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FEATURES OF EXPERIMENTAL STUDIES WITH USE OF ETHANOL ON FEMALES OF CONVENTIONAL WHITE RATS

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Abstract. ***Introduction.*** In fundamental medical and biological toxicological research, including the study of the effects of ethanol intoxication on living organisms, a wide variety of animals are used, such as rodents, birds, cats, dogs and even primates. The present study ***aimed*** to investigate the effects of ethanol intoxication on female outbred albino rats, with a focus on pregnancy dynamics and offspring quantity, to optimize experimental planning in ethanol-related research. ***Material and methods.*** The experiment utilized 250 conventional female albino rats and 580 newborn offspring. Throughout pregnancy, all experimental groups were provided with standard feed and a 15% ethanol solution as the sole source of fluid (administered semi-voluntarily). Statistical analyses were applied to evaluate mortality rates under semi-voluntary ethanol administration, gestational duration, and litter size. ***Results.*** The results demonstrated that ethanol exposure induced significant mortality among experimental subjects. During the first month, female mortality reached 25%; cumulative mortality over the subsequent three months did not exceed 3%, while 10% of females died during the fifth and sixth months. Additionally, ethanol exposure prolonged gestational duration from 21 days under physiological conditions to 26 days following six months of pregravid ethanol administration. A reduction in litter size was observed, decreasing from 11 to 7 newborns per litter. ***Conclusion.*** Thus, ethanol intoxication in female albino rats increases mortality, prolongs pregnancy, and reduces offspring quantity, with the severity of effects correlating with the duration of pregravid ethanol exposure. For experimental studies modeling chronic ethanol exposure, it is critical to adjust animal cohort sizes: a 30% increase in initial sample size is recommended for studies lasting up to four months, and a 40% increase for longer durations. Furthermore, experimental designs investigating antenatal ethanol effects must account for extended gestational periods (up to 26 days after six months of pregravid exposure) and diminished offspring yield.

Keywords: ethanol, rat, pregnancy, mortality, offspring

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ОСОБЕННОСТИ ЭКСПЕРИМЕНТАЛЬНЫХ ИССЛЕДОВАНИЙ С ИСПОЛЬЗОВАНИЕМ ЭТАНОЛА НА САМКАХ КОНВЕНЦИОНАЛЬНЫХ БЕЛЫХ КРЫС

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Резюме. ***Введение.*** В фундаментальных медико-биологических токсикологических исследованиях, в том числе и для изучения влияния этаноловой интоксикации на живой организм, используются самые разнообразные животные, такие как грызуны, птицы, кошки, собаки и даже приматы. Настоящее исследование выполнено с ***целью*** изучить некоторые особенности влияния этаноловой интоксикации на самок беспородных белых крыс, а также особенности течения у них беременности и на количество их потомства для оптимального планирования исследований с использованием этанола. ***Материалы и методы.*** Работа выполнена на 250 самках конвенциональных белых крыс и 580 новорожденных крысах. Экспериментальные группы животных получали комбикорм, а в качестве единственного источника жидкости — 15% раствор этанола во время беременности (полудобровольное введение). С использованием статистических методов исследования изучены смертность крыс при полудобровольном введении этанола, продолжительность беременности и количество новорожденных крыс. ***Результаты.*** Показано, что воздействие этанола привело к гибели части экспериментальных животных. В течение первого месяца эксперимента смертность самок достигала 25%; в последующие три месяца смертность крыс в совокупности не превышала 3%; за пятый-шестой месяцы эксперимента погибло 10% самок. Помимо этого наблюдалось увеличение продолжительности беременности с 21 дня в физиологических условиях до 26 суток после шестимесячного прегравидарного воздействия этанола и уменьшение числа новорожденных крыс в помете с 11 до 7 соответственно. ***Заключение.*** Таким образом, воздействие этанола на самок белых крыс приводит к увеличению смертности и продолжительности беременности экспериментальных животных, а также уменьшению количества новорожденных крысят в помете. Выраженность изменений зависит от длительности прегравидарного воздействия этилового спирта. Выполняя экспериментальные исследования с использованием этанола, необходимо принимать во внимание, что при моделировании его хронического воздействия продолжительностью менее четырех месяцев следует увеличивать количество животных на 30%, а более — на 40% от изначально планируемого. Планируя исследование по изучению антенатального влияния этанола важно учитывать пролонгацию беременности (до 26 суток после 6 месяцев прегравидарного воздействия этанола) и уменьшение количества полученного потомства.

