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Renal and testicular up-regulation of pro-inflammatory chemokines (RANTES and CCL2) and cytokines (TNF-α, IL-1β, IL-6) following acute edible camphor administration is through activation of NF-kB in rats

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ABSTRACT

Camphor-induced oxidative stress and histopathological changes (in brain, lung, liver, kidney and testes) have been reported. We therefore investigated the effect of various doses of camphor in an acute study, on renal and testicular levels of some pro-inflammatory mediators in male wistar rats. Twenty rats divided into four groups of five rats each were used in this study. Group 1 served as control and was administered 6 mL/kg olive oil, the vehicle for camphor, while groups 2, 3 and 4 were orally administered 1000, 2000, and 4000 mg/kg body weight camphor, for seven days. Compared with control, levels of tumor necrosis factor alpha (TNF-α), interleukin 1 beta (IL-1β) and interleukin 6 (IL-6) were significantly increased kidney and testes by 2000 and 4000 mg/kg body weight, while interleukin 10 (IL-10) was only significantly increased by 1000 mg/kg body weight of camphor in both tissues. Also compared with control, all doses of camphor administered resulted in a significant increase in the expressions of renal and testicular nuclear factor kappa B (NFkB), cyclooxygenase 2 (COX-2), regulated upon activation normal T cell expressed and secreted (RANTES), and monocyte chemo-attractant protein 1 (MCP-1). Conclusively, use and consumption of camphor should be with caution as it could trigger renal and testicular inflammation through activation of NF-kB and up-regulation of pro-inflammatory markers.

1. Introduction

Camphor ($C_{10}H_{16}O$) is a white transparent, crystalline, and waxy solid substance, with a potent aromatic odor. Camphor is a ketone body obtained from *Cinnamomum camphora*, a large evergreen tree seen in Asia [1–3], and can also synthetically be gotten from wood turpentine. Exposure to camphor is through inhalation, ingestion or dermal routes [4]. Reports have shown that camphor containing substances have anti-implantation [5], antiestrogenic [6], anticonvulsant [7], antitussive [8], uterotrophic [9], nicotinic receptor blocking [10], and estrogenic [6,11–13] activities. Nausea, vomiting, contraction of heart muscles, colitis, dizziness, delirium, difficulty in breathing, blurred vision, seizures and death are reported symptoms of oral camphor poisoning [14]. It is commonly used as an active ingredient in some old drugs, a fragrance in cosmetics, flavoring food additive, scenting agent in a variety of household products, and intermediate in the synthesis of perfume chemicals [15]. Metabolism of camphor is controlled by cytochrome P_{450} [16], a group of heme-containing monooxygenases majorly and widely distributed in human and animal cells [17]. Hydroxylated products of camphor following cytochrome P450 (CYP_{450}) action are conjugated with glucuronate and then excreted in urine [18]. Organic or chemical filters that are derivatives of camphor have been reported to be two of the active ingredients in sunscreen and cosmetic products with neurotoxic effects [19]. These compounds are 4-methylbenzylidene camphor (4-MBC) and 3-benzylidene camphor (3-BC) [19]. 4-MBC is a component that is highly lipophilic and can be absorbed through the skin and consequently found in placenta and other human tissues [20]. 4-MBC has a toxic activity as estrogenic endocrine disruptor [21–23]. Some studies done *in vivo* have suggested that 4-MBC affects the thyroid axis [24], while the effects of 4-MBC on developing neuroendocrine system have been reported [25–27]. Similar to 4-MBC is 3-benzylidene camphor (3-BC), which is also a lipophilic compound [19]. It is a component of sunscreen products in European Union, at a maximal concentration of 2% [28]. Following topical exposure to rats for 65 days at the dose of 60–540 mg/kg/day, 3-BC was detected in brain (concentration 0.13–1.2 μg/g) and other analyzed tissues, suggesting a

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similar disposition and distribution in humans [29]. 3-BC was found in human placenta [20], and has also been reported to be an estrogenic disruptor [22,30].

