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Toxopathological effects of camphor on some organs of female rats

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Objective The effects of camphor in the histological change on some organs of female rats. In Iraq, camphor in the form of tablets was added to wash dead bodies, and thus putting washers in great risk, especially female washers. Though, the literature concerning its histological toxicity has not been documented. It has long been used in dead bodies washed. In our study, we used high dose of camphor to investigate the effect of camphor in histological change on some organs.
Methods The rats were divided into three groups ($n = 6$), the first group (A) was a control group and treated with distilled water, the second and third groups (B and C) treated with 50 mg camphor/kg and 75 mg camphor/kg, respectively. At the end of the first month of treatment, animals were anesthetized; liver, kidney, spleen, and ovary were removed for serial sections, and histological staining.
Results The results showed vaculation in hepatocytes, and infiltration of inflammatory cells loss of most of the architecture and cell boundaries in liver of group (B), while in group (C), there was congestion in area of Portia, detachment of some hepatocytes, and inflammatory cells. Kidney of group B showed edematous glomerulus, glomerular lobulation, and increased number of nuclei. Also, there were swollen glomerulus, increase number of nuclei, and congestion of blood cells in kidney of group C. Spleen of groups B and C revealed non-active lymphoid tissue and degenerated lymphocytes cells, congestion in lymphoid tissue, and depletion of white pulp mainly neutrophils and macrophage. Also, the results showed congestion in follicles (B) and hemorrhage (C), detachment in oocytes in treatment groups compared with control group.
Conclusions The oral administration of varying concentration of camphor solution to the animal rat has a cytotoxic effect on organs, such as the liver, kidney, spleen, and ovary.
Keywords camphor, *Rattus norvegicus*, liver, kidney, spleen, ovary

Introduction

Camphor ($C_{10}H_{16}O$) is a ketone body from camphor laurel wood *Cinnamomum camphora*.[1,2] It is an herbal medicine has many various physiological effects. It affects the respiratory system, circulatory system, skin, reproductive system, liver, and kidney.[3,4] On the other hand, camphor has exposure and toxicity in human: The main target organs of camphor exposure are the central nervous system (CNS) and kidneys. Convulsions, depression, apnea, asystole, gastric irritation, colic, nausea, vomiting, diarrhea, anxiety, excitement, delirium, and severe post-convulsive coma may occur after intake of camphor. The symptoms may appear 5–90 min after ingestion depending on the product ingested (solid or liquid).[5] Poisoning by camphor is associated with an initial excitatory phase, with vomiting, diarrhea, and excitement, followed by CNS depression and death. Toxic effects appear after the ingestion of approximately 2 g of camphor. It is not clear whether camphor toxicity is due to the parent compound, a metabolite (secondary alcohols, including borneol, and isomers of hydroxy-camphor), or both. Camphor is used exclusively because of its local effects, such as anesthetic effect, which may be followed by numbness. When ingested in small amounts, it creates feelings of warmth and comfort in the stomach, but given in large doses it acts as an irritant. In chronic ingestion, CNS findings may or may not be present, depending on the dosage. Gastrointestinal symptoms may include nausea, vomiting, epigastric pain, and hepatic enzymes elevation. Pathologic hepatic changes often include findings, such as granulomatous hepatitis and fatty metamorphosis.[5] However, camphor ingestion may lead to abortion and/or a death of the fetus because camphor crosses the placenta and fetuses lack the enzymes needed to hydroxylate and conjugate with glucuronic acid.[6] Animal studies: Carcinogenicity tests in animals have been negative. Neuronal necrosis produced experimentally in mice by administration of multiple doses. Mild and transient hepatic derangements may occur and widespread hemorrhages are described in a fatal case.

