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Effect of edible camphor administrations on levels of steroid and thyroid hormones in male wistar rats

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ABSTRACT

Background: Edible camphor (EC) is one of the ingredients in the preparation of local infusion for the management and treatment of pile, back pain, and erectile dysfunction, which are practices commonly in the south-western part of Nigeria. Conversely, 2 and 4 g/kg EC significantly increased the serum level of FT3 but was significantly reduced by 6 g/kg EC, and finally, EC administrations did not have any significant effect on serum PSA.
Methods: Thirty rats 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, while groups III, IV, V, and VI animals were orally administered 1, 2, 4, and 6 g/kg EC for seven days.
Results: Following EC administrations, there was significant ($p < 0.05$) decrease in serum total cholesterol (TCHOL) by 4 and 6 g/kg body weight EC. Furthermore, luteinizing hormone (LH), testosterone (TST), and thyroid stimulating hormone (TSH) levels were significantly reduced by the various doses. Conversely, 2 and 4 g/kg EC significantly increased the serum level of free triiodothyronine (fT3), but was significantly reduced by 6 g/kg EC, and lastly, EC administrations did not have any significant effect on serum prostate specific antigen (PSA).
Conclusion: We therefore concluded that the use of EC should be with caution, and may contribute to thyroid and hormonal disruption in rats.

ARTICLE HISTORY

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KEYWORDS

Camphor; thyroid hormone; reproductive hormone; prostate specific antigen

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. Exposure to camphor is through inhalation, ingestion or dermal routes [4]. Findings have shown that camphor containing substances have antitussive [5], anticonvulsant [6], uterotrophic [7], anti-implantation [8], antiestrogenic [9], nicotinic receptor blocking [10], and estrogenic [9,11-13] activities. Blurred vision, nausea, vomiting, colitis, dizziness, delirium, contraction of heart muscles, difficulty in breathing, seizures and death are reported symptoms of oral camphor poisoning [14]. It is commonly used 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 [15]. Metabolism of camphor is mediated by cytochrome P_{450} [16], a class of heme-containing monooxygenases widely distributed in humans and animals cells [17]. The resulting hydroxylated metabolites of camphor following cytochrome P_{450} (CYP_{450}) action are conjugated with glucuronic acid and excreted in the urine [18].

In Nigeria, herbal infusions containing camphor as an ingredient are widely used to treat pile, back pain, erectile dysfunction, and as an aphrodisiac especially in preparation for sexual intercourse especially by men. Normal thyroid function is important

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to maintain normal reproduction. Thyroid dysfunction can affect sperm production leading to infertility. Apart from that, it can affect sexual performance leading to low libido, easy fatigue, and erectile dysfunction. We, therefore, investigated the effects of various doses of edible camphor (EC) on the serum levels of testosterone (TST), luteinizing hormone (LH), free triiodothyronine (FT_3), thyroid-stimulating hormone (TSH), and prostate-specific antigen (PSA) in male Wistar rats.

Materials and Methods

Test materials and kits

Edible camphor (96% purity) is a product of Zhejiang Chemicals Import and Export Corporation, China. Total cholesterol (TCHOL) kit used, is a product of Cypress Diagnostics, Langdorp, Belgium, while testosterone, LH, FT_3, TSH, and prostate specific antigen enzyme immunoassay (EIA) test kits were products of Bio-Inteco Diagnostic Limited, Beechwood Road, England.

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 once per day for seven (7) days. Oral median lethal dose (LD_{50}) of EC in rat is above 5 g/kg [19], and has been confirmed by us in another study to be actually 9487 mg/kg [20], which prompted the tested doses above.

Sample collections and preparations

The rats were sacrificed by cervical dislocation 24 h after the last administrations. They were handled and used in accordance with the international guide for the care and use of laboratory animals [21]. Blood samples were collected from the abdominal artery into clean plain tubes, and were allowed to stand for 20 to 30 minutes; followed by centrifugation at 3,000 rpm for 10 min. Serum was separated and aliquoted into clean 1 mL Eppendorf tubes, and stored at −18°C until the analysis.