In a recent study conducted by Somade et al. [31], serum activity of lactate dehydrogenase (LDH) was significantly increased, as well as tissues (kidney, testes, liver and lung) levels of endogenous antioxidant parameters (reduced glutathione, catalase, and superoxide dismutase) and malondialdehyde (MDA) were significantly altered following administration of camphor in rats. Also, kidney histopathology revealed moderate to severe congestion of the renal cortex, moderate interstitial congestion, a severe interstitial cellular infiltration, mild haemorrhage into the interstitium, and many tubules have protein casts in their tubular lumina, while majorly in testis there was no visible lesions [31]. No substantial reports associating or relating camphor administration to renal or testicular inflammation have been documented. Inflammation is controlled by a variety of soluble factors including a group of secreted polypeptides known as cytokines. Inflammation, the response of tissue to injury, is characterized in the acute phase by increased vascular permeability and blood flow along with the accumulation of fluid, leukocytes, and inflammatory mediators such as cytokines and chemokines [32]. Therefore, we investigated the effect of camphor on renal and testicular levels of some pro-inflammatory chemokines (RANTES and CCL2) and cytokines (TNF-α, IL-1β, IL-6), as well as anti-inflammatory cytokine (IL-10) in male wistar rats.

2. Materials and methods

2.1. Test materials, kits and chemicals

Camphor ($C_{10}H_{16}O$; CAS# 76-22-2; 96% purity), is edible and a product of Asia Camphor Manufacturing Co., China. Rats TNF-α, IL-1β, IL-6, and IL-10 enzyme linked immunosorbent assay (ELISA) kits are products of Cusabio Technology llc, Houston, TX, USA. Rat NF-kB, RANTES, COX-2, and CCL2 monoclonal primary antibodies were purchased from Abcam UK; rat monoclonal secondary antibodies were purchased from Dako (Agilent Technologies, USA). All other used chemicals and reagents were of analytical grade, and were products of Sigma Chemical Co., Saint Louis, MO, USA or BDH Chemical Ltd, Poole, England.

2.2. Experimental animals and study design

Twenty (20) male wistar albino rats of an average weight of 200 g and 2 months old, used for this study were obtained from the animal house of the College of Veterinary Medicine (COLVET), Federal University of Agriculture Abeokuta (FUNAAB), Nigeria. They were sheltered in steel metal cages in the animal house of our Department and were served food and water *ad libitum*. Approval to use the animals was granted by the Institution's Animal Ethical Committee. After 2 weeks of acclimatization, the rats were divided randomly into four groups of five animals each. Group I animals served as control and were orally administered 6 mL/ kg olive oil, the vehicle for EC, while groups II, III and IV animals were orally administered 1000, 2000 and 4000 mg/kg EC respectively based on calculated mean lethal dose (LD_{50}) in previous study conducted by Somade et al. [31].

2.3. Sample collections and preparations

Camphor was administered for seven days, and 24 h after; animals were sacrificed by cervical dislocation. They were treated in accordance with the international guide for the care and use of laboratory animals [33]. Kidney and testes were harvested, washed in ice-cold saline (0.9% w/v) solution, blotted dry, and weighed. A section of the kidney and testes was cut and fixed in 10% phosphate buffered formalin for immunohistochemistry. Another section of the kidney and testes was suspended in ice-cold 0.1 M phosphate buffer (pH 7.4) for disruption using a tissue homogenizer. Homogenization was followed by centrifugation at 5000 rpm for 10 min. The supernatant was aliquoted into Eppendorf tubes and used for the estimations of IL-1β, IL-6, TNF-α and IL-10.

