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RESEARCH ARTICLE
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Study of embryotoxic and teratogenic properties of Medicine No. 60 and evaluation of its effect on the reproductive function of rats

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ABSTRACT

Intramuscular administration of medicine No. 60 at a dose of 1.281 mg/kg (30 times the estimated highest daily dose for humans) when diluted with 1:5 saline solution to pregnant rats from Day 1 to Day 19 of pregnancy does not affect the indicators of pre- and postimplantation death of baby rats. The body weight of the rats exposed to the medicine No. 60 during the prenatal period of development did not differ from the indicators in the control group. The development of offspring in the experimental group during the entire observation period took place without deviation from the terms characteristic of the normal physiological development of animals of this species. As a result of the studies conducted, it was found that intramuscular administration of drug No. 60 at a dose of 1.281 mg/kg in a 1:10 dilution with saline solution, which was 30 times the estimated maximum daily therapeutic dose for humans, did not affect the sexual activity of animals, reproductive indicators (number of live fetuses, body weight of embryos, their craniocaudal size, number of yellow bodies, implantation sites, resorption), or the neonatal development of baby rats. Thus, there was no effect of medicine No. 60 in the test dose of 1.281 mg/kg on the reproductive function of healthy mature rats and does not exhibit embryotoxic and teratogenic activity.

Keywords: *complex medicinal product; embryotoxic and teratogenic properties; Medicine No. 60; Pletnev drops No. 60; reproductive function.*

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INTRODUCTION

A prerequisite for the use of new drugs for the treatment of humans is the conduct of toxicological studies on animals. The more thoroughly the toxicity in animals is studied, the fewer the adverse reactions that may occur in clinical trials.[1]

Conducting preclinical toxicological studies of new original medicines is necessary to predict their safety for human health.

Therefore, it is relevant to determine the toxicological characteristics of the combined medicinal herbal preparation No. 60, for which the fruits of common cumin, columns with stigmas of common corn and roots of burdock, are used.

LITERATURE REVIEW

Tincture of cumin fruits has antimicrobial[2,3,4] and fungicidal activities,[2,5] hepatoprotective effect,[6,7] and exhibits anti-inflammatory properties.[8,9]

Tincture of cumin fruits has a pronounced antioxidant effect.[8,9]

During toxicological examination, it was found that the tincture of cumin fruits with a single intraperitoneal administration to mice is less toxic. The LD_{50} index of the drug in this case is 480 mg/kg by dry residue.[10]

The administration of cumin fruit tincture at a dose of 30 ml per day for 3 months in 70 overweight and obese women showed that the drug significantly reduces body weight, body fat percentage, and waist circumference.[11]

Under the influence of tincture of cumin fruits at a dose of 30 ml per day, there is a marked decrease in appetite and body weight of patients.[12]

Tincture of columns with corn stigmas exhibits antimicrobial activity,[13] has anti-inflammatory[14] and antioxidant properties.[14]

During toxicological examination, it was found that the tincture of columns with corn stigmas with a single intragastric administration to rats is practically nontoxic, and LD_{50} value in terms of dry matter is 14.500 mg/kg.[15]

Burdock root tincture exhibits pronounced antioxidant[16,17] and antimicrobial activities.[18,19]

Burdock root tincture exhibits fungicidal properties,[18] has hepatoprotective,[19,20] anti-inflammatory,[19,21] hypoglycemic,[16,17] hypolipidemic,[17,22] as well as neuroprotective effects.[23,24]

MATERIALS AND METHODS

Study of embryotoxic and teratogenic properties of drug No. 60

Studies on the embryotoxicity and teratogenicity of drug No. 60 were conducted on 36 pregnant Wistar rats.

Drug No. 60 was diluted 1:10 with sterile saline solution and administered intramuscularly to rats from Day 1 to Day 19 of pregnancy at a dose of 1.281 mg/kg once per day.

