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Original article

A comparative study of the effect of the oral gavage and the intraperitoneal administration of Cinnamomum camphora on the number of embryos in mice

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Abstract

Background: According to a popular Iranian belief, Camphor can not only suppress libido but also decrease the function of the reproductive system. In this regard, this study was conducted to evaluate the role of Cinnamomum camphor in the number of embryos in mice.

Methods: In this study, 40 male and 80 female albino mice, weighing 30-35 g, were randomly divided into 8 groups, including a control group, a sham group that received the vehicle only (olive oil), and six experimental groups that received Camphor. Three experimental groups received camphor by p.o. (gavage) and the other three experimental groups received camphor by i.p. (intraperitoneal) in doses of 10, 20, and 40 mg/kg for 15 days. Mating was confirmed after coupling a male with a female mouse. Vaginal plaque was also considered for mating. Embryos were examined during the period of gestation.

Results: The mean number of embryos in control, sham, and received Camphor (10, 20, and 40 mg/Kg) by o. p. were 9.7 ± 4.6, 7.3 ± 4.3, 9.9 ±4.7, 8.6 ± 5.2, and 8.3 ±4.8, respectively. The mean number of embryo in received Camphor (10, 20, and 40 mg/Kg) by i. p. were 7.6 ± 5.4, 8.3 ± 3.4, and 7.3 ± 6.2, respectively. There were no significant differences in the number of embryos between control, sham, and experimental groups in both groups.

Conclusion: Further investigations with more focus on the effect of Camphor on sexual behavior in mice need to be carried out.

Keywords: Camphor, Fertility, Embryo, Route of administration

Introduction

Camphor is a ketone body, which is naturally obtained from cinnamomum camphora – a plant from Lauraceae family or Laurales order. The synthetic form of Camphor is made up of pinene, which is regarded as the most important constituent in terebanthine essence. The plant is a large, evergreen one, which can grow in East Asia and the Mediterranean region (1). Lavender and zhumeria majdae, thymus daenensis (2), chicory

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and chamchameh, and palm fiber (3) are plants containing a considerable amount of natural camphor. Camphor is believed to have a mild analgesic effect. It can lead to mild skin blush and a local increase in blood flow. It is also known for its anesthetic and antiseptic properties (4). Furthermore, it is used as a preservative agent in some pharmaceutical and cosmetic products (5). Camphor is also suggested as a neurological pain-relieving agent, and is further used for the treatment of a number of diseases such as fibrosis and epilepsy in some cases (6, 7). In addition, it is applied to improve mild burns, relieve insect bites, cool the skin, and protect against the sun (6). It is worth mentioning that Camphor-induced seizures were used for the treatment of psychotic patients in the past (8). Some studies have indicated that Camphor can cross the placenta and can easily enter the body tissues (9). Through hydroxylation in the liver, it is possible to metabolize it as camphor hydroxyl, which can be excreted through the urine in conjugation with glucuronic acid (5). The toxic effects may occur after the administration of approximately 2g of camphor. The lethal dose has been estimated to be 4g for adults and about 1g for children (10). Camphor can enter the body through oral cavity, skin, eye contact, and inhalation (6). The bacterial metabolism of camphor can result in the synthesis of toxins in the body (11)(11). It is also believed that camphor can play a role in decreasing libido (12).

According to the traditional medicine practice in Iran, camphor can function as a libido suppressant and can affect the reproductive system (13, 14), although its negative effect on the reproductive system has not been fully identified so far. Some investigations have suggested the contribution of camphor-containing compounds in the inhibition of cytochrome P450B1; therefore, along with reduction in cytochrome enzymes, the activity of dismutase and 17-hydroxylase is also inhibited and the testosterone level is likely to be reduced (15).

Delayed sexual maturity and the reduced size of male and female reproductive organs are amongst the cases that can be derived from camphor derivatives (15). Some studies have also shown that the use of camphor-containing creams has no effect on the level of gonadotropins and can perhaps have an insignificant effect on testosterone level (16). Nonetheless, the injection of camphor-containing drugs may reduce the luteinizing hormone (LH) or the follicle-stimulating hormone (FSH) at low doses (17). The synthetic camphor may also have estrogenic effects on laboratory animals and/or humans (18). Decreases concerning sperm density in tubules and the mean weight of body and testes have been observed in groups of animals receiving different amounts of camphor-containing extracts of palm fibers (2, 19). According to different views on the effect of camphor on the level of the hormones encouraging ovulation, we strove to investigate the effect of different doses of Camphor administered by gavage and intraperitoneal injection on the fertility rate and the formation of embryos in albino mice.

Materials and Methods

This experimental study was approved by the Ethics Committee of Babol University of Medical Sciences, Babol, Iran. It was performed at the Department of Research and Technology in Babol University of Medical Sciences.

Albino mice (40 males and 80 females), weighing 30-35g, were obtained from the Production and Breeding Center of Laboratory Animals at Babol University of Medical Sciences. The light-dark cycle of 12:12 h was considered. Also, the 23±1 °C temperature and the optimum conditions were provided for keeping the animals. The animals were given ad libitum access to food and water

Mice were randomly divided into 8 groups, including the control group, the sham group, and six experimental groups, which received camphor (three groups received camphor by p.o. and the other three groups received camphor by i.p. in doses of 10, 20, and 40 mg/kg). Five male and 10 female mice were assigned to each group. Male and female mice were housed in separate cages. The camphor groups received camphor dissolved in olive oil (as camphor vehicle) at the doses of 10, 20, and 40 mg/kg, given by p.o. or i.p.. The sham group received the vehicle only (olive oil) either by p.o. or i.p., while the control group received no treatment. The treatments continued for 15 consecutive days.