Ключевые слова: этанол, крыса, беременность, смертность, потомство

INTRODUCTION

Despite positive trends in alcoholism registration, about 2% of the world's population, despite the political and economic situation in a country, is affected by this disease. Recent studies show that losses (demographic, medical-social, financial-economic and moral-ethical ones) from alcoholism remain high [1, 2]. Fundamental medical and biological toxicological studies, including studies of the effects of ethanol intoxication on living beings, use a wide variety of animals, such as rodents, birds, cats, dogs, and even primates [2, 3]. Nevertheless, in a large number of works performed on this subject, the experimental subject is rodents, in particular rats [4–8], which, in turn, have been universal classical laboratory animals for about 150 years [9].

Currently, there are more than 100 outbred stock and inbred lines of laboratory rats, among which rats of the Bio Breeding Wistars, Bio Breeding Sprague-Dawley, Canadian Breeding Wistars and Canadian Breeding Sprague-Dawley lines are most often used to study the toxic effects of ethanol [1]. For example, Bio Breeding Wistars prefer ethanol, which accounts for 53% of total fluid intake, and Bio Breeding Sprague-Dawley rats prefer water (26% of fluid intake). Among conventional rats, ethanol is preferred by 23% of animals, 25% consume ethanol and water in equal amounts, and 52% completely reject alcohol.

There are three main methods of ethanol administration, which have their pros and cons [1]. The first method is forced, in which ethanol is administered through a gastric catheter. This method offers the advantage of accurate dosing of ethanol, while the disadvantage is additional stress, which can significantly distort the results, especially when studying prenatal effects of ethanol, as well as the organs of the immune system, which are known to be characterized by extremely labile morphological and functional organization [10, 11].This method does not involve traumatic manipulations, whereas it lacks the ability to accurately record ethanol consumed when several rats are in the same cage. Finally, the third method is a voluntary method where rats can choose between ethanol solution and water. The positive side of this method is also the absence of additional stressors, and the negative side is the impossibility to track the volume and concentration of ethanol ingested when rats are kept in groups.

In recent decades, the problem of prenatal exposure to ethanol has become particularly relevant due to the fetal alcohol syndrome described in 1968 by P. Lemoine et al. [12]. In our opinion, the most optimal way to administer ethanol to rats to study its effect on the developing organism is semi-voluntary one.

AIM

The research was undertaken to explore some specific effects of ethanol intoxication on female mongrel white rats, as well as peculiarities of their pregnancy and the number of their offspring for optimal planning of studies using ethanol.

MATERIALS AND METHODS

The work was performed on 250 adult sexually mature female mongrel white rats and 580 newborn rats. Female rats were divided into 8 groups: one control group and seven experimental groups (Table 1). The daily diet of animals in the control group included dry mixed fodder and drinking water. All experimental groups of animals also received mixed fodder, and 15% ethanol solution during pregnancy as the only source of liquid.

The amount of consumed liquid and dry feed was recorded once a day at the same time.

Males were hooked to females on day at the estrus or proestrus stage, which was determined by vaginal swabs. The first day of pregnancy was counted after the detection of spermatozoa in the vaginal swab. All groups of animals were kept in the same conditions favorable for normal pregnancy.

Statistical processing of the obtained data was performed using SPSS 16.0 program for Windows. Average arithmetic mean and its standard error were calculated. Significance of differences between two related age groups was assessed by unpaired Student's criterion. Normal distribution was determined by descriptive statistics using the Shapiro-Wilk criterion. Levene's criterion was used to test for equality of variance. Differences were considered significant at $p \leq 0.05$.

RESULTS AND DISCUSSION

Features of drinking and food intakes. The study showed that administration of 15% ethanol solution to nonlinear rats in the first three days causes a decrease in drinking and food intakes up to 75–80%. Starting from the fourth day the volumes of dry feed and liquid intake increase and by the tenth day they recover to the initial level.

Mortality of female rats. Semi-voluntary administration of ethanol to mongrel female rats resulted in death of a number of experimental animals. The highest mortality

