Urology Journal
UNRC/IUA

Vol. 1, No. 4, 268-272 Autumn 2004
Printed in IRAN

Miscellaneous

The Effect of Camphor on the Male Mice Reproductive System

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ABSTRACT

Purpose: In Iranian traditional medicine there is a belief that camphor is a suppressor of sexual activity. Based on this idea and since there are few studies on this issue, we evaluated the effect of camphor on histopathological changes of reproductive system in young male mice of balb/c racial type.

Materials and Methods: Thirty-six premature male balb/c mice, were divided into 3 paired groups of experimental, control, and sham (n = 6). Experimental groups 1 and 2 received 30 mg/kg camphor dissolved in olive oil (orally) for 10 and 20 days, respectively. The control groups received the same volume of olive oil during the same periods of time, and no intervention was done in sham groups. All groups were kept in the same environmental condition. At the end of exposure time, each group was anesthetized and their testes were removed for obtaining serial sections, and histological staining.

Results: Comparing to the control groups less vascularization in testis tissue of experimental groups was seen. Furthermore, using stereological methods demonstrated that internal diameters of seminiferous tubules in experimental groups were significantly smaller than those in control groups ($P < 0.005$). Also, the number of released sexual cells was lower in experimental groups ($P < 0.005$). No meaningful difference was seen between controls and sham groups.

Conclusion: Administration of camphor and its effects on male mice reproductive system may result in significant structural changes, including vascularization and proliferation of sexual cells. This can affect maturation of seminiferous tubules and subsequently, reproductive function of testes in mice.

KEY WORDS: camphor, male reproductive system, balb/c mouse

Introduction

Although camphor, a natural substance, which was known by the Asian nations since ancient times, is derived from Cinnamomum Camphora tree, its synthetic form is now available, being produced for medical, sanitary, and industrial usage.[1-3] As it is believed by the ancients, camphor is used not only as an aromatic material, but also for different purposes such as stimulation of circulatory and respiratory system, psychological stimulation, and cosmetics (as a reddener) for external use.[4-5] In addition, due to an olden belief, camphor can be used for modulating sexual activity, contraception, inducing abortion, and reducing milk production in lactating

Received October 2003
Accepted May 2004
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women.[6-10] Accordingly, camphor may affect sexual activity and although not documented, studies in different parts of the world are in agreement with this belief. Administration of 100 mg/kg of camphor to mice, which have been under gamma rays, has modulated spermatogenesis in their testes.[10] Camphor derived oxidant substances have been traced in umbilical cord, blood, and fetal tissues (including brain, liver, and kidneys) and it has been shown that camphor can easily pass placental barrier and affect fetal development.[11] In spite of the strong belief regarding the effect of this substance on male reproductive system, there is no documentation. Thus, sketching this theory that camphor affects spermatogenesis in animals, it can be postulated that in human model it may have the same effect. We designed this study in order to evaluate the effect of this substance on the development of seminiferous tubules and differentiation of spermatocytes (sexual cells) in male mouse.

Materials and Methods

Experimental Animals and Route of Administration

According to the fact that seminiferous tubules in mouse testis take 40 days to reach full differentiation after birth,[12] 36 twenty-day-old mice of balb/c racial type were selected and divided into 6 groups (2 experimental, 2 control, and 2 sham groups) and kept under standard condition of animals' hutch. Then experimental groups 1 and 2 received camphor dissolved camphor in olive oil,[13] 30 mg/kg/day, orally as gavage for 10 and 20 days, respectively. The control groups received only the same volume of olive oil during the same periods of time. The sham groups were kept in animals' hutch under similar condition, with no intervention.

Sampling and Tissue Preparing

At the end of each period, anesthesia was made for mice in each group, using chloroform and then their testes were removed for sampling and primary fixation with the use of ventricular perfusion and the exploit of formalin 10%. Removed testes were transferred to codified glasses containing formalin 10%, as fixative, for the final fixation. In the next step, fixation and tissue preparation were performed with conventional histological methods and serial horizontal cuts of 7 micron thickness were obtained from tissue blocks. Out of each 5 obtained sections related to each sample, one was selected randomly and stained with hematoxylin and eosin for further study.

Measurement of Tissue Elements

In order to determine volume density of specified parts in the structure of testicular tissue in different groups it was attempted to measure internal and external diameters of seminiferous tubules based on morphometric studies,[14] and to count free and lining cells, using dissector technique.[15] For this purpose, the obtained serial sections from testes of each group were studied with light microscope. The method used was as follows: By putting a scaled square over the subjective lens of the microscope, a specific unit for measuring microscopic field was designed. Afterwards, one field out of each four fields was studied by displacing the samples under microscope. As well as counting sexual cells, each two cells were counted as one for those cells which were situated on the edge of the fields. In addition, the internal and external diameters of one seminiferous tubule out of each four were measured and the results were recorded.

Results

The results were obtained from more than 200 fields in the prepared sections of each case and along with determination of the mean of the measured parameters in each mouse, a total mean for each group (table 1) was calculated and compared with the other groups.

