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ADVERSE EFFECTS OF LAPROL-604 ON PREGNANT WISTAR RATS*

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У статті представлені результати дослідження впливу Лапрола-604 на загальну вагу та вагу печінки вагітних щурів популяції Вістар, їх режим харчування та споживання води у період вагітності. Вагітним щурам (три групи по 25 тварин у кожній) вводили Лапрол-604 у дозі 0,125; 1,25 і 12,5 мг/кг один раз на добу за допомогою шлункового зонду з другого по двадцятий день вагітності. Контрольну групу склали 25 вагітних щурів, які перебували на стандартному раціоні віварію без введення Лапрола-604. Токсична дія Лапролу-604 відзначена дефіцитом ваги вагітних щурів, зниженням споживання їжі, збільшенням питного режиму і зростанням ваги печінки тварин, що спостерігали в 1-ій, 2-ій та 3-ій експериментальних групах. Ефект дії Лапролу-604 на експериментальні тварини був дозозалежним. Відставання у збільшенні ваги особливо відмічали серед вагітних щурів 2-ої і 3-ої груп. Вагітні щури 2-ої (1,25mg/ кг) та 3-ої (12,5 мг/ кг) групи мали дефіцит ваги до останнього тижня вагітності. Повільне збільшення ваги відповідало значному зниженню споживання їжі щурами під час вагітності. Вага печінки щурів, що отримали Лапрол-604, за винятком 1-ї групи, відрізнялися від контролю в значній мірі. Слід зазначити, в той час як був дефіцит ваги щурів, відмічали відносне збільшення ваги печінки тварин всіх трьох груп, що отримали Лапрол-604. Протягом експерименту відмічено зниження вживання їжі та збільшення водного режиму серед тварин 2-ої та 3-ої груп в порівнянні з контрольними самками.

Ключові слова: Лапрол-604, поліоли, поверхнево-активних речовин, репродуктивна токсичність, вагітні щури, день гестації, вага печінки, споживання їжі, споживання води..

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 occupation and home activities in which humans can be exposed to them [4; 14]. Surfactants are also subjected to biological and environmental transformations that may form other compounds [6; 7].

Once released into the environment, the more persistent surfactants can be carried by air and water currents to remote locations and many can be biomagnified through food webs to high levels in humans and other top predators [11; 12]. Transfer of surfactants and their products of biotransformation from the pregnant female to the developing fetus through the placenta and to offspring in mothers' milk also occur in both wildlife and humans [8].

Laprol-604 is non-ionic group of surfactants, including Laproxide-303, methycellosolve, methylcarbitol, polyol P-373-2-20 et cetera. Laprol-604 is constituent of paints, glues and is used to produce plastic materials [14]. The assessment some of animal health effects of exposure to non-ionic group of surfactants has been done. It is known the results of numerous toxicological researches dealing mainly with allergologic, immunologic effects and impact on metabolisms of lipids, proteins and nucleic acids. Some studies report that Laproxide-303, polyols P-373-2-20 and P-5003-AC may produce detectable reproductive impairments of experimental animals [9; 10; 13]. However the influence of Laprol-604 on reproductive system, as well as, the quantitative data on exposure to Laprol-604 has not been available to allow the dose-response relationship to be characterized.

The weight of pregnant rats, their food and water consumption, as well as their liver weight have been examined with the goal of researching the reproductive toxicity of Laprol-604.

Materials and methods

Laprol-604 was provided 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.

According to biologic characteristic of Wistar rat, the placenta is considerably more porous. This property may increase the chance of fetal exposure to an administered test material.

One hundred pregnant Wistar rats (body weight, 180±30 g at study start) bred within a 4-h period in the afternoon and overnight. Those animals with spermatozoa in a vaginal smear were considered to be at gestation day (GD) 0. They were randomly divided into four groups (25 animals in each group). Laprol-604 was administered to pregnant dams once daily by gavage at doses of 0,125; 1,25 and 12,5 mg/kg, respectively is the 1-st; 2-nd and 3-rd group from GD 2 until GD 19. The 4-th group (controls) consisted of 25 intact animals without Laprol-604 administration. The pregnant rats were kept individually in polypropylene cages with heat-treated pine shavings for bedding and tap water ad libitum.

