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

Fatma Al-Qudsi and Sabah Linjawi

Biology Department, Science Faculty, King bdulaziz University, Jeddah, Saudi Arabia
falqudsi@kau.edu.sa

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 watery 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.

[Fatma Al-Qudsi and Sabah Linjawi. **Histological and Hormonal Changes in Rat Endometrium under the Effect of Camphor.** Life Science Journal 2012; 9(2):348-355]. (ISSN: 1097-8135). http://www.lifesciencesite.com. 55

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 (Oluyemi *et al.,* 2007). Camphor is taken from the Camphor tree *Cinnamomum camphora* family Lauracae , 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 vaposteam, Bayer Muscle and Joint cream and many other medicines (Ashby *et al.,* 2004). Camphor oil contains many compounds such as camphor, safrol, eugenol, terpeniol, cineol 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 (Mühlbauer *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

(Ford *et. al*., 2001) and Camphor can be detected in the mother blood after 15 minutes from swallowing and through delivery after 36 hours. It was also detected in the amniotic fluid, the umbilical cord and the fetus blood where the fetus was unable to breathe after delivery (IPCS, May 1989).

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 1OO 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 laparatomy 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 12145383122, Elecys 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-Smimov 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).

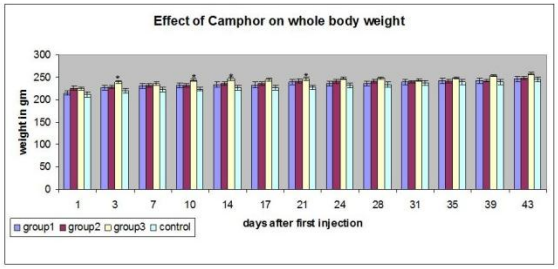

Figure. (1): Showing the effect of Camphor on the whole body weight. Values are means ±SD, n =5. *P<0.05

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).

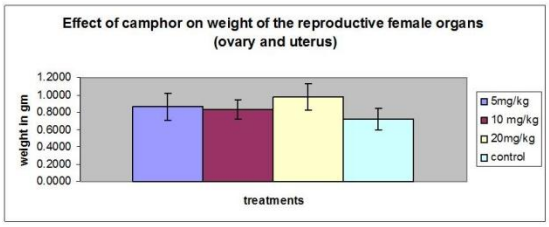

Figure (2): Showing the effect of Camphor on the female reproductive system weight. Values are means ±SD, n =5.

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).

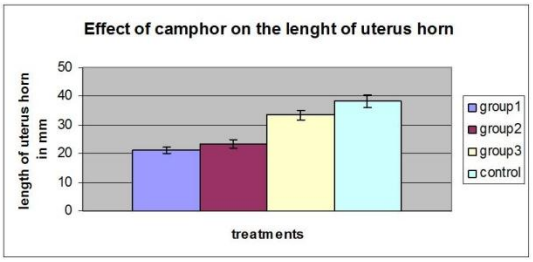

Figure (3): Showing the effect of Camphor on the length of uterine horn. Values are means ±SD, n =5.

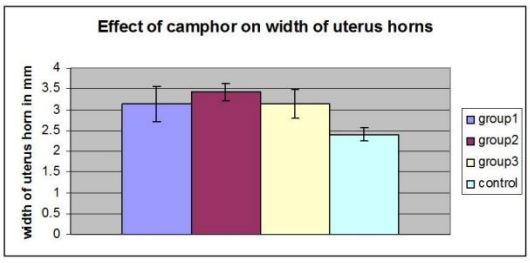

Figure (4): Showing the effect of Camphor on the width of uterine horn. Values are means ±SD, n =5.

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).

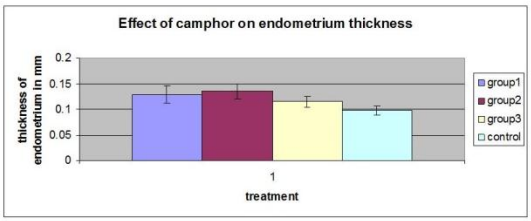

Figure (5): Showing the effect of Camphor on the endometrium thickness. Values are means ±SD, n =5.

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).

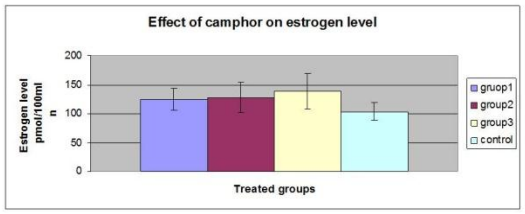

Figure. (6): Showing the effect of Camphor on estrogen level in blood. Values are means ±SD, n =5.