Comparisons of the groups' samples showed significant differences between the two experimental and control groups; the main proportion of seminiferous tubules in the experimental group 1 (fig. 1) was not canalized and only in a small proportion, canalization had been initiated. Non-canalized tubules were solid with a high concentration of cells and microscopic assessment showed that the interstitial tissue of the tubules had developed less than that in the control group

TABLE 1. *Mean (± SD)* of changes in tubules' characteristics and sexual cells in experimental and control groups*

Mean changes/groups†	Experimental 1	Control 1	Experimental 2	Control 2	P values
External diameter of tubules	62.21 ± 1.42	64.18 ± 2.28	76.11 ± 1.24	79.19 ± 2.71	>0.05
Internal diameter of tubules	26.31 ± 1.56	42.38 ± 2.19	48.13 ± 2.67	56.81 ± 2.12	<0.005
Lining cells	32 ± 1.11	17 ± 2.23	21 ± 2.41	13 ± 1.68	<0.005
Released sexual cells	17 ± 3.71	28 ± 3.92	34 ± 1.78	61 ± 4.31	<0.005
Vascular cross-sections	8 ± 0.13	11 ± 0.04	6 ± 0.23	9 ± 0.02	<0.05

*tubules diameters are reported in μm and cell and vascular cross-sectional count in mm^3.
†the results of sham group are not included because of their proximity to controls.

1. Also, the first signs of release of sexual cells were seen in few spaces developed in some tubules, while this process was seen more prominent in the control group one (fig. 2). There was no significant difference between the mean external diameters of seminiferous tubules, but the difference was meaningful between the internal diameters (P <0.005) (table 1).

In the experimental group 2, as shown in figure 3, the internal space of seminiferous tubules was not fully developed, while complete development was seen extensively in the similar samples of the control group 2 (fig. 3). Here, also mean external diameters were not significantly different in the two groups, but internal diameters were different (P <0.005).

Although tubular wall thickness and the number of cell layers were less in the control group as compared with the experimental group (fig. 3,4), the presence of cells derived from germinal layer, containing large round hyperchromatic nucleus indicated active mitosis in this area, but in the experimental samples, the concentration of cells in tubular wall was more and the nuclei were smaller. In this condition the amount of released sexual cells were different in the experimental group as compared with controls (P <0.005). It seems that spermatocytes'

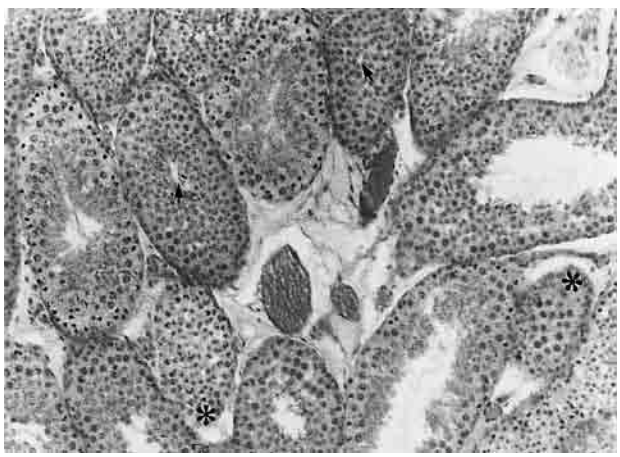

Fig. 1. A cross-section of seminiferous tubules from a sample of experimental group 1, showing the initiation of canalization in some of the tubules (arrows). In this stage, some of the tubules (stars) are still non-canalized and cells are compressed to each other(× 40).

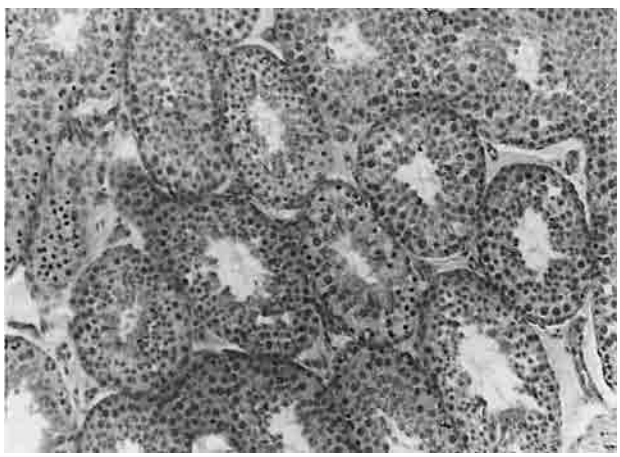

Fig. 2. A cross-section of seminiferous tubules from a sample of control group 1, showing canalization in all tubules. But, small internal area of each tubule and condensed layers of cells in tubular wall indicates that tubular maturation is not complete (× 40).

