Experimental and Toxicologic Pathology 69 (2017) 99–108

Contents lists available at ScienceDirect

Experimental and Toxicologic Pathology

journal homepage: www.elsevier.de/etp

Oral acute toxicity study as well as tissues oxidative stress and histopathological disorders in edible camphor administered rats

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ARTICLE INFO

Article history:
Received 28 September 2016
Accepted 1 December 2016

Keywords:
Edible camphor
Acute toxicity
Oxidative stress
Oral
Histopathology

ABSTRACT

In the South-western part of Nigeria, edible camphor (EC) infusions are used to treat pile, back pain, and erectile dysfunction, especially in preparation for sexual intercourse. We therefore carried out oral acute toxicity study, and then investigated the effects of various doses of EC on the activities of lactate dehydrogenase (LDH), catalase (CAT), and superoxide dismutase (SOD), as well as reduced glutathione (GSH) and malondialdehyde (MDA) levels in wistar rats. Oral LD_{50} of EC was estimated to be 9487 mg/kg body weight. Based on this, thirty animals were divided into six groups of five rats each, and were orally administered various doses of EC (1, 2, 4, and 6 g/kg body weight) for seven days. Comparing all results with control, EC significantly increased serum LDH activity (4 and 6 g/kg), liver (6 g/kg) and kidney (4 and 6 g/kg) MDA levels, as well testis GSH levels (1 g/kg). CAT activities were significantly decreased in liver, kidney, and testis, and also lung GSH level by all the tested doses. For SOD, activities were significantly increased in liver and lung, but significantly decreased in kidney (2, 4, and 6 g/kg). Various pathological disorders were also seen following the various doses of EC administered, especially in liver, kidney and lung. Therefore, from our findings, it is evident that incessant, misuse or overconsumption of EC could lead to oxidative tissue damage in rats.

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1. Introduction

Camphor is a constituent of a various types of foods. It sources are herbs, such as basil, coriander, marjoram, rosemary and sage (Maarse and Visscher, 1989). Camphor has many applications in various countries especially the Asian countries. In herbal medicine, it serves as antiseptic and anti-cold (Chatterjie and Alexander, 1986; Liu et al., 2006), as a fragrance in cosmetics, scenting agent in a variety of household products, active ingredient in some old drugs, flavoring food additive, and intermediate in the synthesis of perfume chemicals (Leikin and Paloucek, 2002).

Administration of camphor has been used in cumulative doses to induce convulsion in psychiatric patients (Pearce, 2008). It also reduces cytochrome P_{450} B1 activity that interferes with 17-α hydroxylase, a key enzyme in the testosterone synthesis. By reducing cytochrome, the function of the enzyme reduces, resulting into diminishing testosterone levels (Mojab and Nickavar, 2003; Chen et al., 2004; Mokhtari et al., 2007; Barzegari and Mirhosseini, 2012).

Metabolism of camphor is mediated by cytochrome P_{450} (Collins and Loew, 1988), a class of heme-containing monooxygenases widely distributed in humans and animals cells (Boxenbaum, 1984). The resulting hydroxylated metabolites of camphor following cytochrome P_{450} action are conjugated with glucuronic acid and excreted in the urine (Sweetman, 2005).

In Nigeria, herbal infusions containing camphor as an ingredient are commonly and widely used to treat pile, back pain, erectile dysfunction, and as an aphrodisiac especially in preparation for sexual intercourse especially among men. To the best of our knowledge, no substantive work has been carried out to investigate the effect of EC on tissue oxidative stress. We therefore carried out oral acute toxicity study, and then investigated the effects of various doses of EC on tissues oxidative stress in wistar rats.

2. Materials and methods

2.1. Test materials, chemicals, and kits

Edible camphor (96% purity) is a product of Zhejiang Chemicals Import and Export Corporation, China. All chemicals and reagents were of analytical grade, products of Sigma Chemical Co., Saint Louis, MO, USA or BDH Chemical Ltd, Poole, England. LDH assay kit is a product of Bio-Inteco Diagnostic Limited, Beechwood Road, England.

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http://dx.doi.org/10.1016/j.etp.2016.12.001
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2.2. Oral acute toxicity of EC

The oral median lethal dose (LD_{50}) of EC was determined as described by Lorke (1983). The study was conducted in three phases. In the first phase, three groups of three rats each were orally administered 5000, 6000, and 7000 mg/kg body weight of EC respectively. We administered these doses based on the study conducted by Opdyke (1978) that LD_{50} of EC in rats is above 5000 mg/kg. The rats were observed for signs of toxicity and possible deaths for a week. In the second phase, another three groups of 1 rat each were orally administered 10,000, 12,000 and 15,000 mg/kg body weight of EC respectively. They were monitored as in phase one for toxicity signs and deaths, while in the third phase, two groups of 1 rat each were orally administered 8000 and 9000 mg/kg body weight of EC, based on outcomes of the second phase and were also monitored as in phase one and two. From the outcomes of the 3 phases, LD_{50} was determined.