Fetal death resulted/after camphor ingestion by mother.[7] Oral administration of different concentrations of camphor solution to rabbits for a period of 10 days, resulted in mild edema glomerulonephritis, glomerular lobulations, tubular necrosis, and congestion of the blood cells.[8]

Materials and Methods

Animals and chemicals

Female Wistar rats (body weight 200–280 g; $n = 18$) were obtained from the animal house of Biology Department, Science Collage, Thi-Qar University. They were maintained at 22°C with a 12 h light/dark cycle and allowed to consume standard rat pellet chow (Rodent Laboratory) and water *ad libitum* prior to treatments. After 10 days of adaptation, the experiment was initiated. The animals were divided into three groups (six for each group). The first group (A) was a control, treated with normal saline for 1 month, the second group (B) was treated with 50 mg/kg/day dose of camphor for 1 month. The third group (C) was treated with 75 mg /kg/day dose of camphor for 1 month. The tissues (liver, spleen, kidney, and ovary) were isolated to section histological study.[9]

Results

Histological changes

In control group, the liver cellularity was within the normal limits (Fig. 1A). In the animals which treated with low dose of

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camphor, there was dilation of sinusoids and vacuolation of hepatocytes with rarification and loss of most of the architecture of the liver tissue and loss of cell boundaries (Fig. 1B). The animals treated with high dose of camphor also revealed congestion in the area of Portia, detachment of some hepatocytes and inflammatory cells, swelling in the sinusoids (Fig. 1C). Figure 2A shows section for normal kidney (especially cortical tubules and glomeruli) with normal limits from the control animal. After exposing the rat to 50 mg/kg dose of camphor for 1 month, kidneys showed mild lesions included hemorrhage in the interstitial tissue with enlargement of epithelial cells lining renal tubules, minimum dilatation of renal cortical tubules and edematous glomerulus, glomerular lobulation, and increased numbers of nuclei (Fig. 2B). But after administrated 75 mg/kg/dose of camphor, the lesions were showed more hyalinization of some renal tubules with necrosis of other tubules, patches of hemorrhage, and inflammatory cells mainly mononuclear cells (Fig. 2C). The microscopic examination of spleen in the control group as white pulp was within normal limits (Fig. 3A), but section of the spleen from the animals in group B treated with 50 mg/kg of camphor showed non-active lymphoid tissue and degenerated lymphocytes cells

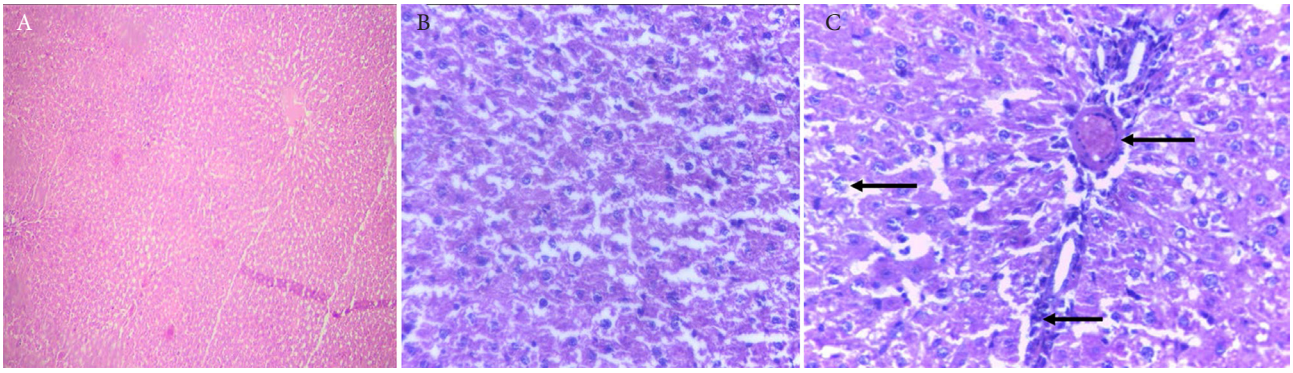

Fig. 1 **(A) Histological sections of liver stained with (H & E), examined under microscope (x200). Liver untreated normal section considered as control group compared with section (B, C) which treated with doses (50, 75 mg/kg/day), (B) Section of the liver from the animals in group treated with 50 mg/kg of camphor. Vaculation in hepatocytes, and infiltration of inflammatory cells loss of most of the architecture of the liver tissue and loss of cell boundaries, (C) Section of the liver from the animals in group treated with 75 mg/kg of camphor shows congestion in area of Portia, detachment of some hepatocytes and infiltration of inflammatory cells and vaculation in hepatocytes.**