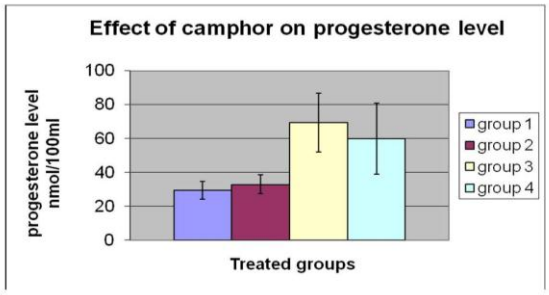

Figure (7): Showing the effect of Camphor on progesterone level in blood. Values are means ±SD, n =5.

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 increases 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 Ttinwell *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-Wuttke *et al.*, 2006).

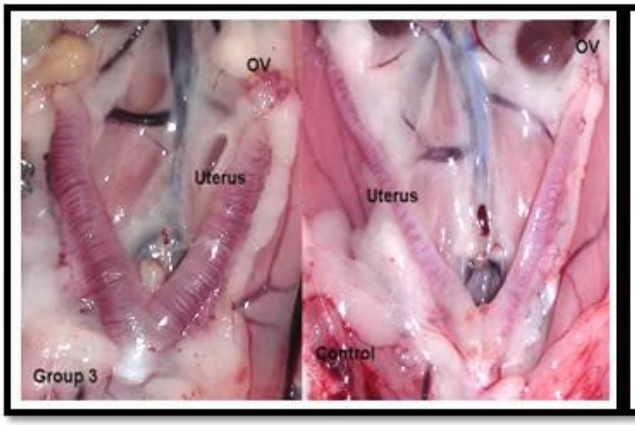

Figure (8): Photographs of female reproductive system, showed the increase in uterine width in response to Camphor in G3 (20 mg/kg) compared to the control.

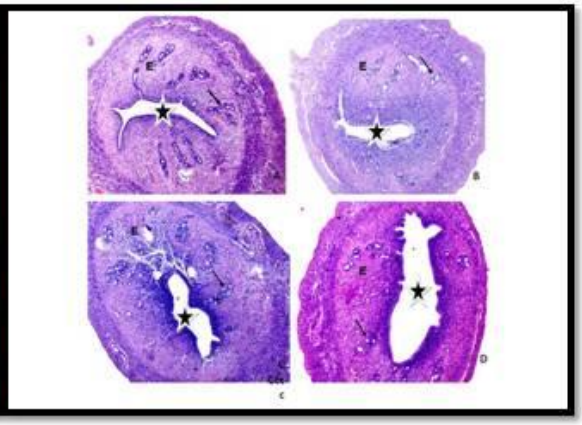

Figure. (9): Showing a difference in the pro-estrous uterus cavity (*) between the control and treated groups. (A) Control, (B) G1(C) G2 (D) G3. As seen the lumen cavity becomes wider and a significant lower in the uterine glands (arrow) with more treatment compared to the control. G2 shows the most increase in the endometrial thickness (E) (X40) (H&E).

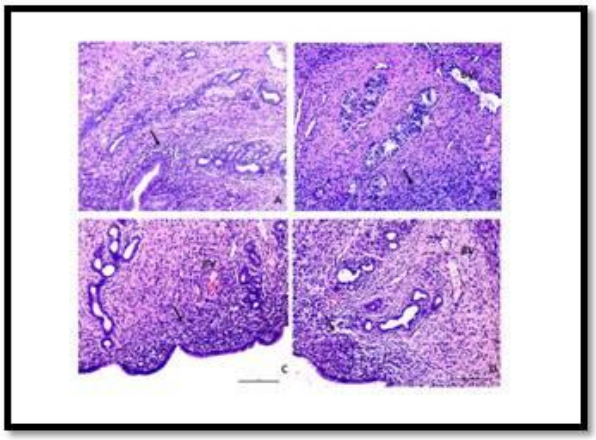

Figure. (10): Showing a difference in the pro-estrous endometrium between the control (A) and treated groups, G1 (B) G2 (C) G3 (D). In the basal portion of the endometrium the vascular channels (BV) were distended, the stroma became denser (arrows) with more treatment compared to the control (X100) (H&E). 2