Determination of total cholesterol (TCHOL) concentration

Serum TCHOL was determined according to the method described in Cypress Diagnostics Kits, Langdorp, Belgium. Briefly, cholesterol esterase hydrolyzed cholesterol esters to release free cholesterol which was oxidized by cholesterol oxidase, and the resulting hydrogen peroxide (H_2O_2) reacted with 4-aminophenazone and phenol to form a red quinonimine dye, whose color intensity is proportional to the cholesterol concentration.

Estimations of serum levels of TST, LH, FT_3, TSH, and PSA

Estimations of serum levels of TST, LH, FT3, TSH, and PSA were carried out as described in Bio-Inteco Diagnostic EIA test kits, based on antibody-antigen reactions. As a result, the developed color intensities which are directly proportional to the concentrations in the test samples were measured spectrophotometrically at 450 nm using BioTek ELx800 Microplate reader (Northstar Scientific Limited).

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 ± standard error of mean. Bars having different letters are significantly different, taking $P < 0.05$ as the significant level.

Results

Effects of EC administrations on serum TCHOL concentration

Following the administration of EC to the rats, only 4 and 6 g/kg body weight showed significant difference (Fig. 1) when compared to the control ($p < 0.05$).

Effects of EC administrations on serum levels of TST and LH

Following the various EC administrations, there was significant ($p < 0.05$) decrease in serum LH (Fig. 2) and TST (Fig. 3) and when compared with control. Furthermore, 2, 4, and 6 g/kg body weight of EC significantly ($p < 0.05$) reduced LH levels when compared with 1 g/kg body weight (Fig. 2).

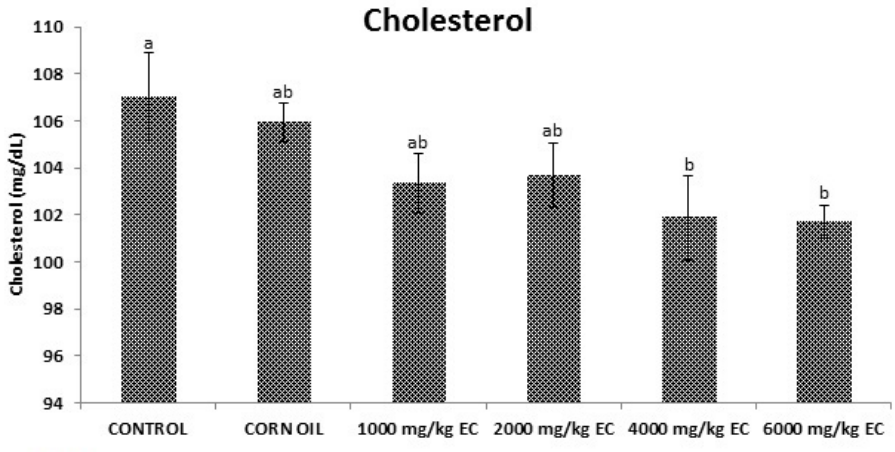

Figure 1. Effects of EC administrations of serum TCHOL concentrations. Values are expressed as mean +/− SEM (n = 5). Bars with different letters are significantly different ($p < 0.05$). EC = edible camphor.

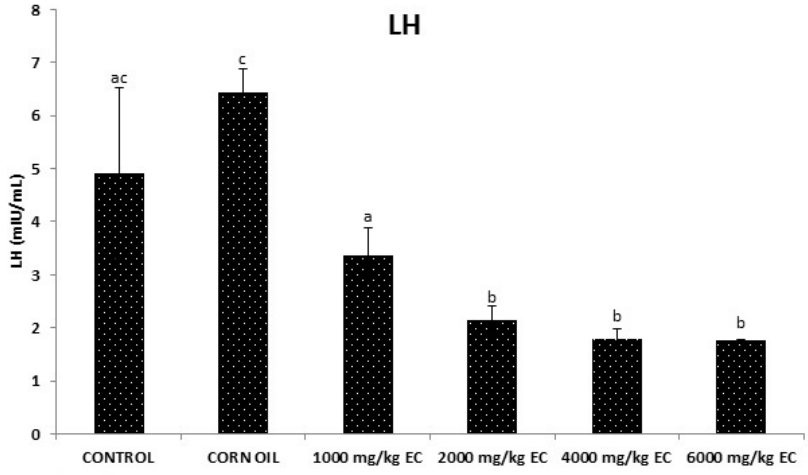

Figure 2. Effects of EC administrations of serum levels of LH. Values are expressed as mean +/− SEM (n = 5). Bars with different letters are significantly different ($p < 0.05$). EC = edible camphor.