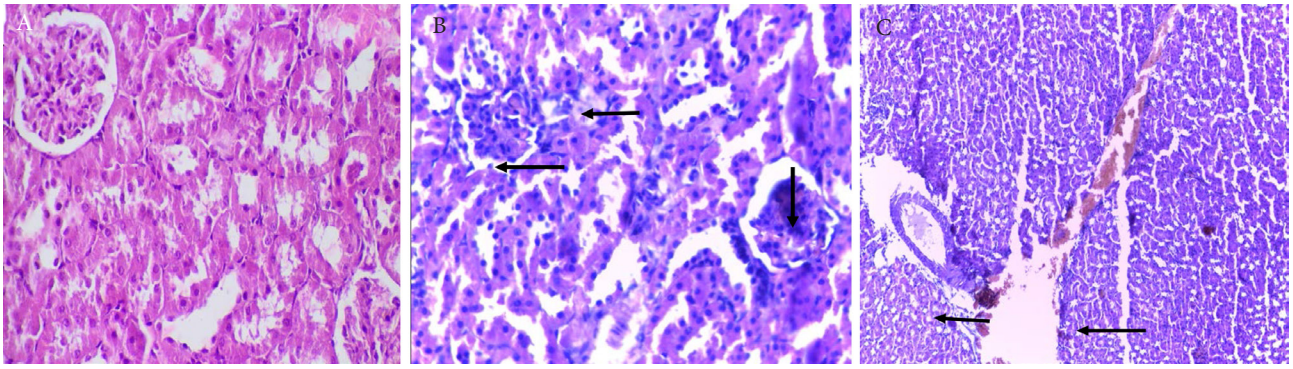

Fig. 2 **(A) Section of liver with normal limits (H & E 800X). (B) Section of the kidney from the animals in group B treated with 50 mg/kg of camphor. (H & E 800X) (EG = edematous glomerulus, LG = glomerular Lobulation, and *N* = Increased numbers of nuclei). (C) Section of the kidney from the animals in group C treated with 75 gm/kg of camphor). (H & E 200X (G = Swollen Glomerulus, *N* = Increased numbers of nuclei, B = Congestion of blood cells.**