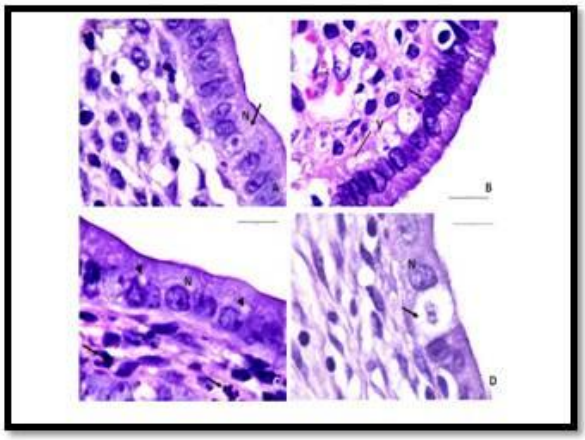

Figure. (11): Showing a difference in the pro-estrous endometrium between the control (A) and treated groups, G1 (B) G2 (C) G3 (D). In control (A) the uterine lumen is lined by tall columnar epithelium (arrow) with oval nucleus(N), in G 1(B) many of the luminal epithelial cells undergo vacuolar degeneration (V) 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 esinophilic infiltration (arrow),in G3 lytic 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 Aspiliaafricana which have anti-fertility effects on the function of isolated uterus from female rats, he found a decrease in the weight of the uteri which could be due to the ability of the extract to contract smooth muscle fibers as reported by Dimo *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 vacuolar degeneration and necrosis as mentioned before (Radi 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 (Seidlovă-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 Camphotr possess negative influences on histo-architecture 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.

Acknowledgements:

The Authors would like to thank King Abdulaziz University/ Science faculty for funding the research project**.**

Corresponding Author:
Dr. Fatma Al-Qudsi,
Biology Deparment, King Abdulaziz University, Saudi Arabia. Email falqudsi@kau.edu.sa

References

1. Ashby J, Tinwell H, Odum J, Lefevre P, Natural Variability and the Influence of Concurrent Control Values on the Detection and Interpretation of Low-Dose or Weak Endocrine Toxicities. Environ Health Perspect 2004;112(8): doi:10.1289/ehp.6862
2. Daniel D. Carson,1 Indrani Bagchi, Sudhandsu K. Dey,, Allen C. Enders,§ Asgerally T. Fazleabas, Bruce A. Lessey, and Koji Yoshinaga Embryo implantation. DevBiol,. 2000;223(2): 217-37.
3. Caserta D, Maranghi L, Mantovani A, Marci R, Maranghi F, Moscarini M. Impact of endocrine disruptor chemicals in gynaecology. Human Reproduction Update 2008; 14: 59-72.
4. Chen B, Pan H, Zhu L, Deng Y, Pollard JW. Progesterone Inhibits the Estrogen-Induced Phosphoinositide 3-Kinase->AKT->GSK-3{beta}->Cyclin D1->pRB Pathway to Block Uterine Epithelial Cell Proliferation. Mol Endocrinol,. 2005;19(8): 1978-1990.

