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Original Research

Edible camphor-induced histopathological changes in hippocampus and cerebral cortex following oral administration into rats

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ABSTRACT

Introduction: Raw edible camphor (EC), and as component of herbal infusions are widely used to treat pile, back pain, erectile dysfunction, and as an aphrodisiac especially in preparation for sexual intercourse by men. It has been traced in umbilical cord, blood, fetal, adipose, and other tissues including brain, where it bioaccumulates. **Methods:** The study, therefore, investigated the possible histopathological changes in brain, heart, and spleen that may result following EC administration in rats. Thirty animals were used for the study and were divided into six groups of five rats each. Group I animals served as normal control, Group II animals served as vehicle control and were orally administered 6 mL/kg corn oil daily for 7 days, while Groups III-VI animals were orally administered 1, 2, 4, and 6 g/kg EC for 7 days daily. **Results and Conclusions:** Following the administrations of various doses of EC, the histopathological changes seen in the cerebral cortex of the brain include mild submeningeal spongiosis, mild diffuse spongiosis of the parenchyma, a very mild diffuse gliosis, and presences of gitter cells, while in hippocampus, there were mild diffuse gliosis and disruption of the progression of the hippocampal horns, as well as foci of spongiosis around the hippocampal horns, and neuronal cells have open faced nuclei. No effect was seen in heart and spleen except 4 g/kg of EC that revealed moderate diffuse congestion in spleen only. In conclusion, EC may not have any toxic effects on the cardiac and splenic cells, but had toxic effects on the brain hippocampus and cerebral cortex, and may lead to brain cell damage.

KEY WORDS: Camphor, cerebral cortex, heart, hippocampus, histopathological changes, spleen

INTRODUCTION

Camphor ($C_{10}H_{16}O$) is a waxy, transparent, white crystalline solid substance, with a strong aromatic odor. It is a ketone body gotten from *Cinnamomum camphora*, a large evergreen tree found in Asia [1-3], and can also be synthetically formed from wood turpentine. It is a constituent of a variety of foods, and typical sources are herbs, such as basil, rosemary, sage, coriander, and marjoram [4].

Camphor is used for different purposes such as stimulation of circulatory and respiratory systems, psychological stimulation, and cosmetics for external use [5]. In addition, it is used for modulating sexual activity, inducing abortion, contraception, and reducing milk production in lactating women [6,7]. Exposure to camphor is through inhalation, ingestion or dermal routes [8].

Following exposure, metabolism of camphor is mediated by cytochrome P_{450} [9], a class of heme-containing monooxygenases widely distributed in humans and animals cells [10]. The resulting hydroxylated metabolites of camphor following cytochrome P_{450} action are conjugated with glucuronic acid and excreted in the urine [11].

Oral toxic doses in adults are in range 50-500 mg/kg. In general, 2 g causes serious toxicity and 4 g is potentially lethal and has been reported as an irritant of the sexual organs [12-14]. Signs of toxicity have been reported in rats orally administered acute doses of camphor. Consumption of food which reduced in a dose-dependent manner, weight reduction, convulsion, and piloerection was reported signs of toxicity [15]. In primary culture of chick embryo liver cells, camphor caused enhanced porphyrin accumulation ranging from 5 to 20-fold [16]. According to Enaibe *et al*. [17], kidney of rabbits administered various doses of camphor revealed mild edema with glomerulonephritis, tubular necrosis, glomerular lobulations, and congestion of the blood cells, suggesting a cytotoxic effect on the organ. Furthermore, camphor exposures resulted into significant structural changes including thickness of myometrium with a large expansion in the uterus cavity,

reduction in the size of epithelial cells, and an increase in inactive chromosomes in the uterus of pregnant rats [18]. Still according to Linjawi [18], in the uterus, there were significant increases in cellular infiltration, the appearance of epithelial cells and nucleuses degradation, disappearance of nuclear envelope, as well as significant increase in numbers of white blood cells during pregnancy period. Similarly, with respect to the study of Al-Qudsi and Linjawi [19], the number of uterine glands was fewer with a concomitant increase in the endometrial thickness and enlargement of the lumen cavity, endometrial epithelium showed cubical epithelium, cytoplasmic vacuolation, marked decrease in the height of the uterine epithelium, and large lumen and increased endometrial thickness as a result of endometrial stromal cell proliferation with very small endometrial glands compared with uterus of control animals.

