from Science and Production Joint Stock Company "Sintez PAV" (Shebekino, Russian). Laprol-604 was reported to be 96% pure by the supplier. For all studies, Laprol-604 was diluted in deionized water and prepared fresh daily.

Forty healthy and fertile adult male Wistar rats were 100 days old. They had 180±200g body weight at study start. Males were randomly divided into four groups (10 animals in each group). Laprol-604 was administered to male rats once daily by gavage at doses of 1/10, 1/100 and 1/1000 LD50 (median lethal dose) – 12.5g/kg, respectively is the 1-st; 2-nd and 3-rd group for 60 days (one cycle of spermatogenesis). The 4-th group (controls) consisted of 10 intact animals without Laprol-604 administration. Animals were maintained at a room temperature (20-22^{0}C) and relative humidity (50-60%) and kept under a 14 hours light/ 10 hours dark cycle. Pelleted diets were presented to the rats in wide mouthed jars with lids and fresh water was provided ad-libitum throughout the study.

Male rats were clinical observed twice daily during the study as an assessment of their general health and effect of Laprol-604 administration. They have been weighed weekly to see any change in the body weight. All the procedures were performed at Kharkiv Medical Academy of Postgraduate Education, according to Ukrainian and International guidelines for the use of animals in research (Law of Ukraine of 21.02.2006 № 3447-IV «On protection of animals from cruelty» // Supreme Council of Ukraine and European convention for the protection of vertebrate animals used for experimental and other scientific purposes. Strasbourg, 18.03.1986.

At the end of Laprol-604 administration, each male from each group was tested for sexual behavior. All sex testing took place during the rats' dark cycle (13:00-15:00) in the soundproof room and under dim red lighting. The test environment was open field (100 cm square) divided by sheets to create the four individual chambers, which were linked in the center. The chambers had open tops. Pelleted diets were presented in the first chamber; fresh water was provided in the second chamber; plastic house was in the third chamber. All male rats did not have a sexual experience. The male rat was taken into the test room, placed in the fourth chamber and allowed to adapt to the test environment for 20 minutes. Then an intact estrous adult female was placed into the center of test environment with each male. Female was left with male for one hour, during which time the experimenter recorded mount latency (ML), intromission latency (IL), ejaculatory latency (EL), number of mountings, number of intromissions and number of ejaculations. Mount latency was time from exposure to a female to the first mount (with or without intromission). Intromission latency was time from exposure to a female rat to the first intromission (with or without ejaculation). Ejaculatory latency was time interval between the intromission and ejaculation. Male sexual behavior was assessed during one hour; rats were observed by a single experimenter, blind to conditions, who recorded all behavior. Between trials, the test environment was thoroughly cleaned with 70% ethanol and the bedding was replaced. Each male rat interacted with only one female rat.

According to the aim of study, at the end of the experiment, the male rats were weighed, euthanized under light or anesthesia. Trunk blood was obtained by decapitation. Serum samples were prepared and stored at –20°C. To determine serum level of follicle stimulating hormone (FSH), luteinizing hormone (LH) and testosterone in male rats was used standard ELISA technique. The male rat reproductive organs were removed, weighed on electronic balance and processed for detailed sperm dynamical, biochemical and hormonal studies. The total number of sperms was assayed by the method of V.P. Mamina and Y. Ban, in a male rat suspension of homogenized testes [2; 6]. The spermatozoa from the tubules of cauda epididymis were released into physiological saline (0.9% NaCl). The motile and immotile sperms were counted in ten separate and randomly selected fields. The results were represented such as percent motility.

The data were statistically analyzed using one-way analysis of variance (ANOVA) followed by Dunnet's multiple comparison test $p< 0.05$ was considered significant. For all values, the means ± standard error mean (SEM) was calculated.