Pelleted diets were presented to the rats in wide mouthed jars with lids. Animal facilities were controlled for temperature (20-22°C) and relative humidity (50-60%) and kept under a 12-hr light/ 12-hr dark cycle.

All the procedures were performed in the Kharkiv Medical Academy 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. 2006; 27:230 and European convention for the protection of vertebrate animals used for experimental and other scientific purposes. Strasbourg, 18.03.1986, http://conventions.coe.int/treaty/en/Treaties /Word/123.doc) [3].

Pregnant rats routinely monitored during study as an assessment of their general health and to effect of Laprol-604 administration. Daily observation was performed in the afternoon to assess the health of the dams. In this observation, behavioral status, respiratory signs, skin, eyes, and excretory products were noted. Pregnant rats have been weighed on the 0, 6, 9, 12, 15, 18, 20-th days of gestation. Throughout rat gestation food and water consumption were recorded.

On 20-th gestational day 10 pregnant rats from each group were euthanized for humane reasons to prevent autolysis and tissue loss.

During the necropsy, an external examination of the pregnant rats was done; all organs were examined, in situ. The livers were removed, weighed and immediately frozen on

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dry ice and stored at - 80°C for investigation. Liver weights were recorded.

Statistical analysis of the data was performed using GraphPad Prism 5. Student's t test was used to detect differences between independent groups of normally distributed variables; difference between groups was considered statistically significant at $p<0.05$.

Results

The influence of Laprol-604 on weight gain in pregnant rats was 25,10±1,280g (controls), 22,10±1,26g, 18,20±1,26*g and 12,63±1,29*g (1-rst, 2-nd and 3-rd group, respectively) on the 6-th gestational day (Fig.1) .

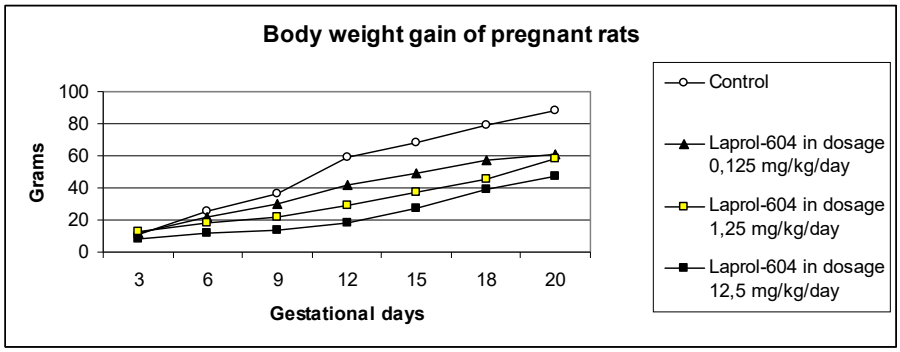

Fig. 1. Impacts of Laprol-604 on weight gain in pregnant rats.

On the 12-th gestational day, the weight gain in pregnant dams were 59,80±2,31g (controls), 42,7±2,96g, 29,30±2,18*g and 18,93±1,37*g (1rst, 2nd and 3rd group, respectively). On the 18-th gestational day, the weight gain in pregnant rats were 79,70±2,68g (controls), 57,5±2,68g, 45,10±2,26g* and 39,30±2,09*g (1rst, 2nd and 3rd group, respectively). The results of weighting of rats were 88,30±3,52g (controls), 61,11±1,67*g, 58,70±2,93*g and 47,80±2,03*g (1rst, 2nd and 3rd group, respectively) on the 20-th gestational day. A treatment effect of Laprol-604 was significantly different indicated in 3-rd and 2-nd groups compared with controls, with the exception of the 1-rst group (0,125 mg/kg) group. Significant variations from controls for the 12,5 mg/kg dose group beginning at 6 gestational day (GD), the 1,25 mg/kg dose group at from GD 12 to GD 18.

It showed be noted, the significant reduction of food consumption (Fig.2). and, in the same time, the water intake was increased in these groups of pregnant rats (Fig.3).