Most of the previous histopathological studies on the effects of EC administrations focused on the kidney and reproductive organs. We, therefore, investigated the effects of various doses of EC administrations on the histopathology of brain, heart, and spleen in rats.

MATERIALS AND METHODS

Test Materials and Chemicals

Edible camphor tablets (96% purity) were supplied by Zhejiang Chemicals Import and Export Corporation, China. Wesson® Corn oil (100% natural), the vehicle for EC is produced by ConAgra Foods, Omaha, NE, USA. All chemicals and reagents were of analytical grade, products of Sigma Chemical Co., Saint Louis, MO, USA or BDH Chemical Ltd, Poole, England.

Experimental Animals and Study Design

Thirty male Wistar albino rats of an average weight of 250 g used for this study were obtained from the animal house of the College of Veterinary Medicine, Federal University of Agriculture, Abeokuta, Nigeria. They were housed in steel metal cages in the animal house of our department and were served food and water *ad libitum* throughout the duration of the study. Permission to use the animals was approved by the Institution's Animal Ethical Committee. After 3 weeks of acclimatization,

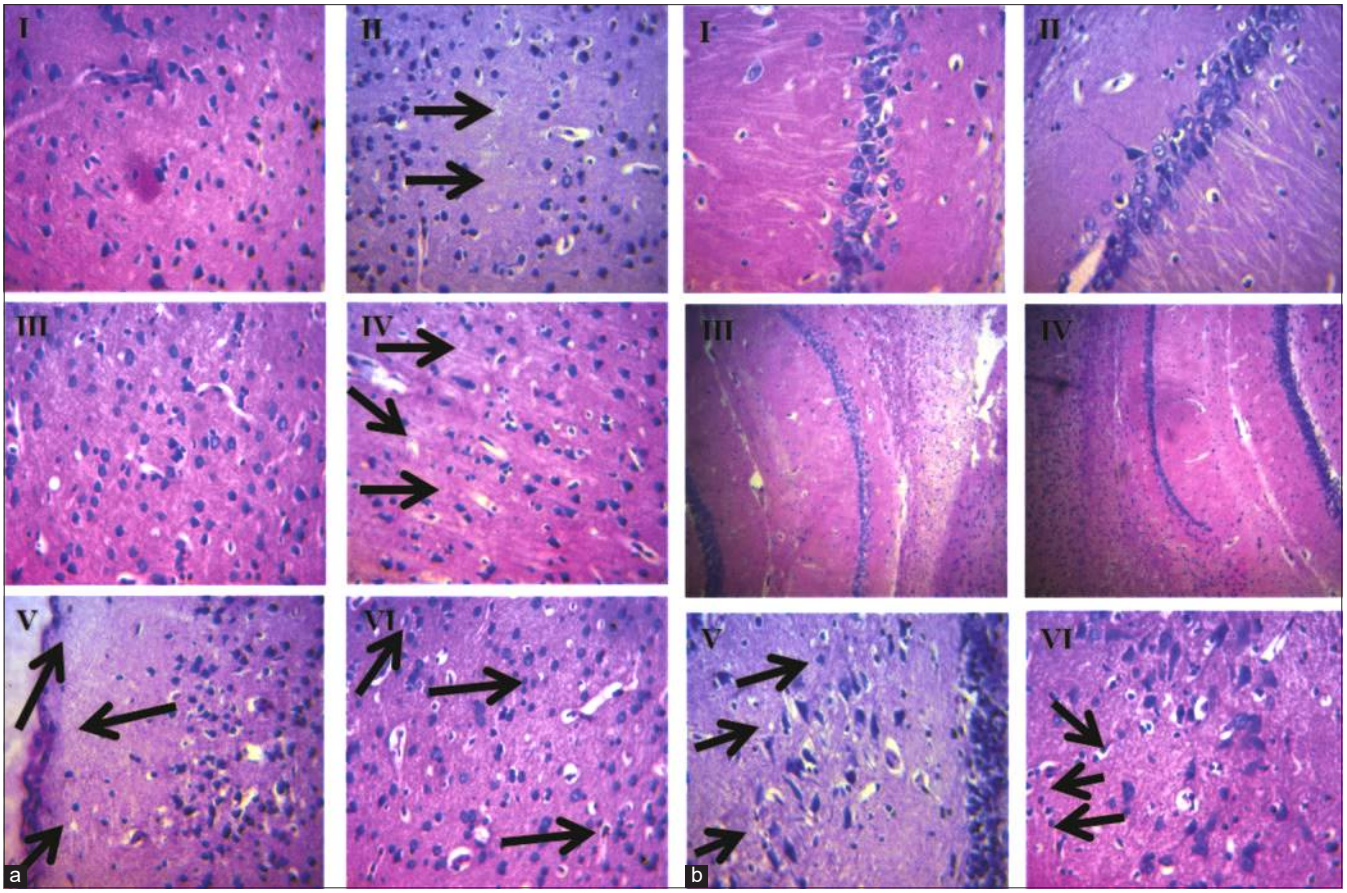

Figure 1: (a) Selected microphotographs of brain cerebral cortex sections (×100) following administration of various doses of EC. Control (I): No visible lesions seen, Corn oil (II): There is a mild diffuse spongiosis of the parenchyma, 1 g/kg EC (III): No visible lesions seen, 2 g/kg EC (IV): There is a very mild diffuse gliosis, 4 g/kg EC (V): There is a mild submeningeal spongiosis, 6 g/kg EC (VI): There is a mild diffuse gliosis and presences of gitter cells, (b) Selected microphotographs of brain hippocampus sections (×100) following administration of various doses of EC. Control (I): No visible lesions seen, Corn oil (II): No visible lesions seen, 1 g/kg EC (III): No visible lesions seen, 2 g/kg EC (IV): No visible lesions seen, 4 g/kg EC (V): There are foci of spongiosis around the hippocampal horns and the neuronal cells have open faced nuclei, 6 g/kg EC (VI): There is a mild diffuse gliosis and the progression of the hippocampal horns appear disrupted

