Effect of Camphor on Uterus Histology of Pregnant Rats

Sabah A. Linjawi, PhD
Department of Biology, Faculty of Science
King Abdulaziz University, Jeddah, Saudi Arabia
s_linjawi@hotmail.com

Abstract. Camphor has long been used in dead bodies washed in Saudi Arabia. Experiments were conducted to study the effects of camphor on the Sprague–Dawley pregnant rat uterus histology. Pregnant rats (n = 36) were divided into 4 groups (n = 9); 3 groups were given intraperitoneal injection by different doses of water camphor solution (5, 10 and 20 mg camphor/kg body weight); the control group was given the same doses of distilled water. All groups were kept in constant temperature rooms (22 ± 2 °C), and 12 h light/12 h dark photoperiod. At the end of 1, 2 and 3 weeks of gestation, 3 animals from each group were anesthetized, their uterus were removed for serial sections, and histological staining. The results obtained show less uterus glands and degeneration of luminal epithelium in all groups. Group 3 (20 mg camphor/kg b. w.) showed a considerable dilated of the blood vessels with a bleeding; the luminal epithelium cells contains dark round nucleuses with abnormal mitosis; large number of white blood cells and vacuolated cytoplasm. The present study shows that camphor effects on female rat reproductive system may result in significant structural changes. This can affect reproductive function and fertility of exposed animals.

Keywords: Camphor, Pregnant rats, Histology-uterus.

Introduction

Camphor is a white crystalline substance, obtained from the tree Cinnamomum camphora commonly known as Camphor tree, Camphor wood or Camphor laurel. Camphor has been used for many centuries as a culinary spice, as a component of incense, as a bug-repellant and as a flea-killing substance. Plus, it has also been used in medicine[2].

Nowadays, camphor is synthetically produced from turpentine oil and is present in many non-prescription medicines such as Tiger Balm,
Vick's vapor-steam, Bayer Muscle, and joint creams; in addition to many other medicines\textsuperscript{[1]}. Camphor oil contains many compounds such as camphor, safrol, eugenol, terpeniol, cineol and ligans\textsuperscript{[2]}.

Although camphor is a natural substance, it was known by the Asian nations since ancient times. Its synthetic form is now available and is being produced for medical, sanitary, and industrial usages\textsuperscript{[3,4]}. As it is believed by the ancient people, camphor is used not only as an aromatic material, but also for different purposes such as stimulation of circulatory and respiratory systems, psychological stimulation, and cosmetics (as sun protection) for external use\textsuperscript{[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 women\textsuperscript{[6,7]}.

Accordingly, camphor may affect sexual activity and although not documented, studies in different parts of the world are in agreement with this belief\textsuperscript{[5]}.

Administration of 100 mg/kg of camphor to mice, which have been under gamma rays, has modulated spermatogenesis in their testes\textsuperscript{[6]}.

Camphor derived oxidant substances that have been traced in umbilical cord, blood, and fetal tissues (including brain, liver and kidneys). It has been shown that camphor can easily pass placental barrier and affects development\textsuperscript{[8]}. Most severe cases are associated with the ingestion of camphorated oil, either deliberately or by mistake as other medication e.g. castor oil\textsuperscript{[9]}.

In Saudi Arabia, camphor in the form of tablets was added to wash dead bodies, and thus putting washers in great risk, especially female washers. Though, literature concerning its reproductive toxicity has not been documented.

Thus, the main objective of this study is to evaluate the effects of camphor on uterus histology in female rats.

**Materials and Methods**

1 - **Camphor Substances**

Camphor tablets were obtained from the traditional medicine market acquired in China (Deer Brand, Rec. Trade mark. Made in China).
2 - Experimental Animals and Route of Administration

Adult male (body weight, 150-200 g) and virgin female (body weight, 120-150 g) of Sprague-Dawley rats were obtained from the Animal House at King Fahad Medical Research Centre and maintained in constant temperature control rooms (22 ± 2°C). Animals received food and water on a 12 h light/12 h dark photoperiod. After one week of acclimatization, untreated females and males mated by overnight cohabitation (one male to three females). Females examined for the presence of a vaginal plug the following morning. The next day, a dropped of copulatory plug was found; designated as day zero of gestation.