2.4. Estimations of kidney and testes levels of TNF-α, IL-1β, IL-6 and IL-10

Protocols in the purchased Cusabio ELISA kits (Cusabio Technology llc, Houston, TX, USA) were followed. Briefly, 100 µL of samples and standards were added into the wells already pre-coated with antibody specific for IL-1β, IL-6, TNF-α, or IL-10 and incubated for 2 h at 37 °C. Unbound substances were removed, and 100 µL of biotin-conjugated antibody specific for IL-1β, IL-6, TNF-α, or IL-10 was added to the well. After washing, 100 µL of avidin conjugated Horseradish Peroxidase (HRP) was added to the wells and incubated for 1 h at 37 °C, followed by addition of 90 µL of TMB substrate solution, followed by incubation for 15–30 minutes at 37 °C to give a color proportional to the amount of IL-1β, IL-6, TNF-α, or IL-10 bound in the initial step. Stop solution was added to each well, plate was gently tapped for thorough mixing, and intensity of the color is measured at 450 nm.

2.5. Kidney and testes immunohistochemistry

This was run as described by Ajayi et al. [34]. Paraffin infiltrated tissue was placed in a mold with a small volume of liquid paraffin. It was briefly cooled to immobilize the tissue and the base of a cassette was placed on top of the mold, filled with liquid paraffin and then cooled. Thin slices of about 4–6 µm was cut on a microtome and sections were floated in a water bath. Sections were mounted on to charged slides and dried overnight to enable sections to adhere to the slide. Paraffin wax was removed by placing sections in three containers of xylene for 5 min each. Poly-L-lysine charged slides were rehydrated in xylene as well as decreasing concentration of ethanol (100-50%). Heat-induced epitope retrieval was done in citrate buffer (pH 6.0) for 20 min followed by immersion in cold water for 10 min. Sections were marked with paraffin pen (PAP) pen and the endogenous peroxidase activities in the tissues were blocked with 5% hydrogen peroxide for 5 min in a dark cupboard. The sections were then incubated overnight at 4 °C with primary antibodies. The slides were washed with Tris buffer saline and then incubated with Horseradish peroxidase labeled secondary antibodies (Dako, Agilent Technologies, US). Immune complexes were visualized using 0.05% 3, 3-diaminobenzidene (DAB), countered stained with hematoxylin and the slides were visualized under light microscope. A slide for each rat was prepared, and 1000 cells were counted per sample. From the number of positive cells counted, percentage of tissue-stained positive cells was scored.

2.6. 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. P values less than 0.05 were considered statistically significant.

3. Results

3.1. Effect of camphor on kidney and testes relative weights

Compared with control, administration of 1000 and 4000 mg/kg body weight camphor significantly ($p < 0.05$) decreased testes relative weights (Fig. 1), while there was no significant difference ($p > 0.05$) in the kidney relative weights (Fig. 1).

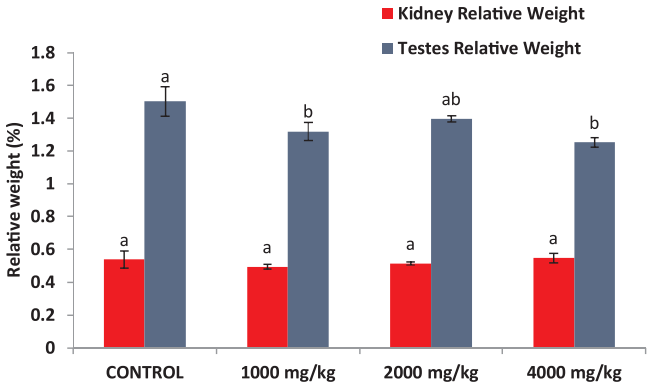

Fig. 1. Effect of camphor administration on renal and testicular relative weights in rats. Values are expressed as mean ± standard error of mean ($n = 5$). Bars labeled with different superscript are statistically significant ($p < 0.05$).