the rats were divided randomly into six groups of five animals each. Group I animals serve as normal control and were fed only rat chow and water only, while Group II animals served as vehicle control and were orally administered 6 mL/kg corn oil daily in the morning for 7 days. EC tablets were dissolved in corn oil, and were administered orally to Groups III-VI animals at the doses of 1, 2, 4, and 6 g/kg, respectively, daily in the morning for 7 days. Oral median lethal dose$_{50}$ of EC in rat is above 5 g/kg [20].

Sample Collections and Preparations

Twenty-four h after the last administrations, animals were sacrificed by cervical dislocation. They were handled and used in accordance with the international guide for the care and use of laboratory animals [21]. Brain, heart, and spleen were harvested; they were washed in ice-cold saline (0.9% w/v) solution and blotted dry. The tissues were then fixed in 10% formal-saline for histopathology.

Histopathological Analysis

Brain, heart, and spleen sections fixed in 10% formal-saline solution were washed in 10 mmol/L phosphate buffer pH 7.4 at 4°C for 12 h. After dehydration, the tissue was embedded in paraffin, cut into sections, stained with hematoxylin-eosin dye on glass slides and was further processed into micrographs. The photomicrographs were obtained at ×100 magnification and read by a pathologist at the University of Ibadan, Ibadan, Nigeria.

RESULTS

Effects of EC Administrations on the Histopathology of Brain's Cerebral Cortex Sections

Following the administrations of various doses of EC, the histopathological changes seen in the cerebral cortex of the brain include mild submeningeal spongiosis, mild diffuse spongiosis of the parenchyma, a very mild diffuse gliosis, and presences of gitter cells [Figure 1a].