The present study was conducted to investigate the effect of camphor on the rat uterus histology during pregnancy period (embryos data will be presented in another study). The pregnant females were divided into four groups; each group consisted of 9 pregnant females. The three experimental groups received intra peritoneal injections of camphor solution dissolved in distilled water. The dose has been chosen from a study by Jamshidzadwh and Sajedianfard [10]. They were studying the effect of camphor on the male rat reproductive system. The doses given were (5, 10 and 20 mg camphor/kg body weight / 5 days / week respectively). Control group (9 rats) were injected by distilled water with the same volume.

At the end of the 1st, 2nd and 3rd weeks of gestation, three animals from each group were anesthetized. Anesthesia was made by using chloroform, then their uteri were removed for sampling examined and primary fixed. The uteri samples; fixed in 10 % neutral buffered formalin then processed for histological examination; 7 micron thickness paraffin sections were cut and stained with hematoxylin-eosin; and photographed using digital camera connected to computer. Sections were examined to evaluate the effect of camphor on the uterus histology.

Results

A histological examination for control group uterus (one week gestation) was performed by using the light microscope which showed similar histological structure to rodent uterus at one week gestation. The uterus consists from inside to outside respectively: Endometrium containing a spongy layer filled with blood vessels and uterine glands
were lined by epithelial cells. It showed active mitosis and myometrium, thus containing thick inner circular layer and thin outer longitudinal layer (Fig. 1 & 2).

![Image: Endometrium (E) contains large number of uterine gland (arrows), myometrium (M) longitudinal and crossing muscles (arrow), and blood vessels (BV). (H&E) (x 100).](image1)

**Fig. 1.** Shows a cross-section for control rat uterus (one week). Endometrium (E) contains large number of uterine gland (arrows), myometrium (M) longitudinal and crossing muscles (arrow), and blood vessels (BV). (H&E) (x 100).

**Fig. 2.** Higher magnification of (Fig. 1) showed the luminal epithelial (LP), from outside simple columnar cells (C), notice the active mitosis (arrow). (H&E) (x 1000).

A microscopic examination of the cross-sections of pregnant female rats uterus that were treated by a dose (5 mg camphor / kg bw), for one week gestation, showed that the histological structure of endometrium and myometrium are similar to the control group uterus. On the cellular level, there was a clear shortness in the length of the columnar cells in some regions with more rounded nucleuses, and a decrease in thickness of the visceral layer compared to control (Fig. 3). By using the high power of the microscope it was found; abnormal mitosis in the columnar cells, vacuolated cytoplasm and a decrease in nucleuses size (Fig. 4).

Moreover, the examination of one week uterus for group two (10 mg camphor / kg bw) showed similarities in the number of uterine glands and the thickness of the internal layer as well as the number of blood vessels in control group. The cellular level showed the emergence of the narrow epithelial cells which gives a wavy internal surface with dark abnormal nucleuses and increase inactive chromosomes. The uterine gland showed decomposition of the cytoplasm with dark inactive chromosomes in the nucleuses (Fig. 5).
Fig. 3. A cross-section for one week gestation (5 mg camphor/kg b. wt.) showed a significant lower in the uterine glands (arrows), endometrium (E) and myometrium (M) similar to natural. (H&E) (x 100).

Fig. 4. Higher magnification of (Fig. 3) showed the cells with rounded nucleuses, abnormal mitosis (arrow), dilated blood vessels (BV) and vacuolated cytoplasm (head-arrow). (H&E) (x 1000).

Fig. 5. Cross-section for one week uterus (10 mg camphor/ kg b.w.) showed the wavy surface with dark abnormal nucleuses (N), vacuolated cytoplasm (head-arrow) and uterine gland with degraded cytoplasm (G).

Fig. 6. Cross-section for one week uterus (20 mg camphor/kg b.w.) showed the endometrium (E), uterine glands (G), bleeding blood vessels and lining short columnar cells (arrow). (H&E) (x 100).