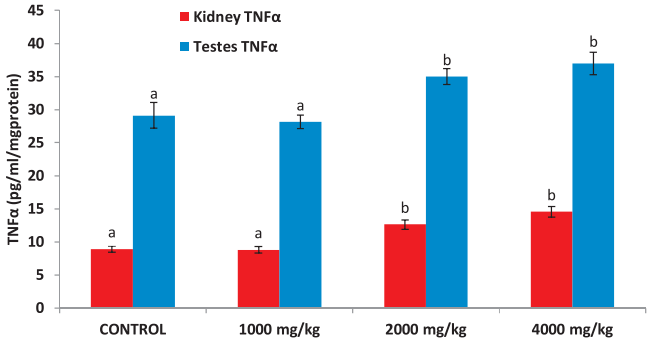

Fig. 2. Effect of camphor administration on renal and testicular levels of TNFα in rats. Values are expressed as mean ± standard error of mean. Bars labeled with different superscript are statistically significant ($p < 0.05$).

3.2. Effect of camphor on renal TNF-α, IL-1β, IL-6, and IL-10

Renal levels of TNF-α (Fig. 2) and IL-1β (Fig. 3) were significantly ($p < 0.05$) increased in a dose dependent manner following administration of 2000 and 4000 mg/kg body weight camphor compared with control, while IL-6 (Fig. 4) level was significantly ($p > 0.05$) increased by all tested doses. For IL-10 (Fig. 5), the level was only significantly ($p < 0.05$) increased by 1000 mg/kg body weight camphor.

3.3. Effect of camphor on testicular TNF-α, IL-1β, IL-6, and IL-10

Similarly in the testes, levels of TNF-α (Fig. 2), IL-1β (Fig. 3) and IL-6 (Fig. 4) were significantly ($p < 0.05$) increased also in a dose dependent manner by 2000 and 4000 mg/kg body weight camphor

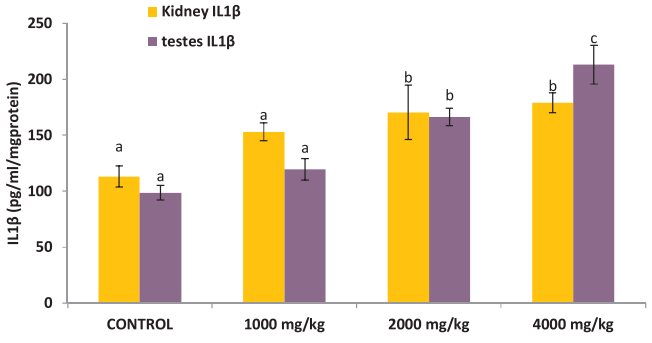

Fig. 3. Effect of camphor administration on renal and testicular levels of IL1β in rats. Values are expressed as mean ± standard error of mean. Bars labeled with different superscript are statistically significant ($p < 0.05$).

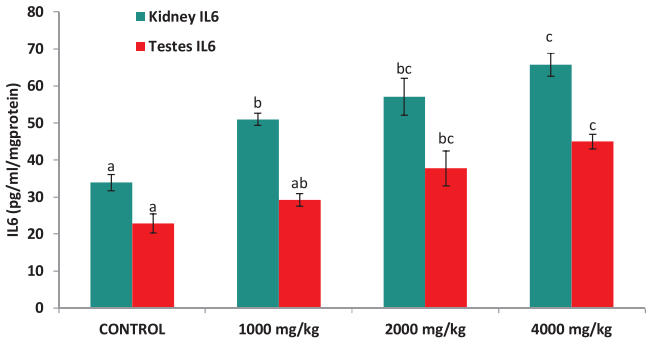

Fig. 4. Effect of camphor administration on renal and testicular levels of IL6 in rats. Values are expressed as mean ± standard error of mean. Bars labeled with different superscript are statistically significant ($p < 0.05$).