Effects of EC Administrations on the Histopathology of Brain's Hippocampus Sections

In the hippocampus, results revealed mild diffuse gliosis and disruption of the progression of the hippocampal horns, as well as foci of spongiosis around the hippocampal horns, and neuronal cells have open faced nuclei [Figure 1b].

Effects of EC Administrations on the Histopathology of Heart Sections

Administrations of EC did not have any effect on heart. All the various doses administered revealed no visible lesions [Figure 2].

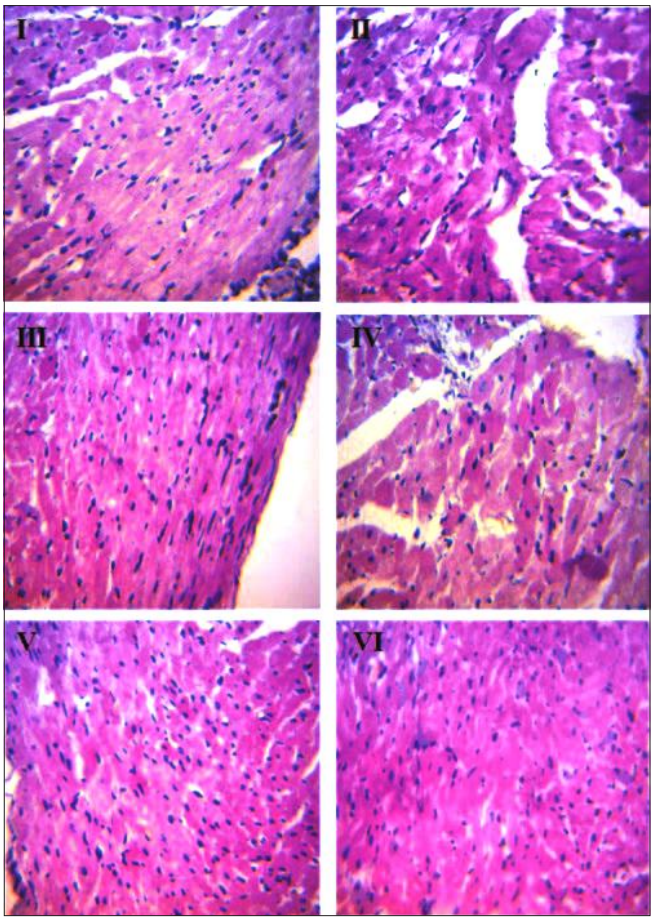

Figure 2: Selected microphotographs of heart sections (×100) following administration of various doses of EC. Control (I): No visible lesions seen, corn oil (II): No visible lesions seen, 1 g/kg EC (III): No visible lesions seen, 2 g/kg EC (IV): No visible lesions seen, 4 g/kg EC (V): No visible lesions seen, 6 g/kg EC (VI): No visible lesions seen

Effects of EC Administrations on the Histopathology of Spleen Sections

Furthermore, administrations of EC did not have any effect on spleen. Almost all the various doses administered revealed no visible lesions, except 4 g/kg that revealed moderate diffuse congestion [Figure 3].

DISCUSSION

Adverse toxic effects may occur from misuse of essential oils and other herbal medicinal plants widely used in alternative and traditional medicine, as well as cosmetics [22]. Therefore, this study evaluated the effect of edible camphor administrations on the histopathological changes in the brain, heart, and spleen.

From the information gathered in this study, EC did not have effects on heart and spleen cells, as most of the previously reported toxicities were seen in fetal blood, brain, liver, kidney and amniotic fluid [23], and reproductive organs [18,19].