On the 20th day of pregnancy, 70% of pregnant rats were euthanized by dislocation of the cervical vertebrae, followed by examination of the bone skeleton and internal organs of fetuses, as well as determination of indicators of preimplantation and postimplantation deaths according to the formulas:

$$\text{Preimplantation death, \%} = \frac{\left(\text{Number of yellow bodies} - \text{Number of implantation sites}\right)}{\text{Number of yellow bodies}} \times 100;$$

$$\text{Postimplantation death, \%} = \frac{\left(\text{Number of implantation sites} - \text{Number of live fetuses}\right)}{\text{Number of implantation sites}} \times 100$$

After assessing the viability of the fruits, determination of their weight, and analysis of external anomalies (subcutaneous hemorrhages, swelling of subcutaneous tissue) and malformations (curvature of the limbs and vertebral column, changes in eyeballs, auricles) were performed. After external examination of the fruits, one group of fruits (about 2/3) was fixed in 96% alcohol and after clarification with an alkali solution, rinsed with water, stained

with alizarin, dehydrated in various mixtures of glycerin with 96% alcohol and used to study the bone skeleton according to Dawson. Another group of fetuses (approximately 1/3) was fixed in Buena's fluid and used to study internal organs on Wilson–Dyban microanatomic sections (at the level of vibrissae, eyeballs, lateral and fourth ventricles of the brain, above and below the front paws, at the level of the heart and lungs, stomach, liver, kidneys), the bladder, ureters and organs of the reproductive system were also examined. Thirty percent of the total number of pregnant females were left for childbirth, after which, during the first month of the postnatal period, mortality, body weight dynamics, and physical development of baby rats were studied.

Evaluation of the effect of drug No. 60 on the reproductive function of rats

The study of the effect of drug No. 60 on the reproductive function of rats was carried out on female and male Wistar rats. The females of the experimental subgroup were intramuscularly injected with drug No. 60 daily for 2 weeks (3–4 estrous cycles) at a dose of 1.281 mg/kg, which was 30 times the estimated highest daily dose for humans. Before administration, drug No. 60 was diluted 1:10 in saline solution.

Males of the experimental subgroup were injected intramuscularly with drug No. 60 daily for 10 weeks (2–3 cycles of sperm maturation) at a dose of 1.281 mcg/kg, which was 30 times the estimated highest daily dose for humans. The animals of the control subgroups received the appropriate amount of saline solution at the same time.

After the end of the drug administration, three groups of animals were formed: the first, 20 control females were hooked to 10 control males; the second (drug No. 60), 20 experienced females were hooked to 10 control males; the third (drug No. 60), 20 control females were hooked to 10 experienced males.

During two estrous cycles, vaginal smears were examined. The stages of proestrus, estrus, diestrus, and metestrus were noted. The detection of sperm in vaginal smears was considered the first day of pregnancy. After 10 days, two subgroups were created from pregnant females in each of the three groups. The first subgroups of females were euthanized on the 20th day of pregnancy. The number of fetuses, resorption, implantation sites in the uterine cavity, and yellow bodies in the ovaries were counted. Based on the data obtained, the indicators of preimplantation and postimplantation death were calculated according to the previously indicated formulas, as well as the fertility index (the ratio of the number of females hooked to males and the number of females in whom sperm was detected in vaginal smears) and the pregnancy index (the ratio of the number of females in whom sperm was detected in vaginal smear and the number of animals who delivered pregnancy). Pathological changes (subcutaneous hemorrhages and swelling of subcutaneous tissue, disorders of the development of the skeleton, eyeballs) were recorded during the examination of the fetuses.

The second subgroups of pregnant females were left to give birth, observing the animals daily, they were weighed once a week and their weight gain was noted. The date of birth, the number of baby rats in the litter, and the body weight of newborn baby rats were recorded. On the 4th, 7th, 14th, and 21st day from the date of birth, the survival rate was calculated, and the total and average weight of the baby rats were noted.

RESULTS AND DISCUSSION

Due to the multicomponent composition of drug No. 60, it was necessary to conduct its preclinical toxicological study.

As a result of the conducted studies, it was found that the combined drug No. 60 increases the resistance of mice to deadly staphylococcal infection, stimulates humoral and cellular immunity, bactericidal activity of mouse peritoneal exudate cells, and human peripheral blood phagocytes, increases

the number of peripheral blood leukocytes of mice and the production of tumor necrosis factor-α by human mononuclear cells, and also exhibits antioxidant activity.

Study of embryotoxic and teratogenic properties of drug No. 60

The study on the embryotoxic and teratogenic properties of drug No. 60 was carried out on 36 pregnant Wistar rats, which were divided into two groups of 18 animals each: Group 1, control, Group 2, drug No. 60 (at a dose of 1.281 mg/kg, which is 30 times the estimated daily dose for humans).

The drug No. 60 was diluted 1:10 with saline solution and injected into rats intramuscularly throughout pregnancy (from Day 1 to Day 19) once per day.

