Histological and Hormonal Changes in Rat Endometrium under the Effect of Camphor

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Abstract: Camphor is prescribed in traditional medicine for the treatment of inflammation-related diseases and skin care products. The present study aims at finding out the effects of Camphor on the endometrium structures of Sprague–Dawley female Rats. 40 animals (3 months old) were divided into 4 subgroups (n = 10), 3 experimental groups were given daily intraperitoneal injection of 5, 10 and 20 mg/kg of Camphor wattery solution and the control group was given distilled water. All groups were kept in the same environmental conditions. At the end of 6 weeks, all rats were killed and their uteri were removed for histological analysis. Comparing with the control group, an increase in the body and reproductive system weight, less uterine glands, degeneration of luminal epithelium and enlargement of uterus lumen were recorded. All the treated groups showed an increase in estrogen concentration.

Furthermore, the highest dose caused an increase in progesterone concentration. The present study showed that Camphor could alter both hormonal and structural aspect of uterus that ultimately reflected on fertility of exposed animals.


Keywords: Camphor, female rats, endometrium, progesterone, estrogen

1. Introduction

Several plants are now being used in part or as a whole to treat many diseases, active components of these plants are now being investigated, extracted and developed into drugs with little or no negative effects or contra-indication (Oluymeni et al., 2007). Camphor is taken from the Camphor tree Cinnamomum camphora family Lauraceae, it is extracted through the oil produced by distillation of the flowers and leaves of the camphor tree. However the bigger portion of camphor is extracted from the wood present in the stem and roots (Health Council of the Netherlands, 2001).

Camphor is used as a secretolytic medicine for relieving respiratory symptoms (Ciuman, 2012).

Nowadays camphor is synthetically produced from turpentine oil and is present in many non-prescription medicines such as Tiger Balm, Vick’s vaporsteam, Bayer Muscle and Joint cream and many other medicines (Ashby et al., 2004). Camphor oil contains many compounds such as camphor, safrol, eugenol, terpeniol, cineole and ligans (Oudi, 2004).

Many studies were performed on animals to study Camphor toxicity, one study showed that the lethal dose of Camphor for dogs was 9-14 grams where this dose caused gradual paralysis of the central nervous system and suffocation. It was also shown that exposing animals to lethal doses of camphor caused nervous convulsions and oedema in the digestive system, kidney and brain (Clarke et al., 1981).

Studies on the effect of camphor during pregnancy did not show any teratological effects, when rats were orally given different doses of camphor (Leuschner, 1997). It was also shown that continuous exposure of mice to camphor caused the appearance of cancer symptoms (Cincinnati, 2001).

In another study it was shown that volatile oils are strong inhibitors for bone metabolism and bone reabsorption in rats (Mühbauer et al., 2003). Jamshidzadeh and Sajedianfard, (2006) showed that several doses of Camphor affected all parts of the rat male reproductive system such as testis, seminal vesicles and vas deference.

Several medical reports have shown toxic effects of camphor on humans, it was shown that a dose of 0.06-4 grams caused vision disturbance, shivering weakness and paralysis (Grant and Charles, 1974). Camphor is easily absorbed through skin, small intestine and the respiratory tract, when taken orally, it causes very high hypertension within 5-10 min) Litovitz et al., 1993). When using dermatological products containing camphor for a long period several symptoms were reported as rash and skin irritation. In cases of large doses acute toxicity might result leading to epilepsy, coma and death might occur of suffocation (Ford et al., 2001).

In children the lethal dose was found to be 0.7-1 gram of camphor where it caused kidney and liver malfunction leading to urinary retention and albuminuria (Gosselin et. al., 1984). Medical report has shown that 9 to 19 children were epileptic 14-120 min after swallowing 0.07 to 0.6 gm of camphor.
Camphor compounds 3-Benzylidene camphor (3-BC) and 4-Methyl benzylidene Camphor (4MBC) are used as UV filters in many products such as hair and skin care products, household products, optical materials, textiles, fabrics and transdermal drug delivery systems. It was demonstrated that 3BC had adverse significant effect on the reproduction of fathead minnows in a dose-dependent manner (Health &Consumer protection directorate-General., 2006).