5. Cincinnati, O.H. American Conference of Governmental Industrial. Hygienis. Documentation of Threshold Limit Values for Chemical substances and Physica Agents and Biological Exposure Indices. 2001
6. Ciuman, R. R. Phytotherapeutic and naturopathic adjuvant therapies in otorhinolaryngology. European archives of oto-rhino-laryngology: Official Journal of the European Federation of Oto-Rhino-Laryngological Societies (EUFOS): affiliated with the German Society for Oto-Rhino-Laryngology - Head and Neck Surgery, 2012;269(2), 389-97.
7. Clarke, M.L.H., D.G. and Humphreys, D.J Veterinary Toxicology. 2nd ed., London: BaillereTindall. 1981; p. 125.
8. Dimo T, Tan PV, Dongo E, Kamtchouing P, Rakotonirina SV. *In vitro* vascular smooth muscle contractile activity of Aspiliaafricana extract on rat aortic preparations. Pharmazie, 2002;57(6): 421-3.
9. Falodun, A., Z.A. Nworgu, and M.O. Ikponmwonsa Phytochemical components of Hunteriaumbellata (K. Schum) and its effect on isolated non-pregnant rat uterus in oestrus. Pak J Pharm Sci,. 2006;19(3): 256-8.
10. Ford, M.D.D., K.A.; Ling, L.J. and Erickson, T. Clinical Toxicology., Philadelphia, PA: W.B. Saunders Company. 2001;p. 345.
11. Gimpl, G. and F. Fahrenholz The Oxytocin Receptor System: Structure, Function, and Regulation. Physiological Reviews,. 2001; 81(2): 629-683.
12. Gosselin, R.E.S., R.P. and Hodge, H.C. Clinical Toxicology of Commercial Products. 5th ed., Baltimore: Williams and Wilkins; 1984;p. III-85.
13. Grant, W.M.a.C., C. Toxicology of the Eye. 2nd ed.: Springfield. 1974; p226.
14. Health & Consumer protection directorate-General Opinion on camphor benzalkonium methosulfatecolipa n S57. European Commission. 2006; SCCP/1015/06.
15. Health council of the Netherlands Committee on updating of occupational exposure limits. Bornan-2-one (camphor, synthetic); Health-based reassessment of administrative occupational exposure limits, The Hague Health council of the Netherlands 2001;2000/15osh/018.
16. IPCS Poisons Information Monograph 095: Camphor. 1989May.
17. Jamshidzadeh, A.a.S., J. Effects of subchronic exposure to camphor on male reproductive tract in rats. Abstracts/Toxicology Letters,. 2006;1645 S1-S324.
18. Leuschner, J. The NOEL for the fetal organism for the rat was above 1000 mg/kg bw, and for the rabbit above 681 mg / kg bw. Arzneimittelforschung, 1997;47 (2): p. 124-8.
19. Litovitze, T.L.C., L. and Soloway, R. A. Annual report of the American association of Poison Control Centers Toxic exposure surveillance system. J Emerg Med, 1993;12:583.
20. Long, J.A.a.E., H. M. (): The estrous cycle in the rat and its associated phenomena. Memories of University of California, 1922; 6: p. 1-148.
21. Manoguerra AS, Erdman AR, Wax PM, Nelson LS, Caravati EM, Cobaugh DJ, Chyka PA, Olson KR, Booze LL, Woolf AD, Keyes DC, Christianson G, Scharman EJ, Troutman WG; American Association of Poison Control Centers. . Camphor Poisoning: an evidence-based practice guideline for out-of-hospital management. Clin. Toxicol (Phila). 2006;44(4): p. 357-70.
22. Marcondes, F.K., F.J. Bianchi, and A.P. Tanno, Determination of the estrous cycle phases of rats: some helpful considerations. Brazilian Journal of Biology, 2002. 62: p. 609-614.
23. Mendoza-Rodríguez CA, Merchant-Larios H, Segura-Valdez ML, Moreno-Mendoza N, Cruz ME, Arteaga-López P, Camacho-Arroyo I, Domínguez R, Cerbón M,.c-fos and estrogen receptor gene expression pattern in the rat uterine epithelium during the estrous cycle. Mol Reprod Dev, 2003. 64(4): p. 379-88.
24. Mühlbauer RC, Lozano A, Palacio S, Reinli A, Felix R., Common herbs, essential oils, and monoterpenes potently modulate bone metabolism. Bone, 2003. 32(4): p. 372-80.
25. Oluyemi KA, Omotuyi IO, Jimoh OR, Adesanya OA, Saalu CL, Josiah SJ., Erythropoietic and anti-obesity effects of Garciniacambogia (bitter kola) in Wistar rats. Biotechnol. Appl. Biochem, 2007. 46(Pt 1): p. 69-72.
26. Oudi, J., The new plant and aromatic pharmacopeias. 1 ed., Lebanon.
27. Radi, Z.A. and N.K. Khan Comparative expression and distribution of c-fos, estrogen receptoralpha (eralpha), and p38alpha in the uterus of rats, monkeys, and humans. Toxicol Pathol, 2006; 34(4): p. 327-35.
28. Schlumpf M, Schmid P, Durrer S, Conscience M, Maerkel K, Henseler M, Gruetter M, Herzog I, Reolon S, Ceccatelli R, Faass O, Stutz E, Jarry H, Wuttke W, Lichtensteiger W., Endocrine activity and developmental toxicity of cosmetic UV filters--an update. Toxicology, 2004;205(1-2): p. 113-22.

29. Margret Schlumpf, Beata Cotton, Marianne Conscience, Vreni Haller, Beate Steinmann, Walter Lichtensteige *In Vitro* and *in Vivo* Estrogenicity of UV Screens. Environ Health Perspect, 2001;109(3).
30. Seidlova-Wuttke, D.B., T.; Christoffel, V.; Jarry, H. and Wuttke, W. Silymarin is a selective estrogen receptor beta (ERbeta) agonist and estrogenic effects in the metaphysic of the femur but no or antiestrogenic effects in the uterus of ovariectomized (ovx) rats. J. Steroid Biocheem. Mol. Biol., 2003;86: p. 179-188.
31. Spornitz, U.M. The functional morphology of the human endometrium and decidua. Adv. Anat Embryol Cell Biol. 1992;124: p. 1-99.
32. Tinwell, H.L., P.A.; Moffat, G.J.; Burns, A.; odum, J.; Spurway, T.D.; Orphanides, G. and Ashby,J. Confirmation of uterotrophic activity of 3-(4-methylbenzylidine) camphor in the immature rat. Environmental health perspectives, 2002; 110 (5): p. 533-536.
33. Wagner, P. Inert reassessment- camphor.. Washington D.C: united States environmental protection agency. 2006;Vol. 20460
34. Williams; P. L.; Bannister, L.H.B., M. M.; Collin P.; Dyson, M.; Dussek, J. E. and Fergusson, M. W. Gray's Anatomy in Reproductive System. 38th Ed. ed., Churchill Livingstone, New York: Bannister L. H. & Dyson, M. Edr. 1999; pp1873-4.
35. Yuan, Y.a.F., G. Female reproductive system. In: Haschek W, Rousseux C, Walling M, editors. Handbook of toxicologic pathology. San Diego: CA: Academic Press. 2002; p. 847–6.

3/22/2012