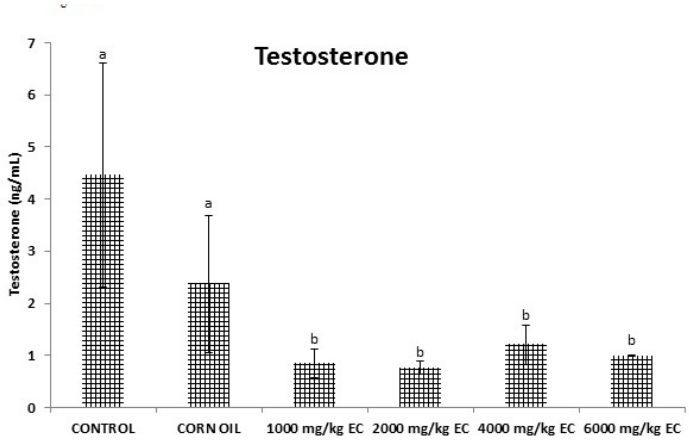

Figure 3. Effects of EC administrations of serum levels of TST. Values are expressed as mean +/− SEM (n = 5). Bars with different letters are significantly different ($p < 0.05$). EC = edible camphor.

Effects of EC administrations on serum levels of TSH and FT_3

TSH level was also significantly reduced ($p < 0.05$) by the various doses of EC, when compared with control (Fig. 4), while 2 and 4 g/kg body weight of EC significantly ($p < 0.05$) increased the serum levels of FT3, but was significantly reduced by 6 g/kg EC (Fig. 5).

Effects of EC administrations on serum levels of PSA

Lastly, EC administrations did not have any significant effect ($p > 0.05$) on serum PSA when compared with control (Fig. 6).

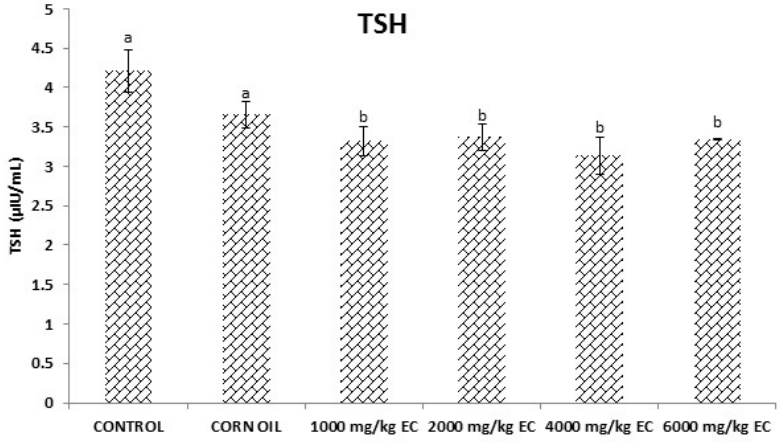

Figure 4. Effects of EC administrations of serum levels of TSH. Values are expressed as mean +/− SEM (n = 5). Bars with different letters are significantly different ($p < 0.05$). EC = edible camphor.