In the females which had been treated by the highest dose (20 mg camphor / kg bw), the microscopic examination showed normal uterine glands with degradation in some regions and a clear shortage in the
length of the epithelial cells; no change in the myometrium layer. However, blood vessels in the internal layer showed a thrombosis with bleeding (Fig. 6). The cellular level examination showed abnormal nucleuses and mitosis in the lining columnar cells, vacuolated cytoplasm above and below the nucleus (Fig. 7).

When examining the control group uterus to the age of three weeks gestation using imaging microscope, it had been found that the tissue structure was similar to the other mammals uterus already explained at the age of one week. But there was a clear shortage in the whole thickness of the wall of the uterus. At the cellular level, the columnar cells appear as cubic cells with secretory cells and large number of defensive white blood cells (Fig. 8).

Using the high power of the imaging microscope, this research showed the epithelial cells containing large round nuclei, surrounded by the nuclear envelope and clear pigmented nucleolus. Moreover, a well developed defensive macrophages cells in various forms, like Monocytes and Polymorphonuclear nucleus. It also showed the increased number of macrophages cells in the visceral layer than the epithelial layer (Fig. 10).

Fig. 7. Higher magnification of (Fig. 6) showed abnormal columnar cells with abnormal nucleuses (N), abnormal mitosis (arrow) and vacuolated cytoplasm above and below the nucleus (head-arrow). (H&E) (x 1000).

Fig. 8. Cross-section for control group uterus (3 weeks gestation) showed decreased endometrium thickness (E) and increased myometrium thickness. (H&E) (x 100).
When examining the cross-sections for the treated groups of rat uterus to the age of three weeks gestation, it was found different tissue damages between the different doses. The tissue structure of the rat uterus (5 mg camphor/kg bw) showed disappearance of uterine glands with the decrease in endometrium thickness, compared to the week I and II gestation. However, in this study it was found an increase in the thickness of myometrium with a large expansion in the uterus cavity (Fig. 11). The cellular level examination showed that the epithelial cells have been reduced in size; appeared as cubic cells with dark non-regular round nucleus and an increase in inactive-chromosomes. The secretory cells between the epithelial cells and dilated congested capillaries with a hemorrhage, and a number of Monocytes white blood cells were also observed; comparing to the control group (Fig. 12).

The examination of the three weeks uterus for group two (10 mg camphor / kg b.w.) showed a small number of uterine glands in the endometrium. This study showed a decrease in the endometrium thickness and an increase in the thickness of myometrium. On the cellular level, the number of epithelial cells increased with decrease in size and the uterine glands containing a number of defensive cells. The cellular infiltration were increased in several areas significantly (Fig. 13). Epithelial cells degradation appeared in (Fig. 14) with vacuolated cytoplasm. The nucleuses were degraded and the nuclear envelope
disappeared. There was also a significant increase in numbers of white blood cells during pregnancy period.

Fig. 11. Cross-section for three weeks gestation uterus (5 mg camphor/kg b.w.) showed uterine glands (G), decrease endometrium thickness (E), increase myometrium thickness (M), peritoneal layer (arrow) and uterus gavity (L). (H&E) (x 40).

Fig. 12. Higher magnification of (Fig. 11) showed the cubical epithelial cells (EC) with dark round nucleus (N), secretory cells (SC), dilated blood vessels with red corpuscles (RBCs) and white blood cells (arrow). (H&E) (x 1000).

Fig. 13. Showed increased abnormal epithelial cells (EC), the uterine glands (G) containing defensive cells (red arrow) and cellular infiltration (detached-line). (H&E) (x 400).

Fig. 14. Cross-sections for three weeks gestation uterus (10 mg camphor/kg BW) showed abnormal small epithelial cells (EC) with vacuolated cytoplasm (head-arrow) and the wavy surface, dilated blood vessels (BV) with red blood corpuscles (RBCs) and white blood cells (arrow). (H&E) (x 1000).
In the females that had been treated by the highest dose (20 mg camphor/kg bw), the microscopic examination showed continuing increase in the number of epithelial cells with a decrease in the size. This gives a wavy surface as well as a decrease in the endometrium thickness with the increase in cellular infiltration of the cell. Muscle layer appeared fragmented with scattered fibers.