Results and discussion

After administration of Laprol-604 the sexual behavior of male rats was studied. The following Laprol-604 effects on male sexual behavior were demonstrated in the table 1

Table 1
The influence of Laprol-604 on sexual behavior of male rats (M± SEM)

Parameters	Groups of animals			
	Control group (n=10)	First group 1/10 LD50 (n=10)	Second group 1/100 LD50 (n=10)	Third group 1/1000 LD50 (n=10)
Mount latency (sec)	131.17 ± 29.56	480.0 ± 49.20 *	315.4 ±27.50*	287.6 ±35.12*
Number of mounts	12.1 ± 1.6	3.1 ± 0.2 *	4.4 ±0.6*	7.5 ±0.8*
Intromission latency (sec)	142.3 ± 27.79	488.0 ± 36.40	329.5 ± 31.70*	286.3 ± 32.80
Number of intromissions	13.2 ± 1.4	3.8 ±0.3*	5.1 ±0.2*	8.2 ±0.6

Ejaculatory latency (sec)	389.5 ± 29.66	-	-	-
Number of ejaculations	1.3± 0.06	0	0	0

Note. ** Significant differences (p<0.05) from control values.*

All control male rats showed sexual activity in the presence of estrous female. The sexual behavior of control male rats consisted of pre-sexual performances, copulation and ultimate ejaculation. In contrast to controls, male rats exposed to Laprol-604 exhibited the incomplete sexual behavior. The following changes in the sexual behavior of male rats treated with Laprol-604 were discovered: the mount latency of males 1-st, 2-nd and 3-rd groups was significantly lengthened by 3.7, 2.4 and 2.2 times, respectively, compared to controls. The number of mountings was significantly reduced by 3.9, 2.8 and 1.6 times in male rats of 1-st, 2-nd and 3-rd groups compared to control animals. Similarly the number of male rat's intromissions were also significantly decreased by 3.5, 2.6 and 1.6 times in the relevant groups compared to control male rats. Intromission latency of male rats 1-st, 2-nd and 3-rd groups was by 3.4, 2.3 and in 2.0 ftimes significantly higher than data of controls. It should be noted, estrous females were provided to male rats treated by Laprol-604, but all of their failed to copulate.

Male rats in all Laprol-604 treatment groups exhibited lower levels of receptivity to the estrous females. The mount latency and intromission latency were differed from control and depended on dose of polyol (Table 1). A more severe disruption of behavioral masculinization occurred when dose of Laprol-604 was 1/10 and 1/100 of LD50. Both of the mount latency and intromission latency were significantly lengthened, by contrast, the numbers of mountings and intromissions were reduced and ejaculations were absent in animals 1-st, 2-nd, and 3-rd groups. The analysis examining mount latency and intromission latency across the Laprol-604 treatment groups showed that they were longer in 1-st and 2-nd groups than 3-rd group.

Both serum luteinizing hormone (LH) and follicle stimulating hormone (FSH) levels were comparable between control group and Laprol-604 treated groups, assessed after 60 days of treatment. The results of this study demonstrated that 10 (100%) control male rats had normal endocrine pattern against 30 (100%) male rats of 1-st, 2-nd and 3-rd groups which had an endocrinopathy. There was statistically lower level of FSH which was 1.11±0.08 IU/ml, 1.74 ±0.13 IU/ml, 2.06±0.25 IU/ml in male rats of 1-st, 2-nd and 3-rd groups, respectively, than 4.98 ± 0.19 IU/ml in control male rats. The luteinizing hormone (LH) mean blood level was 1.83±0.09 IU/ml, 2.21±0.16 IU/ml and 2.89±0.15 IU/ml in male rats of 1-st, 2-nd and 3-rd groups, respectively, that was significant difference compared with mean control value 5.05±0.16 IU/ml. Significantly altered level of serum testosterone has been found in male rats of the 1-st, 2-nd and 3-rd groups (0.91 ±0.04 IU/ml, 1.21±0.12 IU/ml and 1.53±0.18 IU/ml, respectively) compared with control animals (2.07±0.16 IU/ml). The disturbance of sexual behavior may be related to decrease of serum testosterone level in Laprol-604 treatment male rats which needs further analyses.

This study attempted to relate the Laprol-604 treatment effects with testosterone, luteinizing hormone and follicle stimulating hormone levels that are known to influence on the process of sexual behavior.

The biochemical changes in the rat testes obtained after oral administration of Laprol-604 have been shown in the table 2.