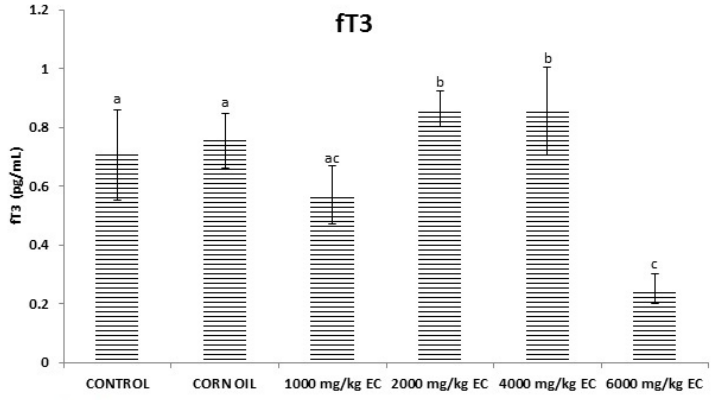

Figure 5. Effects of EC administrations of serum levels of fT3. Values are expressed as mean +/− SEM (n = 5). Bars with different letters are significantly different ($p < 0.05$). EC = edible camphor.

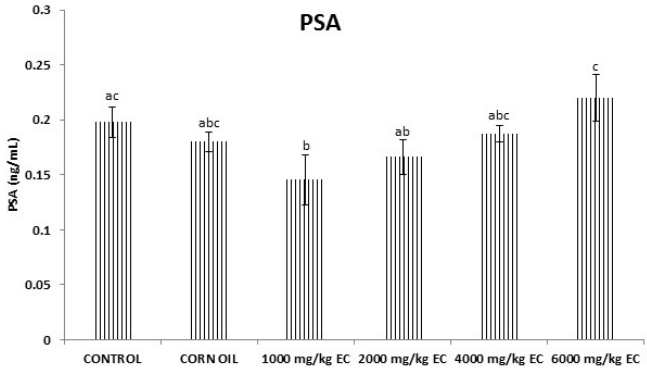

Figure 6. Effects of EC administrations of serum levels of PSA. Values are expressed as mean +/− SEM (n = 5). Bars with different letters are significantly different ($p < 0.05$). EC = edible camphor.

Discussion

Hormones are involved in the regulation of many of the body's biological activities such as metabolism, reproduction, growth and development, and electrolyte balances. The study therefore investigated the effects of EC administration on serum levels of TST, LH, TSH, FT_3, and PSA in male wistar rats.

One of the key functions of cholesterol in animals is to serve as a pre-cursor to the biosynthesis of steroid hormones [22]. The significant decrease in TCHOL concentrations (Fig. 1) following various doses of EC administrations compared with control may be due to cholesterol lowering potentials, or alterations in cholesterogenesis in the animals. EC administrations could also have altered other biological molecules that require cholesterol as precursor (e.g bile acid), and consequently, the availability of the lipid may have been channeled to their synthesis. Our findings are similar to the earlier reports of Mohamed and El-Massry [23], who stated that chronic administration of *Cinnamomum camphora* oil significantly decreased cholesterol concentrations in female albino rats. Still according to Mohamed and El-Massry [23], the hypolipidemic effects of camphor may be due to linalool and cineole, the major oil constituents of *C. camphora* which have cholesterol synthesis inhibitor, anticholelithogenic, cholesterol antagonist, and lipid metabolism regulatory effects. These observations also corroborate the findings of Chelliah [24], while Seidlova-Wuttke et al. [25] and Seidlova-Wuttke et al. [26] also found that camphor treatment reduced serum triglycerides, the size of fat depots and serum leptin, a lipocyte-derived hormone, when compared to control animals.

LH is also referred to as lutrophin [27], and is produced by gonadotropic cells in the anterior pituitary gland. In females, it triggers ovulation, while in males, it stimulates leydig cells to produce TST [28]. Pituitary gland releases LH, and is controlled by actions of gonadotropin releasing hormone (GnRH). Following low levels of TST, hypothalamus releases GnRH, stimulating the pituitary gland to produce LH [29]. In this present study, EC administration significantly decreased serum LH levels compared to control (Fig. 2), suggesting an interference of EC with anterior pituitary functions, leading to lack of LH secretion. Also, it may be due to decreased secretion of GnRH by the hypothalamus, or failure of the anterior pituitary to respond to GnRH stimulation. According to Carou et al. [30], administration of camphor in rats caused slight reduction in levels of LH, FSH, and (GnRH) *in vitro*, while similar findings have also been reported in rats following morphine [31-34], and alcohol exposures [35,36].