Control animals received an appropriate amount of saline solution daily from the 1st day of pregnancy to delivery.

Intramuscular administration of the drug No. 60 to pregnant females at the studied dose of 1.281 mg/kg caused a slight slowdown in the weight gain of pregnant females, statistically unreliable compared with the control (Table 1).

The criteria for assessing the embryotoxicity of drug No. 60 such as the duration of pregnancy, the number of live fetuses, implantation sites, yellow bodies, and embryo body weight and craniocaudal size did not significantly differ from the control. The indices of pre- and post-implantation death in the experimental group also did not differ from those in the control (Table 2).

Macroscopic and microanatomic examinations (standard Wilson–Dybin incisions) of fetuses exposed to drug No. 60 in the prenatal period at a tested dose of 1.281 mg/kg revealed no significant differences in the frequency of registered pathologies in the experimental and control groups (Table 3).

In the analysis of total preparations stained with alizarin to study the development of the bone system in rat fetuses exposed in the prenatal period to drug No. 60 at a tested dose of 1.281 mg/kg,

TABLE 1. Dynamics of body weight of pregnant rats (%).

Animal groups	1st week	2nd week	3rd week
1 – control	108.8 ± 1.8	122.5 ± 2.1	145.7 ± 2.8
2 – preparation No. 60, 1.281 mg/kg	110.2 ± 1.4	119.7 ± 1.8	137.9 ± 2.2

TABLE 2. Indicators of the embryotoxic effect of drug No. 60 at a dose of 1.281 mg/kg when administered intramuscularly to rats from 1 to 20 days of pregnancy.

Investigated indicators	Animal groups	
	1 – control	2 – preparation No. 60, 1.281 mg/kg
Duration of pregnancy, (day)	22.7 ± 0.2	22.9 ± 0.2
Number of embryos per rat	9.5 ± 0.5	9.5 ± 0.6
Number of implantation sites per rat	10.8 ± 0.5	10.3 ± 0.5
Number of yellow bodies per rat	11.1 ± 0.5	10.6 ± 0.5
Preimplantation death, %	2.9	2.7
Postimplantation death, %	9.7	7.3
Craniocaudal fetal size, cm	3.4 ± 0.1	3.2 ± 0.1
Fruit weight, g	2.3 ± 0.1	2.4 ± 0.1

TABLE 3. Anomalies of development and hemorrhages in fetuses on the 20th day of the prenatal period.

Investigated indicators	Animal groups	
	1 – control	2 – preparation No. 60, 1.281 mg/kg
External hematomas, abs.	0	0
Abdominal hemorrhage, abs.	4 (10.5%)	3 (7.9%)
General edema of the embryo, abs.	0	0
Hematoma in the body tissue, abs.	1 (2.6%)	1 (2.6%)
Hydronephrosis, abs.	0.5 (1.3%)	2 (5.3%)
Fetal underdevelopment, abs.	0	1 (6.7%)
Hemorrhage in the pericardium, abs.	2 (5.3%)	3 (7.9%)

TABLE 4. Indicators of fetal skeletal development on the 20th day of prenatal development.

Absence of ossification centers in fetal skeletons	Animal groups	
	1 – control	2 – preparation No. 60, 1.281 mg/kg
Sternum, absolute amount per offspring	2.1	2.2
Hyoid bone, %	2.2	3.8
Forelimb, %		
2nd metacarpal bone	10.1	12.5
3rd metacarpal bone	1.2	3.8
4th metacarpal bone	8.0	7.2
Hind limb, %		
2nd metatarsal bone	8.0	9.4
3rd metatarsal bone	0.5	9.4
4th metatarsal bone	0.5	9.4
Trunk bones		
Sciatica	0	0
Iliac	0	0
Lonnaya	1.8	2.2

skeletal malformations were not detected. However, there was some delay in ossification at the following points: the 3rd and 4th metatarsal bones (Table 4).

With intramuscular administration of drug No. 60 at a dose of 1.281 mg/kg from the 1st to the 19th day of pregnancy, no significant differences were found in the number of rats born in the experimental group. Compared with the control, the percentage of stillborn baby rats, the body weight of live baby rats at birth, and the dynamics of their body weight during the first month of life did not significantly differ from the control indicators. The mortality rates of rats exposed to drug No. 60 in the studied dose in the prenatal period of development also did not differ from the controls (Table 5). The development of offspring during the entire observation period according to other accepted parameters (covering with wool, appearance of incisors, opening of

TABLE 5. Indicators of postnatal development of baby rats exposed in the prenatal period (Day 1 to Day 19 of pregnancy) to drug No. 60 at a dose of 1.281 mg/kg.