Table 2
Biochemical changes in the rat testes after exposure to Laprol-604 (M± SEM)

Parameters	Groups of animals			
	Control group (n=10)	First group 1/10 LD50 (n=10)	Second group 1/100 LD50 (n=10)	Third group 1/1000 LD50 (n=10)
Cholesterol (mg/g)	6.92 ±0.41	4.50 ±0.19*	5.20 ±0.18*	5.96 ±0.25
Protein (mg/g)	261.50 ±8.20	118.68 ±2.55*	173.84 ±5.36*	204.99 ±6.81
Glycogen (mg/g)	3.10 ± 0.12	1.38 ± 0.06**	1.95 ± 0.01*	2.67 ± 0.08
Acid phosphatases (units)	5.59 ±0.28	11.38 ±0.66*	9.80 ±0.27*	6.78 ±0.30
Alkaline phosphatase (units)	61.15±4.26	38.74±5.31*	45.33±4.29*	52.71±5.34

Note. ** Significant differences (p<0.05) from control values.*

Biochemical profile of the testes revealed a significant decline in the contents of protein, testicular cholesterol and glycogen in male rats of 1-st and 2-nd groups compared with control animals. At the same time, the activity of testicular acid phosphatase was significantly increased in 2,0 and 1,8 times, but decreasing alkaline phosphatase activity in 1,6 and 1,4 times was found in 1-st and 2-nd groups, respectively, compared with control group.

The changes of reproductive organ weights of male rats obtained after oral administration of Laprol-604 have been shown in the table 3.

Table 3
Body and reproductive organ weights of male rats after Laprol-604 administration (M± SEM)

Parameters	Groups of animals			
	Control group (n=10)	First group 1/10 LD50 (n=10)	Second group 1/100 LD50 (n=10)	Third group 1/1000 LD50 (n=10)
Body weight (g)	387.83 ± 12.79	266.45±11.26*	295.24 ± 14.46	338.17 ± 10.08
Testis weight (g)	1.87 ± 0.05	0.96 ± 0.08*	1.16 ±0.04*	1.56 ±0.08
Epididymis weight (g)	0.61 ± 0.02	0.45 ± 0.04*	0.49 ± 0.02*	0.51 ± 0.04

Seminal vesicle (g)	1.33 ± 0.08	0.63 ± 0.09*	0.96 ± 0.04	1.17 ± 0.05
Ventral prostate (g)	0.67 ± 0.03	0.39 ± 0.03*	0.47 ± 0.01*	0.53 ± 0.02

Note. ** Significant differences (p<0.05) from control values.*

Body weights of male rats as well as weights of reproductive organs have been diminished by Laprol-604 treatment. Administration of Laprol-604 to male rats has been resulted in reproductive toxicity. Laprol-604 showed dosage dependent reproductive toxicity influence on adult male rats.

The fertility parameters of male rats obtained after oral administration of Laprol-604 are shown in table 4.

Table 4

Sperm density in testes, cauda epididymis and sperm motility in cauda epididymis after Laprol-604 administration

Parameters	Groups of animals			
	Control group (n=10)	First group 1/10 LD50 (n=10)	Second group 1/100 LD50 (n=10)	Third group 1/1000 LD50 (n=10)
Testis (million/ml)	5.8 ± 0.17	2.3 ± 0.19*	3.5 ±0.16*	4.8 ±0.16
Cauda epididymis (million/ml)	21.7 ± 1.15	11.8 ± 1.13*	14.9 ± 1.18*	17.8 ± 1.16
Sperm motility in cauda epididymis (%)	81.3 ±4.8	35.3 ± 3.79*	56.4 ± 4.46*	71.4 ± 4.66