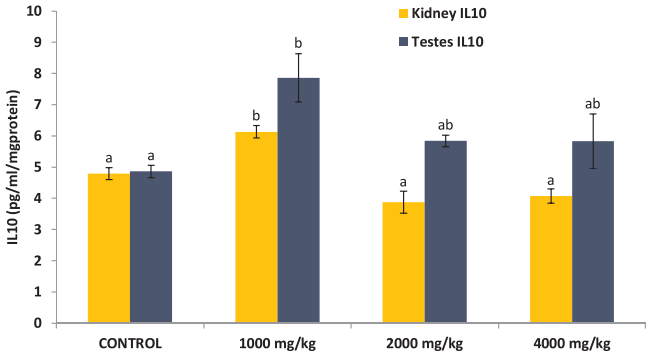

Fig. 5. Effect of camphor administration on renal and testicular levels of IL10 in rats. Values are expressed as mean ± standard error of mean. Bars labeled with different superscript are statistically significant ($p < 0.05$).

compared with control. Also, there was a significant ($p < 0.05$) increase in the levels of IL-1β (Fig. 3) when group administered 4000 mg/kg body weight camphor is compared with other tested doses. For IL-10 (Fig. 5) also, the level was only significantly ($p < 0.05$) increased by 1000 mg/kg body weight camphor.

3.4. Effect of camphor on expressions of renal NF-kB, COX-2, RANTES and CCL2

Compared with control, there was a significant ($p < 0.05$) increase in the renal expressions of NFkB (Fig. 6A), COX-2 (Fig. 6B), RANTES (Fig. 6C), and MCP-1 (Fig. 6D) by all tested doses compared with control. Group administered 4000 mg/kg camphor showed the highest expressions of these parameters. The degree of expressions was 4000 mg/kg > 2000 mg/kg > 1000 mg/kg of camphor.

3.5. Effect of camphor on expressions of testicular NF-kB, COX-2, RANTES and CCL2

Similarly in the testes, all the tested doses of camphor resulted in a significant ($p < 0.05$) increase in the expressions of NFkB (Fig. 7A), COX-2 (Fig. 7B), RANTES (Fig. 7C), and MCP-1 (Fig. 7D) compared with control. The greatest effect was seen following administration of 4000 mg/kg camphor, while the degree of expressions was also 4000 mg/kg > 2000 mg/kg > 1000 mg/kg of camphor.

4. Discussion

Camphor undergoes swift oxidation to 5-exo-hydroxyfenchone that is mainly mediated by human liver microsomal cytochrome P_{450}. Cytochrome P450 2A6 (CYP2A6) is the key enzyme responsible for the hydroxylation of camphor by human liver microsomes [35]. This phase