In men, the largest amounts of testosterone are formed by the testes [37]. In women, it is formed in smaller quantities by the thecal cells of the ovaries, by the placenta, as well as by the zona reticularis of the adrenal cortex and skin [38] in both sexes. It is produced by the leydig cells of the testes [39]. The amount of testosterone formed by leydig cells is controlled by LH, which regulates the expression of 17-beta-hydroxysteroid dehydrogenase [40]. The hypothalamic pituitary-testicular axis controls the amount of testosterone produced [29]. Gonadotropin-releasing hormone (GnRH) is produced by the hypothalamus in response to low testosterone levels, which then stimulates the pituitary gland to release FSH and LH that stimulate the testes to synthesize testosterone [29]. The significant decrease in serum TST (Fig. 3) obtained in this study can be attributed to the significant decrease in serum TCHOL recorded, being a precursor to steroid hormone synthesis [41]. LH stimulates leydig cells of the testes to produce TST [28,29]. Therefore, the decreased level of serum TST recorded may be attributed to the low LH levels. Our findings are corroborated by the studies of Bazzano et al. [42] and Ochiogu et al. [43] who reported low serum TCHOL, LH and TST following monosodium L-glutamate administration in humans and West African Dwarf goats respectively. Also, Manjunath et al. [44] reported low TST and LH levels in streptozotocin-induced diabetic rats. Organic compounds such as camphor reduce cytochrome P_{450} B1 activity, an enzyme that works with one of the key enzymes in the testosterone synthesis called 17-α hydroxylase [14]. By reducing cytochrome, the enzymes functions reduce, so testosterone level diminishes [45-48].

TSH or thyrotropic hormone is a pituitary and glycoprotein hormone synthesized and secreted by thyrotrope cells in the anterior pituitary gland, regulating the endocrine function of the thyroid [49]. TSH stimulates thyroid gland to produce thyroxine (T_4), and triiodothyronine (T_3) which is responsible for the metabolism of virtually all tissues in the body [50]. When there is low level of thyroid hormone in the blood, high thyroid-releasing hormone (TRH) is released by the hypothalamus, so high TSH is secreted by the pituitary [51] to produce thyroid hormones [50]. From our findings, significantly low levels of serum TSH (Fig. 4) may either be due to inability of the hypothalamus to produce TRH that will stimulate pituitary gland

to produce TSH, or direct interference of EC with the functions of anterior pituitary gland by blocking the release of TSH. T_3 is a thyroid hormone that affects almost every physiological process in the body. It is circulated in blood almost completely bound to carrier proteins [52], known as thyroxine binding globulin (TBG) [52]. However, only the unbound portion of T3 (fT_3) is known to be the true hormone responsible for biological actions [50]. Only 20% of thyroid hormone produced is T_3, appreciable amount (85%) of the circulating T_3 is therefore formed from T_4 in the liver and pituitary [50]. Again, the significant increase in serum levels of FT_3 (Fig. 5) by 2 and 4 g/kg EC may be attributed to the corresponding low levels of TSH. The higher the T_3 level, the lower the level of released TSH and vice versa [50,51]. Also, the significant reduction in FT_3 level by administration of 6 g/kg EC (Fig. 5) may be due to hepatic dysfunction or damage that may have altered the activation of T4 to T3, the active form of the hormone responsible for metabolism [50,53].

PSA, also known as KLK-3 is a glycoprotein enzyme, secreted by the epithelial cells of the prostate gland. KLK-3 is needed for the ejaculate, where it liquefies semen in the seminal coagulum, enabling sperm to swim freely [54]. KLK-3 is available in serum of men with healthy prostates in small quantities, but is usually elevated in the presence of prostate cancer or other prostate disorders [55]. From this present study, there was no significant effect in serum PSA levels (Fig. 6) following EC administration. Therefore, EC may not play a role in the etiology of prostate cancer or disorders.

We therefore concluded that the indiscriminate and careless use of EC should be discouraged and avoided, as it may contribute to thyroid and hormonal disruption in rats.

Conflict of Interest

The authors declared no conflicts of interest.

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