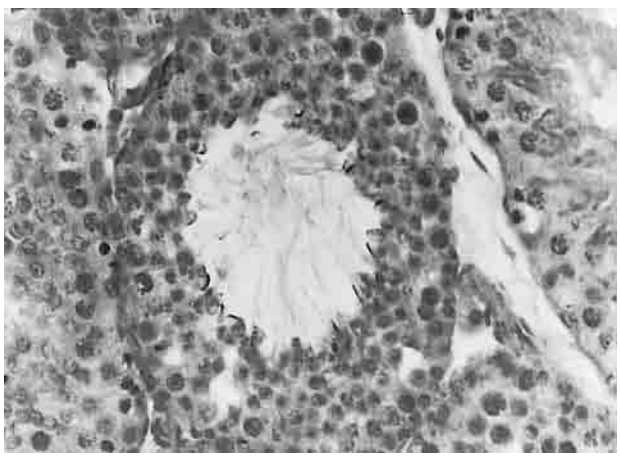

Fig. 3. A cross-section of seminiferous tubules from a sample of control group 2, showing a single tubule. The central canal is fully formed. The cells derived from germinal layer are seen with large hyperchromatic nucleus, showing that cells contain a large amount of chromatin and have active mitosis. Reduced cell wall layer as compared with experimental group's samples (fig. 4), indicates that differentiation and release of spermatocytes is taking place rapidly and a large amount of cells in the terminal stages of differentiation can be traced in the lumen (× 400).

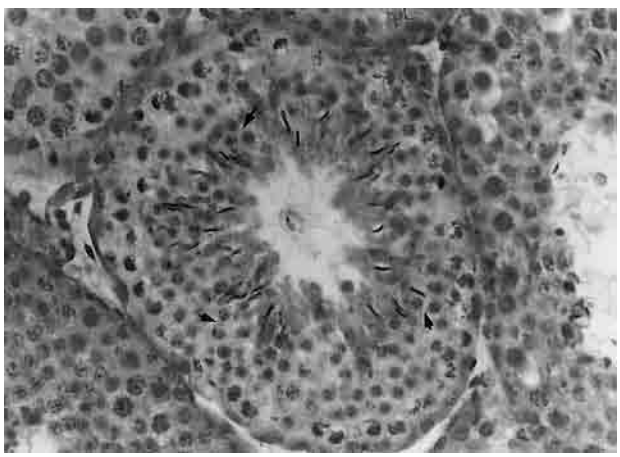

Fig. 4. A cross-section of seminiferous tubules from a sample of experimental group 2, showing a single tubule. Here, although central canal is formed and external diameter of the tubule is maximal, multiplicity and concentration of cell layers in tubular wall and considerable population of spermatocytes (arrows), which have remained in internal layers and have not been differentiated into spermatozoids, indicates delayed spermatogenesis (× 400).

maturity and release had been delayed and they were compressed to layers near the central canal of the tubule.

Finally, no meaningful difference was seen between control and sham groups.

Discussion

The administered dosage of camphor in various experiments is different in published reports. Intraperitoneal injection of 300 to 400 mg/kg; 1, 2 or 3 times, has not shown toxic effects in behavioral or autopsy studies;[16] whereas, it has been reported that administration of 400 to 550 mg/kg of camphor to rats has led to rigor and seizure.[17] On the other hand, administration of 1000 mg/kg of this substance to mouse causes toxicity along with reduced consumption of food and water and salivary secretion,[18] and 2200 mg/kg was the minimum lethal dose in mouse.[16] However, the non-toxic dose of 100 mg/kg has been identified as a dose that affects testicular tissue activity, which could alter the process of spermatogenesis.[10] In this study, we used 30 mg/kg of camphor in time periods of 10 and 20 days and evaluated the probable effects on testicular tissue. With this reduced dosage the probability of toxicity was further diminished. In this regard, in one of the few reports, it is shown that intraperitoneal injection of 100 mg/kg of camphor to 8-week-old mice could reduce the number of primary spermatocytes temporarily, but the difference was not significant after one week.[18] On the other hand, it seems that if the experimental samples are treated for a long period of time, seminiferous tubular structure and probably supporting tissues may be affected as well as camphor's impact on proliferation and differentiation of spermatocytes.[10,18] This study showed that the differences between experimental and control groups were significant as the proliferation and differentiation activity are lower in experimental group. The reason is that the vascular expansion, pertinent to tissue expansion, which is necessary for activity and multiplication of cells, is lacking.[19] Although in control group two less vascularization was observed than in control group one, it should be considered that the testicular tissue in control group one was an immature tissue and had more angiogenesis during its own development and after reaching full development and maturity, angiogenesis would become closer to that in control group two and vascular bed would have limited development.[19] This theory is supported by that, despite this decrease, the significant difference between experimental and control groups remained unchanged.

On the other hand, comparing the figures obtained from different groups shows that the internal diameter of seminiferous tubules in experimental groups is less than that of control groups and this significant difference was also seen between experimental groups one and two. The cells in seminiferous tubules have not been differentiated adequately and therefore, they can not become mature and subsequently release into the lumen. In these circumstances two events happen; first, due to the paucity of the released cells and their immaturity, as shown in the figures, the thickness of seminiferous tubular wall increases and their internal diameter decreases. Second, counting of free cells in tubular lumen showed that they were lesser in experimental groups than in control groups.

Conclusion

It can be concluded that although the exact mechanism of camphor effect is not known to us, one point can not be ignored, and it is that continuous administration of low doses of camphor can affect the development and differentiation of testicular tissue and reduce its spermatogenesis activity.

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