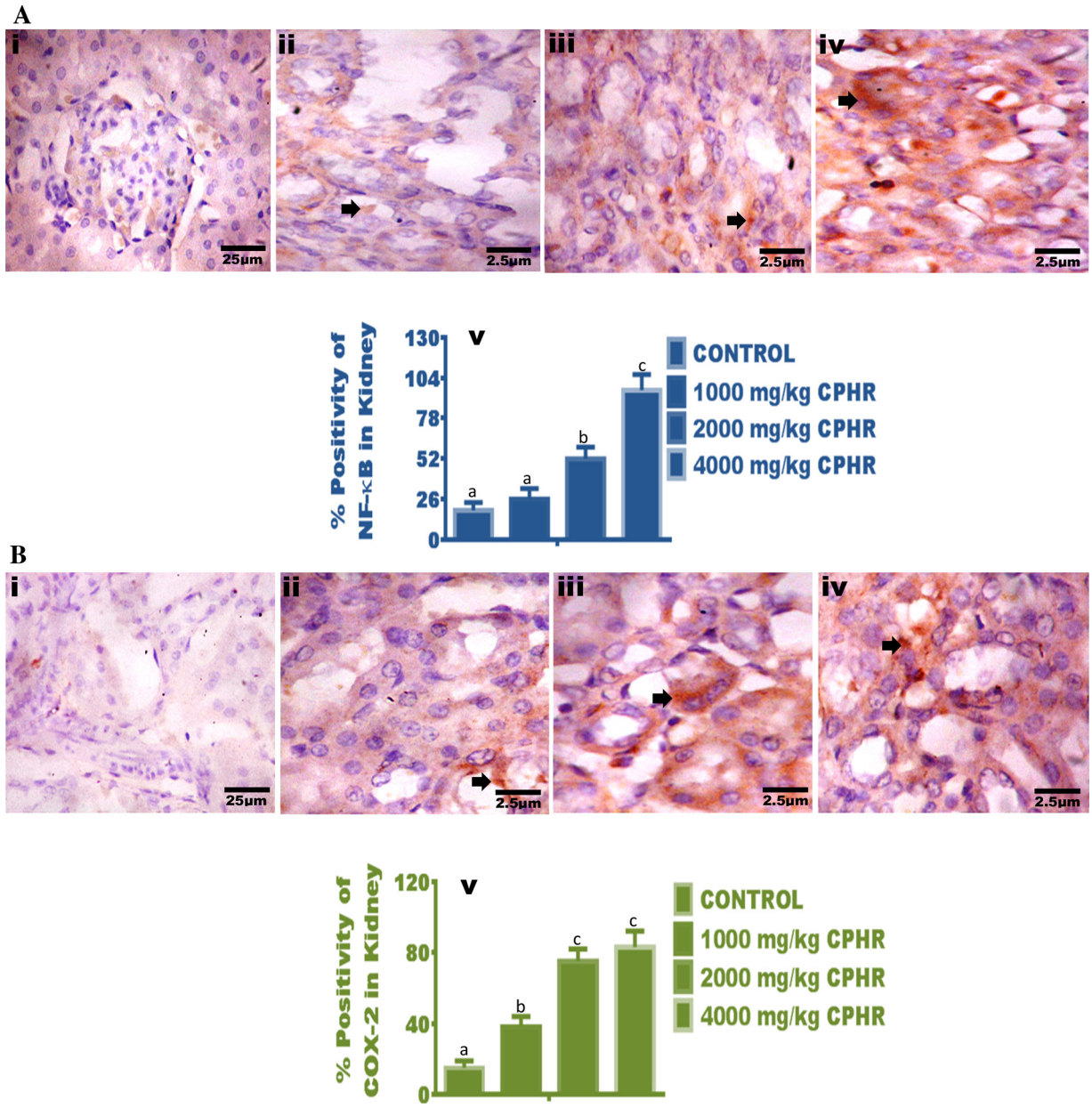

Fig. 6. A. Immunohistochemical expressions of renal NFkB in camphor administered rats (black arrow). i = Section from control rat not administered camphor (IHC x 25µ); ii = Section from rat administered 1000 mg/kg camphor (IHC x 2.5µ); iii = Section from rat administered 2000 mg/kg camphor (IHC x 2.5µ); iv = Section from rat administered 4000 mg/kg camphor (IHC x 2.5µ); v = graph showing percentage positive NFkB cells. Each bar represents mean ± SEM. Bars labeled with different superscript are statistically significant ($p < 0.05$). CPHR = camphor. **B.** Immunohistochemical expressions of renal COX-2 in camphor administered rats (Black arrow). i = Section from control rat not administered camphor (IHC x 25µ); ii = Section from rat administered 1000 mg/kg camphor (IHC x 2.5µ); iii = Section from rat administered 2000 mg/kg camphor (IHC x 2.5µ); iv = Section from rat administered 4000 mg/kg camphor (IHC x 2.5µ); v = graph showing percentage positive COX-2 cells. Each bar represents mean ± SEM. Bars labeled with different superscript are statistically significant ($p < 0.05$). CPHR = camphor. **C.** Immunohistochemical expressions of renal RANTES in camphor administered rats (Black arrow). i = Section from control rat not administered camphor (IHC x 25µ); ii = Section from rat administered 1000 mg/kg camphor (IHC x 2.5µ); iii = Section from rat administered 2000 mg/kg camphor (IHC x 2.5µ); iv = Section from rat administered 4000 mg/kg camphor (IHC x 2.5µ); v = graph showing percentage positive RANTES cells. Each bar represents mean ± SEM. Bars labeled with different superscript are statistically significant ($p < 0.05$). CPHR = camphor. **D.** Immunohistochemical expressions of renal MCP-1 in camphor administered rats (Black arrow). i = Section from control rat not administered camphor (IHC x 25µ); ii = Section from rat administered 1000 mg/kg camphor (IHC x 2.5µ); iii = Section from rat administered 2000 mg/kg camphor (IHC x 2.5µ); iv = Section from rat administered 4000 mg/kg camphor (IHC x 2.5µ); v = graph showing percentage positive MCP-1 cells. Each bar represents mean ± SEM. Bars labeled with different superscript are statistically significant ($p < 0.05$). CPHR = camphor.