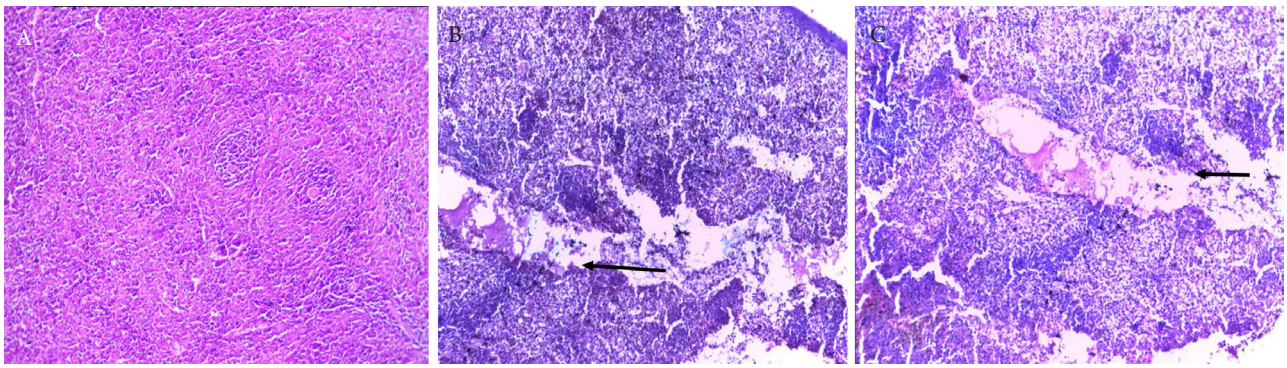

Fig. 3 **(A) Section of spleen with normal limits. (B) Section of the spleen from the animals in group B treated with 50 mg/kg of camphor. (H & E 200) shows non-active lymphoid tissue and degenerated lymphocytes cells. (C) Section of the spleen from the animals in group C treated with 75 mg/kg of camphor. (H & E 200) shows congestion in lymphoid tissue, depletion of white pulp mainly neutrophil and macrophage.**

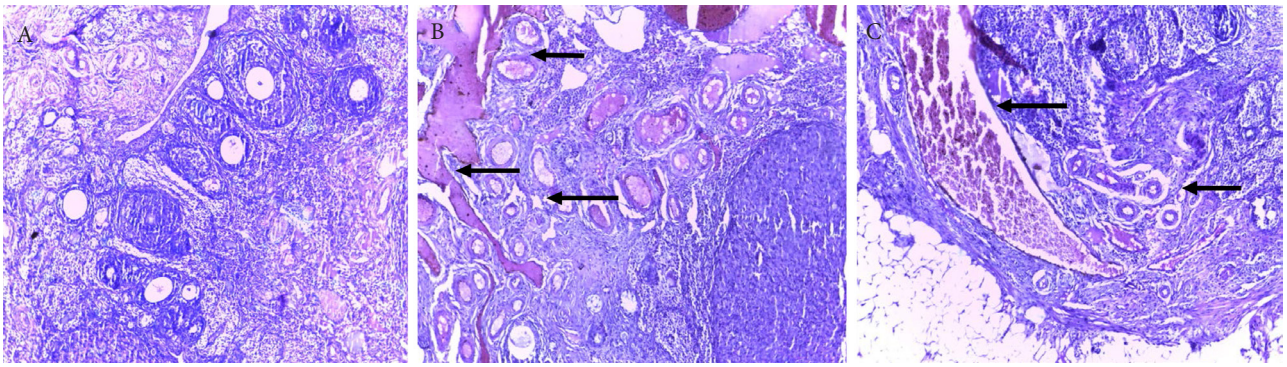

Fig. 4 **(A) Histological sections of ovaries stained with (H & E), examined under microscope (X200). (A) Representative untreated normal section considered as control group compared with section (B, C), which treated with doses (50, 75 mg/kg/day). This also explains effect on all the follicles inhibition (B, C) groups (Ovary non active). Also showed congestion in follicles (B) and hemorrhage (C). Detachment in oocytes in treatment groups. (Arrows) refer area of lesion.**

(Fig. 3B). While section of the spleen from the animals in group C treated with 75 mg/kg of camphor showed congestion in lymphoid tissue and depletion of white pulp mainly neutrophil and macrophage (Fig. 3C). Histological sections of ovaries, examined under microscope representative untreated normal section considered as a control group (Fig. 4A) compared with sections (B, C) which treated with doses (50 and 75 mg/kg/day). This explains the effect on all the follicles inhibition (B, C) groups (ovary non-active) and also shows congestion in follicles (B) and hemorrhage (C). Detachment in oocytes in treatment groups (Figs.4B and 4c).