Investigated indicators	Animal groups	
	1 – control	2 – preparation No. 60, 1.281 mg/kg
Number of baby rats born per rat (stillborn baby rats, %)	9.7 ± 0.5 (0,34%)	7.3 ± 1.0 (4,5%)
Postnatal mortality, %	5.1%	6.8%
Body weight of baby rats, g		
At birth	6.4 ± 0.5	6.1 ± 0.1
7th day of life	15.5 ± 1.0	15.1 ± 0.8
14th day of life	23.9 ± 1.2	23.2 ± 0.8
21st day of life	37.1 ± 2.1	35.5 ± 2.4

the eyes, detachment of the auricles, opening of the vagina, lowering of the testicles, time of maturation of reflexes, etc.) took place without deviation from the terms characteristic of the normal physiological development of animals of this species.

Drug No. 60 in a tested dose of 1.281 mg/kg (30 times the estimated therapeutic dose for humans) when administered intramuscularly to pregnant rats from Day 1 to Day 19 of gestation does not affect the dynamics of body weight of pregnant rats and the duration of pregnancy. The effect of drug No. 60 on indicators of embryotoxicity such as the number of live fetuses, the body weight of embryos, the number of implantation sites and yellow bodies, indicators of pre- and postimplantation death has not been established. Under the influence of the drug, there was a delay in ossification of two metatarsal bones.

Thus, in the tested dose of 1.281 mg/kg (30 times the estimated highest daily therapeutic dose for humans), drug No. 60 when administered intramuscularly to pregnant rats from Day 1 to Day 19 of gestation does not have embryotoxic and teratogenic properties.

Study of the effect of drug No. 60 on the reproductive function of rats

The study of the effect of drug No. 60 on the reproductive function of rats was performed on female and male Wistar rats (initial body weight 180–200 g). A group of females consisting of 60 animals was divided into two subgroups: control (40 animals) and experimental (20 animals). The females of the experimental subgroup were intramuscularly injected with drug No. 60 daily for 2 weeks (3–4 estrous cycles) at a dose of 1.281 mg/kg, which was 30 times the estimated highest daily dose for humans. Before administration, drug No. 60 was diluted in 1:10 saline solution.

A group of males consisting of 30 animals was divided into two subgroups: control (20 animals) and experimental (10 animals). Males of the experimental subgroup were injected intramuscularly with drug No. 60 daily for 10 weeks (2–3 cycles of sperm maturation) at a dose of 1.281 mg/kg, which was 30 times the estimated highest daily dose for humans. The animals of the control subgroups received the appropriate amount of saline solution at the same time.

After the end of the administration of drug No. 60, three groups of animals were formed: the first, 20 control females were planted to 10 control males; the second (drug No. 60), 20 experienced females were planted to 10 control males; the third (drug No. 60), 20 control females were planted to 10 experienced males.

When studying the fertility of rats, there were no significant differences from the control in all indicators, both in the group of females who

TABLE 6. Fertility Indicators of Animals Receiving Intramuscularly Drug No. 60 at a Dose of 1.281 mg/kg.

Investigated indicators	Control	Females preparation No. 60, 1.281 mg/kg	Males preparation No. 60, 1.281 mg/kg
The number of females planted, abs.	20	20	20
Number of fertilized females, abs.	18	18	17
Number of pregnant females, abs.	12	12	12
Fertility index, %	90	90	85
Pregnancy Index, %	66.7	66.7	76.5

TABLE 7. Effect of drug No. 60 with intramuscular administration at a dose of 1.281 mg/kg on the reproductive function of rats.

Investigated indicators	Control	Females preparation No. 60, 1.281 mg/kg	Males preparation No. 60, 1.281 mg/kg
Number of pregnant females, abs.	12	12	13
Number of yellow bodies	10.0 ± 0.8	11.0 ± 0.7	10.9 ± 0.6
Number of implantation sites	9.5 ± 0.8	10.5 ± 0.6	10.7 ± 0.6
Number of live rat fetuses	9.2 ± 1.0	9.7 ± 0.6	10.0 ± 0.6
Number of resorption	0.3 ± 0.1	0.8 ± 0.2	0.7 ± 0.2
Preimplantation death, %	5,2	4.4	1.3
Postimplantation death, %	4.6	7.3	6.6
The weight of the animal's fetus, g	2.6 ± 0.1	2.6 ± 0.4	2.7 ± 0.2
Craniocaudal fetal size, g	31 ± 0.03	3.1 ± 0.01	3.1 ± 0.04

received drug No. 60 before pregnancy, and in the group of females pregnant from males who received the drug at the studied dose of 1.281 mg/kg. The fertility index in both experimental groups practically did not differ from the controls. The data obtained are shown in Table 6.