Note. ** Significant differences (p<0.05) from control values.*

The weight of testis is largely dependent on the mass of differentiated sper-matogenic cells and the reduction in the weight of testis may be due to reduced tu-bule size, decreased number of germ cells and elongated spermatids. It is known that the sperm motility has been affected by altered enzymatic activities of oxida-tive phosphorylation. The normal spermatozoa movement depends on the amount of ATP as well as testosterone level. Testosterone is the principal androgen of the testes and it is essential for sperm production and maintenance [16]. So, the depression of ATP and testosterone amount leads to mortality of sperm which may cause infertility [3]. A direct correlation between testosterone and spermatozoa motility that has been reported [18]. Exposure of Laprol-604 also changes the biochemical parameters of the testes like other surfactants [19]. A decrease in testicular cholesterol, protein and glycogen levels may be due to interference in testosterone synthesis, spermatogenesis and glycogenolysis. Since glycogen is an energy source for general metabolism and constant supply of glucose is essential for proper functioning of testes. Similarly decreasing in testicular cholesterol content may be due to androgen production. The increase in acid phosphates activity may be the result of labialization of lysosomal system [4]. The reduction in serum testosterone demonstrates the inhibitory influence of non-ionic surfactants on the secretion of pituitary luteinizing hormone and follicle stimulating hormone in turn on the testosterone biosynthesis [13]. Laprol-604 treatment caused a significant ($p < 0.05$) decrease in both serum levels of LH and FSH compared to the control group.

Results and discussion

Sexual behavior in male rats is regulated by the hypothalamic-pituitary-testicular axis. Gonadotropin releasing hormone produced by hypothalamus triggers the luteinizing hormone released by adenohypophysis. The luteinizing hormone in turn stimulates the release of testosterone from the testes [8]. Testosterone and its metabolites initiate male sexual behavior by acting on key brain regions [11]. Reducing male testosterone levels after the course of Laprol-604 prolonged exposure to male rats have been found. The effect of Laprol-604 on testosterone levels of adult male rats was dose dependent. The long-term process of stem cells transformation into spermatozoa was disturbed by Laprol-604. Treatment with Laprol-604 changed the biochemical and morphometric parameters of the reproductive tract like other nonionic surfactants [13; 18; 19]. The male rats exposed by Laprol-604 illustrated a decrease in attempted of mountings and intromissions. There was also a drop in serum testosterone leveled off lower than was observed for control males. The significant relationships between the amount of sexual behavior and testosterone levels were found that has not been reported previously. The very low levels of sexual behavior of male rats of 1-rst and 2-nd groups suggest that impact of Laprol-604 to cause a drop in serum testosterone level. Both fertility and sex behavioral parameters showed distinct dose-dependence of the effects of prolonged Laprol-604 exposure.

The testosterone levels were reduced in males of 1-st, 2-nd groups after exposure them to Laprol-604. The testosterone level was higher in male rats of control group than in males of 1-st, 2-nd groups ($p<0.05$). The results of study provide evidence of a relationship between the amount of sexual activity and level of testosterone. Additionally, the lowest testosterone level was found in animal of the 1-st group treated the highest dose of Laprol-604 that is evidenced to nonionic polyols research by Zhukov V.S. et al. (2000), which indicated that rodents had a decrease in testosterone levels depending on the polyol dose [18;19].

Thus, our most important finding was that attenuated sexual interactions with estrous female in adult male rats were caused by prolonged Laprol-604 treatment and there is explanation for this effect. The lower levels of sexual activity (absent ejaculations and reduced number of intromissions) among males that administered Laprol-604 compared to control male rats corresponded to a significant decrease of testosterone, luteinizing hormone and follic stimuleting level. This result is supported by some past studies with rodents [13, 19]. An explanation is that testosterone can increase the sexual activity and an increase in neurogenesis [14, 15]. However, the polyol effects on sexual activity cannot fully explain our results.

Conclusion

1. Altered masculinization was seen in males of 1-st, 2-nd and 3-rd groups, despite the presentce of estrous female. The male rats of the 1-st and 2-nd groups with severely attenuated sexual behavior had the low testosterone level.

2. Prolonged administration of laprol-604 to male rats altered the release of pituitary follicle stimulating hormone and luteinizing hormone, led to reduction of testosterone production, changes the biochemical parameters of testes, epididymis and as a result the reduction of sperm production in the testes.

3. The findings showed the progressive morphometrical and biochemical alterations caused by various doses of Laprol-604 which have direct suppressing effect on the male rat reproductive function.

4. The study results highlight the direct relationship of dose to morphometrical and biochemical alterations.

5. The experiment has demonstrated the changes in sexual behavior of male rats that were paralleled by impaired fertility performance.

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