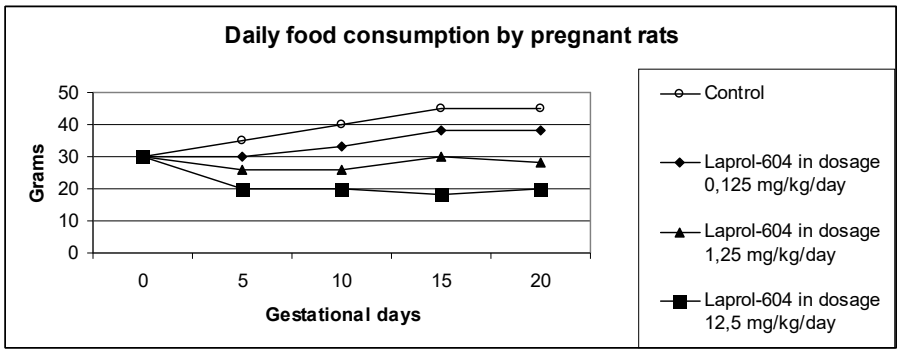

Fig. 2. Daily food consumption by pregnant rats.

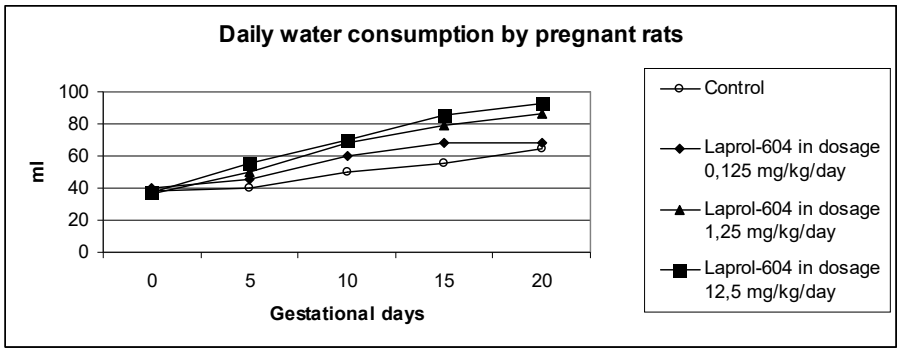

Fig. 3. Daily water consumption by pregnant rats.

Laprol-604 showed dosage dependent developmental toxicity when the pregnant rats were exposed. It led to reduced statistically significant body weight of pregnant dams. In contrast, Laprol-604 administration increased maternal liver weight in a dose-dependent fashion. Indeed, in the highest dosage 3-rd group (12,5 mg/kg), the livers weight almost doubled (16,7±0,44*), compared with those (9,2±0,66) in controls. Indeed, in the highest dosage 3-rd group (12,5 mg/kg), the livers weight almost doubled (16,7±0,44*), compared with those (9,2±0,66) in controls (Table 1).

Table 1
Effect of Laprol on rat maternal liver weight (M±m)

Gestational day	Groups of animals

	Control group (n=10)	First group 0,125 mg/kg (n=10)	Second group 1,25 mg/kg (n=10)	Third group 12,5 mg/kg (n=10)
	Liver weight, g			
20	11.7±0.4	13.1±0.5	15.2±0.4*	16.7±0.44*
20	Relative liver weight, (%)			
	4.0±0.1	5.1±0.1	5.8±0.1*	6.8±0.1*

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

With the 20-day exposure Laprol-604 affected liver weight in pregnant rats (Table 1). The liver/body weight ratio was increased in the 1,25 mg/kg and 12,5 mg/kg dosage groups the most likely reflecting the marked body weight deficit.