On the cellular level, nucleated degradation began with gaps in cytoplasm cells. The number of white blood cells, both Monocytes and Polymorphonuclear nucleus were increased (Fig. 15). Degradation of the epithelial cells which were not clearly defined and a clear shortage in the size of nucleated cells with the disappearance of nuclear envelope were also observed (Fig. 16). The number of white blood cells clearly increased compared to the other groups. Also noted, cytoplasm degradation in the visceral layer with the presence of large distances between the interface cells led to the disintegration of the tissue structure.

**Fig. 15.** Showed increased abnormal epithelial cells (EC), the uterine glands (G) containing defensive cells (red arrow) and cellular infiltration (detached-line). (H&E) (x 400).

**Fig. 16.** Cross-sections for three weeks gestation uterus (10 mg camphor/kg BW) showed abnormal small epithelial cells (EC) with vacuolated cytoplasm (head-arrow) and the wavy surface, dilated blood vessels (BV) with red blood corpuscles (RBCs) and white blood cells (arrow). (H&E) (x 1000).
Discussion

In this study, the histological examination for control group uterus (one week gestation) was performed by using the light microscope showed a similar histological structure to rodent uterus at one week gestation\textsuperscript{11}. This study was focused on the endometrium (the place of the implantation and placenta formation) where many of the changes could occur during pregnancy\textsuperscript{12}.

It was found that the endometrium is composed of the visceral layer (Lamina propria) containing connective tissue with many blood vessels and dense fibrous cells. The uterus cavity, which is lined by simple columnar epithelium showed many divisions and extended tubular endometrial glands into the connective tissue; this observations was reported before in the study\textsuperscript{13}. It was observed that the mucous lining of the uterus cavity is composed of epithelial cells and what distinguishes this layer, that it’s rich by the endocrine glands. This confirms what was reported by Abate et al.\textsuperscript{14} in their study, that proved the importance of the secretory endometrium for the success of the pregnancy.

The cellular level showed clear epithelial cells containing oval nucleuses filled by a large amount of chromosomes and is surrounded by granular cytoplasm with regular shape of the outer edge; this is in agreement with what was previously reported by Conti et al.\textsuperscript{15} and Oluyemi et al.\textsuperscript{16} in their studies.

The examination of the treated female’s uterus for one week age gestation for different doses showed a distinct lack in the number of uterine glands; dilation of blood vessels in the internal layer with a thrombosis and bleeding; abnormal nucleuses and mitosis in the lining columnar cells; vacuolated cytoplasm above and below the nucleus. The presence of the vacuolated cytoplasm above and below the nucleus agree with what was reported by Johnson et al.,\textsuperscript{17} which clarified the effect of cadmium on the endometrium.

Examining the control group uterus to the age of three weeks gestation by the higher magnification showed well developed defensive macrophages cells in various forms; like Monocytes and Polymorphonuclear nucleus and an increased number of macrophages cells in the visceral layer than the epithelial layer. The same results have been found before in the study\textsuperscript{13}, where the presence of macrophages
white cells in the visceral layer is higher than its presence in the epithelial cells in pregnant female rats.

The cross-sections examination for the treated groups of rat uterus to the age of three weeks gestation showed different tissue damage between the different doses. The low dose (5 mg camphor/kg bw) showed disappearance of uterine glands with the decrease in endometrium thickness, compared to the week I and II gestation. The same results were found by Sami et al.\textsuperscript{[18]} in examining the effect of nicotine on pregnant female rats at the end of the pregnancy, which observed a decrease in the number of uterine glands and the thickness of the endometrium and myometrium.

It was also observed; reducing in the epithelial cells size which appeared as cubic cells with dark non-regular round nucleus; an increase in inactive-chromosomes; dilated congested capillaries with a hemorrhage; and a number of Monocytes white blood cells comparing to the control group. Camphor effect was very similar to the effect of food contamination which has been reported by Singh et al.\textsuperscript{[19]}; that explained the food pollutants and clear changes in blood vessels and inflammatory cells in female rats during the end of pregnancy.

This study also showed that the dose (10 mg camphor / kg b.w.) caused less number of uterine glands in the endometrium, a decrease in the endometrium thickness and an increase in the thickness of myometrium. The same result was observed by Sami et al.\textsuperscript{[18]} on the effect of nicotine on the pregnant female uterus.