one reaction of drug metabolism is followed by glucuronidation, a phase two biotransformation reaction where glucuronide acts as the conjugation molecule and binds to a substrate, catalyzed by glucuronosyltransferases. In the present study, the effect of EC administration on kidney and testes levels and expressions of pro-inflammatory markers in rats was investigated. Pro-inflammatory cytokines are produced majorly by activated macrophages and play a predominant role in the up-regulation of inflammatory reactions [36]. Abundant evidence are available that certain pro-inflammatory cytokines like IL-6, IL-1β, and TNF-α are involved in the process of pathological pain [36]. In this study, camphor administration did not have any effect on kidney relative weight, which is in agreement with the report in an earlier study of camphor-induced oxidative stress [31]. On the other hand, the significant decrease in testes relative weight as a result of camphor

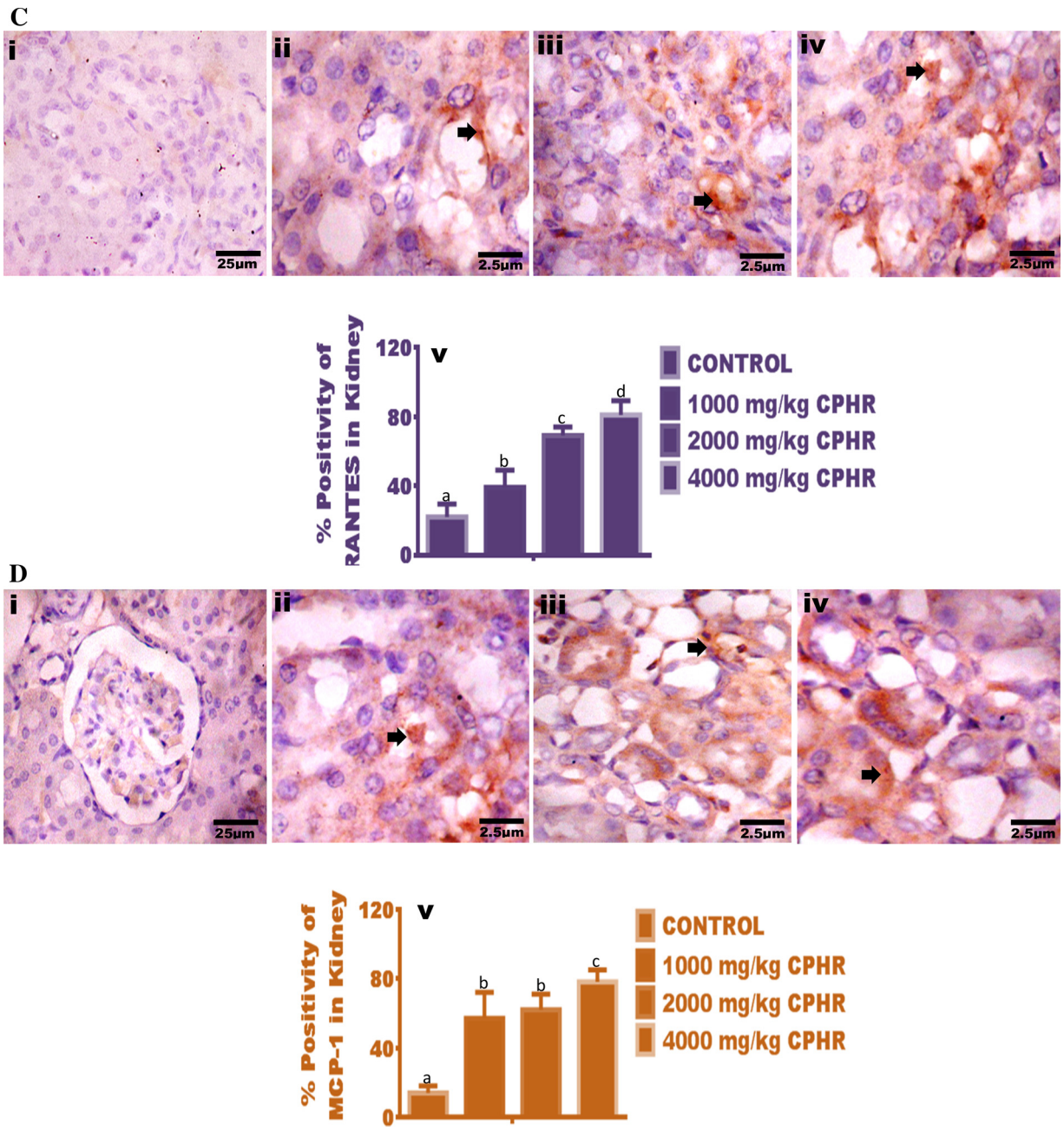

Fig. 6. (*continued*)

administration may indicate a direct toxicity of the substance to the organ, which can affect vital activities including hormonal disruptions. In a recent study, it was reported that camphor administration significantly led to the reduction of testosterone and luteinizing hormone in rats [37].

Cytokines are produced by numerous cell populations, but the main producers are macrophages and helper T cells. Cytokines may be produced in and by peripheral nerve tissue during physiological and pathological processes by recruited and resident macrophages, mast cells, Schwann cells and endothelial cells [36]. In this present study, the