Discussion

In this study, the reason for histochange in the liver, spleen, kidney and ovary are probably because of different doses of camphor used. One of the most important components in camphor is cinnamaldehyde.[9] For camphor toxicity caused for hepatotoxicity, Aliye suggested that hemlolysis caused tissue damage in the liver due to camphor toxicity. Some scientists found because of camphor poisoning caused due to increased serum LDH level, and a high AST–ALT ratio has been observed.[10] Camphor is deeply dependent on the level of dose and probably in some doses causes damage of liver cells.[11,12] Therefore, Mereto suggests that the liver is the preferential target of its undesirable effects by high doses of cinnamaldehyde that may induce genetic alterations at the chromosomal level,[13] but this study is different from Muguruma's theory. He found that the genotoxicity effect of cinnamaldehyde on the liver is very low.[12] Scientists found that camphor affects liver tissue and changes it. In fact camphor causes vasodilation in central liver veins.[15] Damaged hepatocytes are occurred by liver toxicity measured by ALT and AST released into the blood from the liver.[16] The cellular damage to this tissue causes a substantial increase in the blood levels of this enzyme, infact it renders an excellent marker of cellular necrosis. These results are in agreement with our results, which found lesion of liver area, serum ALT activity is frequently associated with hepatotoxic effects but the latter is not always correlated with the histological findings.[17] The main problems about camphor toxicity in humans are connected more to the large availability of camphor-containing products, and their diffused perception as unhazardous medicines rather than in the intrinsic toxicity of camphor. The daily maximum human therapeutic dose is in fact approximately 1.43 mg · Kg^{-1}, which corresponds to a therapeutic ratio of more than 450 for the end point toxicity, reflecting a wide margin of safety.[18] In the present study, we found histochange in the kidney. Camphor causes convulsions by stimulating the cerebral cortex cells. Pathological findings consist of congestion and edematous changes in the gastrointestinal tract, brain and kidneys.[19] As in humans, the majority of drugs administered to animals are eliminated by a combination of hepatic metabolism and renal excretion.[20] Though the kidney plays a major role in drug metabolism, its major importance to drugs is still its excretory function. In humans, camphor is hydroxylated in the liver to yield hydroxyl camphor metabolites, which are then conjugated with glucoronic acid and excreted in the urine.[21] Our results implicate camphor as a precipitant of kidney disease by causing congestion and edema of the kidney. This observation is in consonance with the findings recorded in previous work, where it was noted that camphor administration has a distortion on the hepatocytes of a developing liver of the Wistar rats. This result is in agreement with Enaibe et al. (2007) observed in the kidney of the treated groups revealed edema, glomerular lobulations, congestion of the blood cells and some cells necrosis.[3] The results of histological change of spleen agrees with Hashem (2012),[22] which found in his research on some of plant extract. Camphor and sumac treatments (ethanol extract) showed a significant reduction in body weight, significant increases in the internal organs weight (liver, kidney, heart, brain, spleen and lung), noticeable dark color of the spleen, congestion and bleeding in the kidney, liver, and heart as well as obvious congestion and bleeding in the brain. Components of plant extracts indicated that glycosides, tannins, flavonoides, saponins, triterpenes and sterols may be responsible for the rodenticide effect observed in his study.

Pathologic ovary changes often include such as the effect of camphor on ovary. The present study observed non-active tissue of ovary caused from camphor toxicity, which effect in ovary. In other research on male reproductive system, Nikravesh and Jalali (2004) found that administration of 30 mg/kg of camphor in time period of 10 and 20 days to male mice showed histochanges, which affect maturation of seminiferous tubules and reproductive function of testes in mice.[23] While Jamshidzadwh and Sajedianfard (2006)[24] showed a decrease in

the rat body weight, testis size, sperm number and mobility with all of the experimental doses. This explains that higher doses of camphor caused morphological changes and a toxic effect on sperm and their mobility. Also our study showed detachment in oocytes and inhibition in ovaries, follicle and hemorrhage, administration of high doses of camphor effect of ovary. These results suggest that oral administration of varying concentration of camphor solution to rat have a cytotoxic effect on the liver and kidney. This may have an adverse effect on the histochange of the liver, kidney, spleen and ovary.

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