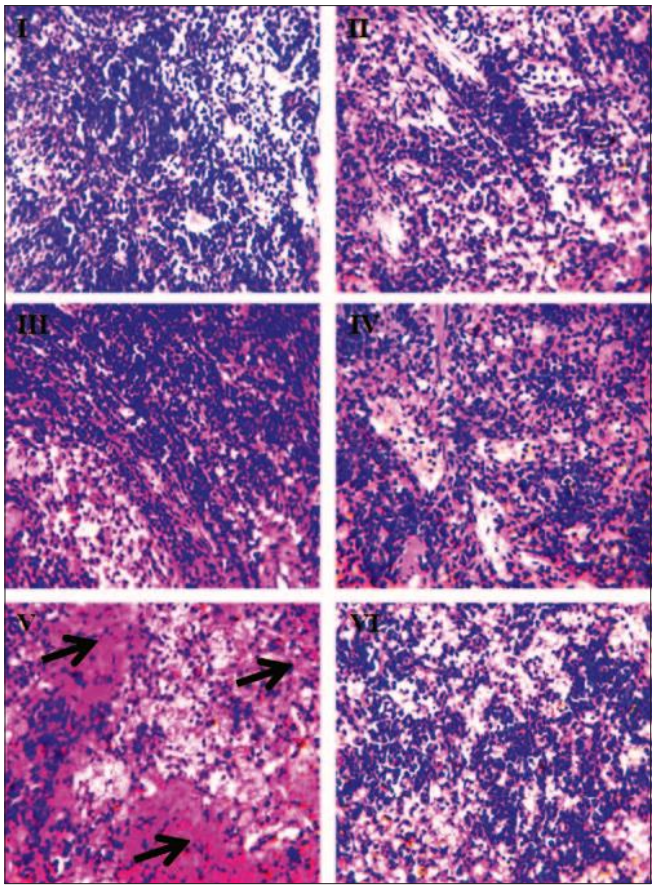

Figure 3: Selected microphotographs of spleen sections (×100) following administration of various doses of EC. Control (I): No visible lesions seen, corn oil (II): No visible lesions seen, 1 g/kg EC (III): No visible lesions seen, 2 g/kg EC (IV): No visible lesions seen, 4 g/kg EC (V): There is a moderate diffuse congestion, 6 g/kg EC (VI): No visible lesions seen

Contrarily, EC significantly affected the hippocampus, the elongated ridges on the floor of each lateral ventricle of the brain, referred to as the center of emotion, memory, and the autonomic nervous system, as well as the cerebral cortex that play an important role in consciousness. The effects on the brain may explain the observed physical symptoms including restlessness, convulsions, epileptic seizures, and paralysis. The brain utilizes mainly glucose as a source of energy, and in the absence of glucose, it opts for ketone bodies because of its permeability to the blood brain barrier. Therefore, EC being a ketone body may have bioaccumulated in the brain, and, therefore, explains the observed histopathological changes. To further corroborate our findings on the effects of EC on the brain, it has been reported that essential oils containing large amounts of monoterpenes such as camphor and cineole could cause the epileptic activity in animals and humans [24-26]. Again, Grbic *et al*. [22] and Grbić *et al*. [27] have reported the changes in rat electro-cortical activity after intraperitoneal administration of camphor oil, through spectral and fractal analysis. This effect is attributed to the highly reactive monoterpenes: Camphor and cineole and their synergistic action, as previously suggested [28]. In a study conducted by Culic *et al*., [29] to investigate the rat brain activity in acute seizures evoked by camphor essential oil or its main constituent 1,8-cineole by wavelet and fractal analysis, higher values of relative wavelet energy of delta frequency band and slight changes of the mean fractal dimension values were recorded in a dose-dependent manner. Profound sex- and region-specific alterations in the regulation of estrogen target genes at brain level following pre- and post-natal exposure to 4-methylbenzylidene camphor (4-MBC) in rat offspring at brain and reproductive organ levels have been reported by Maerkel *et al*., [30] and according to Völkel *et al*., [31] absorbed 4-MBC undergoes extensive first-pass biotransformation in rat liver leading to very low blood levels of the parent 4-MBC. Again, Age-related regulation of mouse brain cortex adrenoreceptors following camphor vapor exposition has been reported [32], while camphor toxicity may clinically mimic Reye's syndrome, due to its hepato-neurotoxic effects [33], and also camphor has been reported to specifically block nicotinic acetylcholine receptors and increases catecholamine secretion [34].

CONCLUSION

The histopathological changes caused by EC on the brain hippocampus and cerebral cortex are indications of its toxic effects, and therefore, may lead to alterations in brain cell functions and damage.

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