The cellular level examination showed an increased number of epithelial cells with decrease in size and the uterine glands containing a number of defensive cells. Also observed increase in the cellular infiltration significantly and epithelial cells degradation with vacuolated cytoplasm. In addition, the nucleuses were degraded and the white blood cells number increased significantly during pregnancy period. In the study by Kramer et al.\textsuperscript{[20]} they reported that any changes and degradation in the endometrium structure may affect the fetus implantation. This explains the negative effect of camphor on the uterus, making it one of the causes of early abortion\textsuperscript{[21]}.

The examination of the three weeks uterus for group three (20 mg camphor / kg b.w.) showed nucleated degradation, increased number of
white blood cells, both Monocytes and Polymorphonuclear nucleus and degradation of the epithelial cells with a clear shortage in the size of nucleated cells with the disappearance of nuclear envelope. Also observed, cytoplasm degradation in the visceral layer led to the disintegration of the tissue structure. In this respect Singh et al.\cite{17} recorded that there is a clear change in the blood vessels and inflammatory cells when examining the impact of food contaminants on the uterus of female pregnant rats during the end of pregnancy.

Concerning the effect of camphor on male reproductive system Nikravesh and Jalali\cite{5} recorded that administration of 30 mg/kg of camphor in time period of 10 and 20 days to balb/c type male mice showed significant structural changes, including vascularization and proliferation of sexual cells, which affect maturation of seminiferous tubules and reproductive function of testes in mice. While Jamshidzadwh and Sajedianfard\cite{10} showed a decrease in the rat body weight, testis size, sperm number and mobility with all of the experimental doses. Also, they added that higher doses (10 and 20 mg/kg) of camphor caused morphological changes and a toxic effect on sperm and their mobility.

The results of this research indicate that there is a direct correlation between the amount of the dose used and the negative impact of camphor on the histo-architecture of the uterus of pregnant rats; suggesting negative influence on the reproductive health of the animals which might cause abortion in animals with higher doses of camphor.

References


تأثير الكافور على التركيب النسيجي للرحم الجرذان الحوامل

صبح_exclude
de absorbing 

قسم علوم الأحياء، كلية العلوم، جامعة الملك عبد العزيز
جدة، المملكة العربية السعودية

المستخلص. يستخدم الكافور منذ القدم في عشيل الموتى في المملكة العربية السعودية. لذا أجري هذا البحث لدراسة تأثير الكافور على التكوين النسيجي للرحم الجرذان الحوامل من نوع - Sprague-Dawley. قسمت الجرذان الحوامل (ن = 32) إلى 4 مجموعات (ن = 9)، وحقنت 3 مجموعات منها في التج Campos البريتوتي بجرعات مختلفة من محلول الكافور المائي (50 و 100 و 200 ملجم كافور/ كجم من وزن الجسم). أما المجموعة القياسية فقد حقنت بجرعات مختلفة من الماء المقطر. حفظت جميع الحيوانات المعالمة في غرف ذات درجة حرارة ثابتة (37 ± 2°م) و تحت فترة ضوئية تتراوح ما بين 12 ساعة ضوء و 12 ساعة ظلام. خردت 3 حيوانات من كل مجموعة في نهاية الأسبوع 1 و 2 و 3 من الحمل ثم أزال الرحم لعمل القطعات النسيجية المختلفة وصيغها. أظهرت النتيجة انخفاض عدد الغدد الرحمية و تحلل النسيج الطلياني للرحم في كل المجموعات التجريبية. ظهر في المجموعة الثالثة (200 ملجم كافور/ كجم من وزن الجسم) تمدداً واضحاً في الأوعية الدموية مع نزف، أنوية مستدفية داكنة مع انقسام كروموسومي شاذ في الخلايا الطليانية، زيادة عدد خلايا الدم البيضاء بشكل كبير مع ظهور تجاويف في السيتوبلازم. تشير هذه الدراسة إلى أن الكافور قد غير التركيب النسيجي للرحم مما انعكس على خصوبة الحيوانات المعالمة.