Discussions

Maternal toxicity of Laprol-604 indicated by deficits in weight gain, reduction of food consumption, increase in drinking regimen and largamente of liver weight were observed in 1-rst, 2-nd and 3-rd groups, during pregnancy. The severity Laprol-604 induced effects were dose-dependent. The lag in weight gain was particularly pronounced in the 3-rd group (the highest dosage group, 12,5 mg/kg), which exhibited significant reduction of food intake but water consumption was increased, duration of pregnancy period. The Laprol-604-induced reductions of weight gain in the pregnant rat seen here are comparable to similar alterations produced by the non-ionic surfactant in the rats and mice [13], indicating that the adverse effect on maternal weight gain may be a common feature of toxicity for the non-ionic surfactants. Liver enlargement might be associated with biochemical disturbances is another feature seen after exposure to Laprol-604 and other non-ionic surfactants [9; 10; 13]. A somewhat similar finding was obtained in another study. An increase of liver weight is generally observed in rodents during pregnancy (by about 24% in rat, Buelke-Sam et al., 1982) [2]. Above and beyond this physiological change, significant elevations of hepatic weight were found in the 3-rd group as much as twofold over the corresponding the rat of control group. Interestingly, the small increase in the relative liver weight in the 0,125 mg/kg dosage group largely reflected the reduction of body weight, rather than a net increase of liver weight. Like as an explanation for these findings may be attributed to the relatively low dosage of Laprol-604. Nonetheless, the high sensitivity to Laprol-604-induced liver toxicity in the rat of 3-rd group should be noted and the liver weight increase estimated at 1,7 times.

The liver being the major organ carrying out metabolic and detoxification processes is unique among the body's vital organs. Organ weight can be the most sensitive indicator of an effect of an experimental compound, as significant differences in organ weight between exposure and un exposure (control) animals may occur in the absence of any morphological changes [1].

The liver is such a frequent target organ in toxicity studies (in fact, the most common) that a discussion of some of the more common lesions that occured in the pregnant rats seems warranted. Observations carried out on the pregnant rats, lead to the following findings: continuous increase of liver weight along pregnancy from 2nd to 20th days, mainly within the 2nd week (organs making), to reach limit value on the 18th day [1; 5].

The overall mother's weight is proportional to liver weight in pregnant rats of control group. All these facts underline metabolic relations between mother and fetus during pregnancy.

Acknowledging the results reported here and the fact that Laprol-604 is frequently used a variety of industry, it is necessary continue research the influence of Laplor-604 on different metabolic pathways of living organisms.

Conclusion

1. Within the experimental model reported here, we have shown that the exposure to Laprol-604 on pregnant Wistar rats reduces the weight gain and the food consumption of animals, moreover, elevates their daily water intake. The adverse effect of Laprol-604 was dose-dependent.

2. Liver weights of the Laprol-604 -exposed pregnant rats, except 1-rd group, were different from controls appreciably. It should be noted, while body weight was deficit, the relative liver weight of all Laprol-604 dosage groups was significantly increased. Indeed, in the highest dosage 3-rd group (12,5 mg/kg), the livers weight almost doubled (16,7±0,44*), compared with those (9,2±0,66) in controls. Indicating the adverse effect on maternal weight gain may be linked with intoxication caused by Laprol-604 a common feature of toxicity for the non-ionic surfactants.

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Summary

The article reports the results of research Laprol-604-induced effects on general weight, liver weight of pregnant rats, and their food and water consumption. Pregnant Wistar rats were administered 0,125; 1,25 and 12,5 mg/kg Laprol-604 once daily by gavage from the second gestation day (GD) to the twentieth GD. Maternal toxicity of Laprol-604 indicated by deficits in weight gain, reduction of food consumption, increase in drinking regimen and largamente of liver weight were observed in 1-rst, 2-nd and 3-rd groups, during pregnancy. The severity Laprol-604 induced effects were dose-dependent. The lag in weight gain was particularly pronounced in the pregnant rats of the 2nd and 3-rd groups. Pregnant rats in the 2nd (1,25mg/kg) and 3-rd (12,5 mg/kg) groups failed to gain any weight until the last week of pregnancy. These weight gain deficits corresponded to significant reductions in food consumption throughout gestation. Liver weights of the Laprol-604 -exposed pregnant rats, except 1-rd group, were different from controls appreciably. It should be noted, while body weight was deficit, the relative liver weight of all Laprol-604 dosage groups was significantly increased.

Key words: Laprol-604, polyols, surfactant, reproductive toxicity, pregnant rat, gestation day, liver weight, food consumption, water intake

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