Camphor is widely used in Saudi Arabia with grave clothes (cerements), and it is traditionally known that pregnant women should not come near dead relatives because of camphor odors and its effect on the mother and embryo. As this information is traditional and there is a dearth of publications on the effects of this plant on the uterine tissues, this research was carried out to prove its reality and to study the effect of pure camphor on the female reproductive system using rats as a model animal.

2. Material and Methods

This study was given approval for the methodology and other ethical issues concerning the work by King Fahad Medical Research Center at King Abdulaziz University.

Camphor blocks were obtained from the traditional medicines market; it's used as aromatic substances added to dead bodies’ wash in Saudi Arabia.

Experimental Animals and Route of Administration:

Forty cyclic female Sprague-Dawley rats (3 month old) (200-300g) were sorted randomly from the animal house of the King Fahad Medical Research Center. The rats were kept in the animal control room and acclimatized for two weeks. The rats were fed on standard rat pellet produced by Bendel Feed and Flour Mills Limited. They were allowed access to water ad libitum and maintained under standard conditions.

The animal room was well ventilated with a temperature range of (22 ± 2 °C) under day/night 12-12 hours photoperiodicity. The rats were randomly grouped into four groups of 10 rats each, G1 (5mg/kg body weight), G2 (10mg/kg body weight), G3 (20mg/Kg body weight) and G4 (control) (Jamshidzadeh and Sajedianfard, 2006). The three experimental groups received intraperitoneal injections of camphor solution dissolved in distilled water, control group were injected by distilled water with the same doses and route of administration.

Methodology:

All rats in this experiment were weighed before the first injection, and every Saturday and Tuesday of each week during the experiment. The estrous cycles were monitored by a method described by Marcondes et al. (2002) where vaginal smear was collected with a glass pipette filled with 10ml of normal saline (NaCl 0.9%). The vaginal fluid was placed on clean glass slide and observed under a light microscope at x 100 and x400 magnification. The three types of cells recognized were epithelial, cornfield and leucocytes cells. The proportions among these cells were used to determine the estrous phases according to Long & Evans (1922).

Histological studies:

At the end of 6 weeks, polyestrous female rats were sacrificed at proestrus stage of estrous cycle, the uteri of the animals from each group were dissected out by laparotomy immediately and both the length and size of them were measured. Also, anatomical photos were taken for them using Nikon coolpix s10 digital camera. The removed uteri were per fused in normal saline, blotted dry and weighed in electronic weighing balance and then fixed in 10% buffered formalin for histological assessment, 7 micron thickness paraffin sections were cut and stained with hematoxylin-eosin, examined and photographed using digital camera connected to computer. The thickness of endometrium was measured from the histological photos.

Hormonal assays:

Animals were slightly anaesthetized and their eyes were bled within two minutes by using a heparinized syringe and blood samples were taken from each rat by the end of the experiment (after six weeks). The blood samples (10 ml) were collected in EDTA tubes, then centrifuged at 1200 rpm for 10 minutes to separated the plasma from the blood and kept at -20 °C. Hormonal assay for estrogen and progesterone serum levels was done using (Estradiol-E2, Elecsys and cobase analyzers, Roche Diagnostic GmbH.D-68298 Mannheim: US Distributor), (Progesterone 1214538312, Elecsys 1010/2010 and MODULAR ANALYTICA E170, Roche Diagnostic GmbH.D-68298 Mannheim: US Distributor) and correlated with histological endometrial changes.

Statistical analysis:

The whole body weight, weight of reproductive system, the length and width of uterus .Also thickness of endometrium and estrogen &
progesterone concentration data were compared at appropriate confidence intervals. Values are recorded as mean ± S.E.M. and all data were statistically analyzed using SPSS 13 for windows. The normality test was done using One way Anova, Test of Homogeneity of Variances, then if data was normally distributed Student-Newman-Keuls test and Tukey test were performed to see if there were any significant differences between the treated and control groups. Whenever the data was not normally distributed nonparametric tests were done and these were Mann-Whitney U test and Kolmogrov-Smirnov Z test to see if there were any significant differences between the treated and control groups. In all cases difference was considered significant if (p<0.05).

3. Results

During the experiment, all animals survived and this meant that the doses administered the laboratory conditions of water, food and shelter was appropriate.

Whole body weight:

We noticed an increase in the whole body weight of all experimental rats and this increase was significant (p<0.05) with the high dose G3 (20 mg/kg) only on the days 3,10,14,21 (p=0.049, 0.036, 0.039, 0.044) of the experiment one compared to the controls (Fig. 1).