Table 1

Animal groups and their characteristics

Таблица 1

Группы животных и их характеристика

Группа животных / Animal groups	Характеристика группы / Group characteristics
Контрольная / Control	Животные получали питьевую воду до наступления беременности и во время нее / The animals received drinking water before and during pregnancy
I	Животные получали питьевую воду до наступления беременности, а во время нее — 15% раствор этанола / The animals received drinking water before pregnancy, and a 15% ethanol solution during pregnancy
II	Животные получали 15% раствор этанола в течение 1 месяца до наступления беременности и во время нее / The animals received 15% ethanol solution during 1 month before pregnancy and during pregnancy
III	Животные получали 15% раствор этанола в течение 2 месяцев до наступления беременности и во время нее / The animals received 15% ethanol solution during 2 months before pregnancy and during pregnancy
IV	Животные получали 15% раствор этанола в течение 3 месяцев до наступления беременности и во время нее / The animals received 15% ethanol solution during 3 months before pregnancy and during pregnancy
V	Животные получали 15% раствор этанола в течение 4 месяцев до наступления беременности и во время нее / The animals received 15% ethanol solution during 4 months before pregnancy and during pregnancy
VI	Животные получали 15% раствор этанола в течение 5 месяцев до наступления беременности и во время нее / The animals received 15% ethanol solution during 5 months before pregnancy and during pregnancy
VII	Животные получали 15% раствор этанола в течение 6 месяцев до наступления беременности и во время нее / The animals received 15% ethanol solution during 6 months before pregnancy and during pregnancy

Table 2

Characteristics of pregnancy duration and the number of newborn rats

Таблица 2

Особенности продолжительности беременности и количество новорожденных крыс

Группа животных / Animal group	Продолжительность беременности, суток / Pregnancy duration, days	Количество новорожденных крыс / Number of newborn rats
Контрольная	21–22	11,1±0,4
I	24–25	8,8±0,4*
II	21–22	9,6±0,3*
III	23–24	9,1±0,5*
IV	24–25	9,0±0,2*
V	23–24	8,7±0,4*
VI	24–25	7,7±0,2*
VII	26–27	7,1±0,2*

* Differences compared to control are statistically significant. / Различия по сравнению с контролем статистически значимы.

is recorded during the first month, reaching 25%. In the following three months, the mortality of rats is insignificant and does not exceed 3% cumulatively. Between the fifth and sixth months of the experiment, 10% of female rats died.

Duration of pregnancy. Under normal conditions, the duration of pregnancy of mongrel white rats is 21–22 days. Depending on the duration of alcoholic influence on females, the duration of pregnancy changed. When females consumed ethanol exclusively during pregnancy (group I), its duration increased by 3 days; after ethanol intoxication for one month before and during pregnancy (group II), there were no significant changes in this parameter compared with the control.Female rats exposed to ethanol for two months before pregnancy (group III) were pregnant for 23 days; exposed for three months (group IV) —

24 days; four months (group V) — 23 days; five months (group VI) — 24 days; six months (group VII) — 26 days (Table 2).

There is no general consensus regarding the change in gestational age in rats under alcohol exposure [13–17]. Data are given both on increasing pregnancy term in rats under ethanol influence during pregnancy, and on the absence of changes in this indicator. This parameter also depended on the duration of pregravid ethanol exposure.

The number of newborn rats also changes significantly due to the effect of ethanol on the organism of females during pregnancy and various terms before its onset. Intact females give birth to an average of 11 rats. In group I animals, 8–9 rats are born. On the average 10 newborns appear in females of group II; females of groups III, IV and V give birth to 8–9 rats. In rats of group VI, 7–8 rats can be found in the litter, and in animals of group VII, 7 cubs are most often found (Table 2). The decrease in the number of newborn rats in the litter is due to the increase in intrauterine mortality, particularly preimplantation mortality, as indicated by various authors [18].

CONCLUSION

Thus, ethanol exposure to female white rats leads to an increase in mortality and duration of pregnancy of experimental animals, as well as a decrease in the number of newborn rats in the litter. The severity of changes depends on the duration of pregravid exposure to ethanol.

When performing experimental studies using ethanol, it is necessary to take into account that when modeling its chronic exposure for up to four months, it is necessary to increase the number of animals by 30%, and more — by 40% of the initially assumed number. When planning a study to investigate the antenatal effects of ethanol, it is important to take into account the prolongation of pregnancy and the number of offspring obtained depending on the duration of pregravid ethanol intoxication.

ADDITIONAL INFORMATION

Author contribution. Thereby, all authors made a substantial contribution to the conception of the study, acquisition, analysis, interpretation of data for the work, drafting and revising the article, final approval of the version to be published and agree to be accountable for all aspects of the study.

Competing interests. The authors declare that they have no competing interests.

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Experiments with animals were carried out in accordance with international rules (Directive 2010/63/EU of the European Parliament and of the Council of the European Union of September 22, 2010 on the protection of animals used for scientific purposes).

ДОПОЛНИТЕЛЬНАЯ ИНФОРМАЦИЯ

Вклад авторов. Все авторы внесли существенный вклад в разработку концепции, проведение исследования и подготовку статьи, прочли и одобрили финальную версию перед публикацией.

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

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