Under coupling condition, one male and two females were placed together in the same cage for one night. Mating was confirmed the next morning after observing the vaginal plaque. Females were then separated and housed in individual cages to be checked for the number of embryos after 21 days of gestation.

After the gestational period and before the delivery, 75 mg/kg of ketamine was intraperitoneally injected to all female mice for surgical anesthesia. An autopsy

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was performed by scalpel dissection, and the embryos formed were counted following the removal of the uterus.

Statistical Analysis

The mean (± SD) number of embryos in every group was calculated using the SPSS version 20, and the statistical significance was evaluated using the Kruskal-Wallis Test. The P-value <0.05 was considered as a statistically significant level.

Results

This study focused on the effect of o.p. in different doses of Camphor on the number of embryo. In Figure 1, the number of embryos in experimental groups and the sham group were compared with that of the control group. The mean belonging to the number embryos in each group was calculated, and then a comparison was made between groups. The mean number of embryos in the control, sham, camphor 10, camphor 20, and camphor 40 mg/Kg were 9.7 ± 4.6, 7.3 ± 4.3, 9.9 ±4.7, 8.6 ± 5.2, and 8.3 ±4.8, respectively. There were no significant differences in the number of embryos between the control, sham, and experimental groups ($p<0.05$).

The study also focused on the effect of i.p. in different doses of Cinnamomum camphora on the number of embryos. In Figure 2, the number of embryos in the experimental groups and the sham group were compared with that of the control group. The means belonging to number of embryos in Camphor 10, Camphor 20, and Camphor 40 mg/Kg were 7.6 ± 5.4, 8.3 ± 3.4, and 7.3 ± 6.2, respectively. There were no significant differences in the number of embryos between the control, sham, and experimental groups ($p<0.05$).

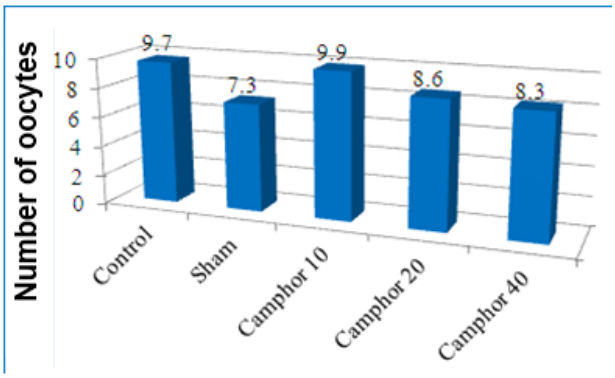

Fig 1: The number of embryos in mice after receiving p.o. of Camphor (10, 20 and 40 mg/Kg) in the three experimental, control, and the sham groups.

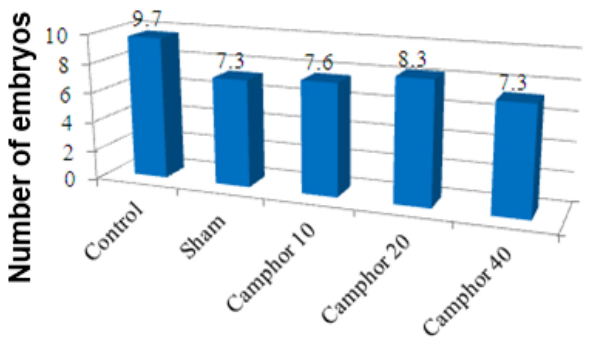

Fig 2: The number of embryos in mice after receiving i.p. injection of Camphor (10, 20 and 40 mg/Kg) in the three experimental, control, and sham groups.

Discussion

Camphor-treatment showed no significant difference in the number of embryos compared with the sham and control groups. Regarding the results of studies conducted so far on the effect of Camphor on the reproductive system, it can be concluded that Camphor is likely to have a negative effect on sperm parameters and/or ovarian tissue, leading to an unwanted infertility. Although the scope of this study is not very broad, there has always been a conception in the community indicating that Camphor or its compounds may lead to sexual function suppressing effect. Camphor in sunscreens has little effect, which could be due to its poor absorption by skin and its lower dose (17). Therefore, it should be accepted that any Camphor-containing materials cannot leave negative impacts. Instead, it depends on the dose and the duration of exposure as well as the mode of the absorption.

With regard to the fact that the dose of Camphor used in the present study was lower than that of the effective dose, it did not turn out to be significantly effective. It should be noted, however, that if Camphor had been effective at the doses used, it would have definitely resulted in decreased fertility and represented the number of offspring born in pregnancy cycles (20).

Finally, given the fact that pure Camphor in the present study was administered by i.p. and o.p. injection, the study condition was different from those of other investigations. Therefore, it seems that in i.p.

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and o.p administrations of Camphor-containing plants as well as compounds with low percentage of Camphor, certain conditions such as the duration and the dose of exposure should be considered to have a long-term affect. Thus, when the dose of Camphor is lower than that of the effective dose, the effect is not likely to be significant. However, further research is still needed to distinguish the probable significant differences between the uses of camphor as a combination of different ointments entering the body, which may last a long time.

In conclusion, the present study demonstrated that the administration of pure camphor through p.o. and i.p. injection had no specific effect on the number of embryos formed. However, further investigations are suggested to be conducted to ensure the results achieved and to complement this study in terms of the effects of camphor on sexual behavior in mice.

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Conflict of interest

None declared.

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