Figure. (1): Showing the effect of Camphor on the whole body weight. Values are means ±SD, n =5. *P<0.05

Whole weight of reproductive system:

The average of reproductive system weight of those animals treated with Camphor dissolved in distilled water was somewhat more than for those given water alone, as there were increase in the whole weight of the reproductive system in all experimental groups but this increase was not significant in the highest Camphor dose G3 (20 mg/kg) compared to the controls (Fig. 2).

Figure (2): Showing the effect of Camphor on the female reproductive system weight. Values are means ±SD, n =5.

The length and the width of uterus horns:

There was a non-significant decrease in the length of the uteri horns in all experimental groups and this decrease was indirectly proportional with the injected doses as the most noticeable decrease was recorded in the lowest Camphor dose G1 (5 mg/kg) compared to the controls (Fig. 3), but the width of these uteri increased non significantly in the experimental groups as the most widest one was G2 (10 mg/kg) compared to the controls (Fig. 4).

Figure (3): Showing the effect of Camphor on the length of uterine horn. Values are means ±SD, n =5.

Figure (4): Showing the effect of Camphor on the width of uterine horn. Values are means ±SD, n =5.

The endometrial thickness:

There was a slight insignificant increase in the thickness of endometrium between the treated and the control groups and this increase was noticeable in G2 (10 mg/kg) agree with the anatomical measurements (Fig. 5).
The level of estrogen and progesterone concentration:

In the present study hormonal assay revealed a significant dose dependant increase in serum estrogen in Camphor injected animals (Fig.6). On the other hand significant decrease in progesterone levels was observed in G1 (5mg/ kg) and G 2 (10mg /kg) while an increase was observed in G3 (20 mg /kg) compared to control group (Fig.7).

Histological analysis:

Upon examining the histological sections of the different parts of the uterus of the treated rats and comparing them with the controls, several remarks were noted as follows:

1- G1 (5mg/kg body weight):

The examination of treated rat uterus histological sections and comparing it to the controls revealed that the number of the uterine glands was fewer with an increase in the endometrial thickness; also the lumen cavity became wider (Fig. 9). In the basal portion of the endometrium the vascular channels were distended and the stroma was denser (Fig. 10). Many of the luminal epithelial cells were undergoing supranuclear vacuolation, exhibited small, hyper chromatic nuclei and contained in-between necrotic cells (Fig. 11).

2- G2 (10 mg/kg body weight):

The lumen of uterus in G 2 became wider in compared with the other treated groups and the control group and the endometrial thickness increased with a significant lower in the uterine glands (Fig.9), also more dilated vascular channels and hyperplasia of the stroma cells in the basal portion of the endometrium were seen (Fig.10). The luminal lining epithelial cells were reduced in height with rounded nuclei i.e. the endometrial epithelium displayed cubical epithelium and there is cytoplasmic vacuolation. Noticeable stroma esinophilic infiltration was indicated (Fig. 11).

3- G3 (20gm/kg body weight):

Sections of G 3 (20 mg/kg) showed large lumen and the endometrial thickness increased as a result of endometrial stromal cell proliferation with very small endometrial glands in compared to the control(Fig. 9). The examination of the basal portion of the endometrium revealed distended vascular channels with stasis of red blood corpuscles and the connective tissue fibers in the stroma were densely packed (Fig. 10). There was a marked decrease in the height of the uterine epithelium, with malformation in the lining cells where lytic cells with degenerated nuclei in between the low height luminal epithelial cells which contained irregular nuclei (Fig.11).

4. Discussions

Camphor is used nowadays as an active ingredient in many substances such as cosmetics especially creams protecting from UV (Schlumph et al., 2001, Tinwell et al., 2002, Schlumph et al., 2004 and Wagner, 2006), medicines, rubs for muscles and colds (Manoguerra et al., 2006). It is also used as moth repellent and an artificial flavoring to give acceptable odor to insecticide (Wagner, 2006).

In this study Intraperitoneal injection of rats with Camphor caused a significant increase in the whole body weight which agrees with the study of Tinwell et al., (2002) where immature female rats were subcutaneously or orally injected with 500 – 800 mg/kg 4MBC, both groups had significantly increased whole body weight compared to the controls. The increase in body weight in response to Camphor involves increase in the reproductive system weight which was explained as due to more
liquid ambition as shown before with 4MBC which caused a slight increase in uterus weight (Seidlová-

Figure (9): Photographs of female reproductive system, showed the increase in uterine weight in response to Camptor in G2 (30 mg/kg) compared to the control.