Drug No. 60 at a dose of 1.281 mg/kg when administered intramuscularly to males and females did not cause significant changes in indicators of the reproductive function of rats such as the number of yellow bodies, implantation sites, live fetuses and resorption, embryo mass, and their craniocaudal size. The indicator of pre- and postimplantation death did not exceed the indicators in the control (Table 7).

The weight gain of pregnant rats, the duration of pregnancy, and the instinct of motherhood in experimental animals receiving drug No. 60 intramuscularly at a test dose of 1.281 mg/kg did not differ from similar indicators in control animals.

When monitoring the postnatal development of offspring, there was no increase in the death of baby rats in both experimental groups of females who received drug No. 60 before pregnancy and pregnant males exposed to the drug compared with the control indicators (Table 8).

The number of baby rats per rat, both in the group of females receiving drug No. 60 and in the group of females pregnant from males receiving the drug, as well as the weight of fetuses at birth and further weight gain of newborn baby rats in the first month of neonatal development, did not significantly differ from the control (Table 8).

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TABLE 8. Indicators of postnatal development of baby rats in the study of the effect of drug No. 60 on the reproductive function of rats.

Investigated indicators	Control	Females preparation No. 60, 1.281 mg/kg	Males preparation No. 60, 1.281 mg/kg
Number of baby rats per female, abs.	9.0 ± 0.7	7.7 ± 0.8	7.8 ± 1.6
Postnatal mortality of baby rats after 3 weeks, %	7.4	8.5	4.3
Body weight of newborn baby rats, g	6.2 ± 0.3	6.2 ± 0.4	6.2 ± 0.5
Dynamics of body weight of baby rats, g			
1st week	13.3 ± 1.3	12.2 ± 0.9	14.8 ± 0.4
2nd week	26.0 ± 1.6	25.8 ± 1.2	27.0 ± 1.0
3rd week	35.0 ± 7.0	37.6 ± 0.9	42.2 ± 2.1

The physical development of baby rats during the 1st month of postnatal development (covering with wool, appearance of incisors, opening of the eyes, peeling of the auricles, opening of the vagina, lowering of the testicles, etc.) did not differ from the terms characteristic of the normal physiological development of animals of this species.

In conclusion, it should be noted that as a result of the studies conducted, it was found that intramuscular administration of drug No. 60 at a dose of 1.281 mg/kg in a 1:10 dilution with saline solution, which was 30 times the estimated maximum daily therapeutic dose for humans, did not affect the sexual activity of animals, reproductive indicators (number of live fetuses, body weight of embryos, their craniocaudal size, number of yellow bodies, implantation sites, resorption), or the neonatal development of baby rats.

Thus, there was no effect of drug No. 60 in the test dose of 1.281 mg/kg on the reproductive function of healthy mature rats.

CONCLUSION

Intramuscular administration of medicine No. 60 at a dose of 1.281 mg/kg (30 times the estimated highest daily dose for humans) when diluted with 1:5 saline solution to pregnant rats from Day 1 to Day 19 of pregnancy does not affect the indicators of pre- and postimplantation death of baby rats. The body weight of the rats exposed to medicine No. 60 during the prenatal period of development did not differ from the indicators in the control group. The development of offspring in the experimental group during the entire observation period took place without deviation from the terms characteristic of the normal physiological development of animals of this species. As a result of the studies conducted, it was found that intramuscular administration of drug No. 60 at a dose of 1.281 mg/kg in a 1:10 dilution with saline solution, which was 30 times the estimated maximum daily therapeutic dose for humans, did not affect the sexual activity of animals, reproductive indicators (number of live fetuses, body weight of embryos, their craniocaudal size, number of yellow bodies, implantation sites, resorption), or the neonatal development of baby rats. Thus, there was no effect of medicine No. 60 in the test dose of 1.281 mg/kg on the reproductive function of healthy mature rats and does not exhibit embryotoxic and teratogenic activities.

COMPETING INTERESTS

The author claims that he has no competing interests.

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