2.3. Experimental animals and study design

Thirty (30) 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. Permission to use the animals was approved by the Institution's Animal Ethical Committee. After a long period of acclimatization, the rats were divided randomly into six groups of five animals each. Group I animals serve as normal control, group II animals served as vehicle control and were orally administered 6 mL/kg corn oil, while groups III, IV, V, and VI animals were orally administered 1, 2, 4, and 6 g/kg EC respectively. The result of oral acute toxicity (LD_{50}) conducted in this study, prompted the choice of the tested doses.

2.4. Sample collections and preparations

Administrations lasted for seven days, and 24 h after, animals were sacrificed. They were handled and used in accordance with the international guide for the care and use of laboratory animals (National Research Council, 1996). Blood samples were collected from the abdominal artery into clean plain tubes, and were allowed to stand for 20–30 min; followed by centrifugation at 3000 rpm for 10 min. Serum was separated and aliquoted into clean 1 mL Eppendorf tubes, and stored at $-18\,^{\circ}C$ until when used. Lung, liver, kidney, and testis were also harvested; they were washed in ice-cold saline (0.9% w/v) solution, blotted dry, and weighed. A section of the tissues were cut and fixed in 10% formal-saline for histopathology, and the rest were suspended in ice-cold 0.1 M phosphate buffer (pH 7.4) for homogenization. Homogenization was followed by centrifugation at 5000 rpm for 10 min. The resulting supernatant was also aliquoted into Eppendorf tubes and stored at $-18\,^{\circ}C$ until when used.

2.5. Determination of serum LDH activity

Serum LDH was determined according to the methods described in Bio-Inteco Diagnostic kit, based on the conversion of pyruvate to lactate, resulting into concomitant oxidation of NADH to NAD, which is directly proportional to LDH activity. Briefly, 1 mL of the working reagent which is made up of 50 mmol/L tris buffer mixed with 0.6 mmol/L pyruvate (buffer/substrate) and 0.18 mmol/L NADH, is added to 0.02 mL of sample. It was mixed and incubated at $25\,^{\circ}C$ for a minute, and absorbance was immediately read at 0, 1, 2, and 3 min.

2.6. Estimation of liver and kidney MDA concentrations

MDA concentrations, a marker of lipid peroxidation (LPO) were determined by the method of Buege and Aust (1978). In this procedure, 0.1 mL of the supernatant was added to 2 mL of trichloroacetic acid–thiobarbituric acid–hydrochloric acid (TCA/TBA/HCl) (1:1:1 ratio) reagent, boiled at $100\,^{\circ}C$ for 15 min, and allowed to cool. Flocculent materials were removed by centrifuging at 3000 rpm for 10 min. The supernatant was removed and the absorbance read at 532 nm against blank. MDA concentration was calculated using the molar extinction coefficient for MDA-TBA complex of $1.55 \times 10^{6}\,M^{-1}\,cm^{-1}$.

2.7. Estimation of liver, kidney, lung, and testis GSH concentrations

Reduced glutathione (GSH) levels were determined by the method of Moron et al. (1979), where the color developed was read at 412 nm.

2.8. Estimation of liver, kidney, lung, and testis CAT activities

Activities of catalase were determined by the method of Aebi (1974). Sample (0.1 mL) was added to quartz cuvette containing 1.9 mL of 10 mM phosphate buffer (pH 7.0). Reaction was initiated by the addition of 1.0 mL of freshly prepared 30 mM hydrogen peroxide (H_2O_2). The rate of decomposition of H_2O_2 was measured spectrophotometrically at 240 nm.

2.9. Estimation of liver, kidney, lung, and testis SOD activities

SOD activities were determined by the method of Misra and Fridovich (1972). The method is based on the ability of superoxide dismutase to inhibit auto-oxidation of adrenaline to adrenochrome at alkaline pH. The unit of enzyme activity is defined as the enzyme required for 50% inhibition of adrenaline auto-oxidation.

2.10. Determination of tissues total protein concentrations

Total protein concentrations in lung, liver, kidney, and testis were determined by the method of Gornall et al. (1949), and were used for the estimations of CAT and SOD activities.

2.11. Histopathological analysis

Lung, liver, kidney, and testis sections fixed in 10% formal-saline solution were washed in 10 mmol/L phosphate buffer pH 7.4 at $4\,^{\circ}C$ for 12 h. After dehydration, the tissue was embedded in paraffin, cut into sections, stained with haematoxylin–eosin dye, and finally observed at $\times 400$ magnification under a Nikon light microscope.

2.12. Statistical analysis

Data were analyzed by one-way analysis of variance (ANOVA), followed by least significant difference (LSD) to test for significant differences among the groups of rats using Statistical Package for Social Sciences program version 17.0. Data were expressed as mean $\pm$ standard error of mean. P values less than 0.05 were considered statistically significant.

3. Results

3.1. Oral acute toxicity test

As early as an hour after EC administrations, animals showed signs of toxicity which include decreased food and water consumption, convulsion, seizure, and death. In phase one,

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