Wuttke et al., 2006).

Figure (10): Showing a difference in the pro-estrus uterus cavity (*) between the control and treated groups (A) Control, (B) G1 (C) G2 (D) G3. As seen the cavity becomes wider and a significant lower in the uterine glands (arrow) with more treatment compared to the control. G2 shows the most increase in the endometrial thickness (E) (X40)(H&E).

Figure (11): Showing a difference in the pro-estrus endometrium between the control (A) and treated groups, G1 (B) G2 (C) G3 (D). In control (A) the uterine lumina is lined by full columnar epithelium (arrow) with oval nucleus (N), in G1 (B) many of the luminal epithelial cells undergo vascular degeneration (N) and necrosis (arrows), in G2 (C) the luminal lining epithelial cells are reduced in height with rounded nucleus (N). There is cytoplasmic vacuolation (head-arrow), the stroma oedematous infiltration (arrow) in G3 luteal cell (arrow) in between the low height luminal epithelial cells with deformed nucleus (N)(X1000)(H&E).
On the other hand, Falodun et al. (2006) reported the toxic effects of Aspilia africana which have anti-fertility effects on the function of isolated uterus from female rats, he found a decrease in the weight of the uterus which could be due to the ability of the extract to contract smooth muscle fibers as reported by Dimeo et al. (2002), they reported that, extract of Aspilia africana increases in vitro vascular smooth muscle contraction in rats’ aortic ring preparations. These effects are most probably due to imbalances in hormonal level caused by high level of saponins and other phytoestrogens found in this plant.

In this study there was a difference in the lumen width of the uterus where it seemed wider with more Camphor treatment although both control and treated uteruses were in proestrus stage when fixed as shown by the vaginal smears. This dilation led to uterus swelling as seen in the anatomical photos of the treated female rats.

Camphor is considered as an endocrine disruptor and an estrogen agonist (Caserta, 2008). Therefore it caused hormonal imbalances which led to increase in endometrium thickness as the thickness of endometrium varies considerably according to the individual's hormonal state (Spornitz, 1992) and hormonal balance is required for implantation and proper development of concepts (William, 1999) and this might explain the toxic effect of Camphor on the histological appearance of the uterus.

During proestrus in rats, the uterine lumen is distended with clear fluid and the lumen is lined by large low columnar cells (Yuan and Foley, 2002) and all cellular components of the uterus respond to steroid hormones such as estrogen, which stimulates DNA synthesis and cellular proliferation in the uterus of mammals (Mendoza-Rodriguez et al., 2003). In the current study, for treated rats in the proestrus stage, the highest degree of cellular proliferation was seen in the stromal cells only and many of the luminal and glandular epithelial cells undergo vascular degeneration and necrosis as mentioned before (Rahi and Khan, 2006).

Mendoza-Rodriguez et al., (2003) illustrated that when progesterone levels drops significantly, there is a corresponding decrease in glandular and luminal epithelial proliferation and increased apoptosis in these cells. In corroboration, our data demonstrated that cellular proliferation was minimal in the glandular and luminal uterine compartments during this study, as shown before when 4MBC inhibited slightly and non-significantly endometrium proliferation (Seidlová-Wuttke et al., 2003) and the luminal lining epithelial cells are reduced in height, the stroma becomes denser, and mitotic activity decreases (Yuan and Foley, 2002).

Studies in several species have addressed the role of progesterone (P4) in modulating estrogen (E2) activity and maintaining the uterus in a state of quiescence or inactivity (Gimple and Fahrenholz, 2001). Progestrone is secreted for only a limited time by the rat unless a leuteotropic signal from the pituitary is received (Carson et al., 2000). Appropriate E2/P4 synergism is governed by co activators and repressors of steroid receptors (Chen et al., 2005). For example, estrogen receptor negative uteri are hypoplastic while progesterone receptor negative uteri are hyperplastic.

The results of this research showed that Camphor possess negative influences on histological structure of the uterus of female rats suggesting negative influences on the reproductive health of the animals. Therefore more studies have to be done on the effect of camphor on the histology of the female reproductive system in mammals, and its effect on placenta formation and pregnancy continuation.

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