significant increase in the levels of renal and testicular TNF-α, IL-1β, and IL-6 is an indication of camphor-induced injuries or infections to these vital organs causing the release and recruitment of these pro-inflammatory mediators, predominantly by the helper T cells and macrophages to the affected site where they promote inflammation and trigger pathological pain. The effect of camphor on renal cells revealed in this study is in line with the report of Ramesh and Reeves [38] that TNF-α and IL-1β were up-regulated in mouse model of cisplatin-induced nephrotoxicity. In a more recent study, up-regulation in the expressions of TNF-α, IL-1β and IL-6 were reported in adenine-induced chronic kidney disease with uraemia in mice [39]. Studies have documented an increase in the pro-inflammatory cytokines IL-1β and TNF-α following ischemia/reperfusion (IR) of the testis, suggesting a role of the pro-inflammatory cytokines as early mediators of IR-injury in testis [40]. As early as 30 min after IR of murine testis, an increase in expression of both IL-1β and TNF-α was observed [40] and this precedes the activation of c-jun N-terminal kinase (JNK). Germ cell-specific apoptosis following IR of testis is dependent on the recruitment of neutrophils to the testis [41], and it has been reported that an increase in pro-inflammatory cytokines after IR of testis is synonymous with activation of signaling pathways eliciting neutrophil recruitment [40]. IL-1 and TNF-α are produced in testis under normal physiological conditions and play a key role in maintaining testicular function. Certain pathologic conditions, such as autoimmune orchitis and testicular

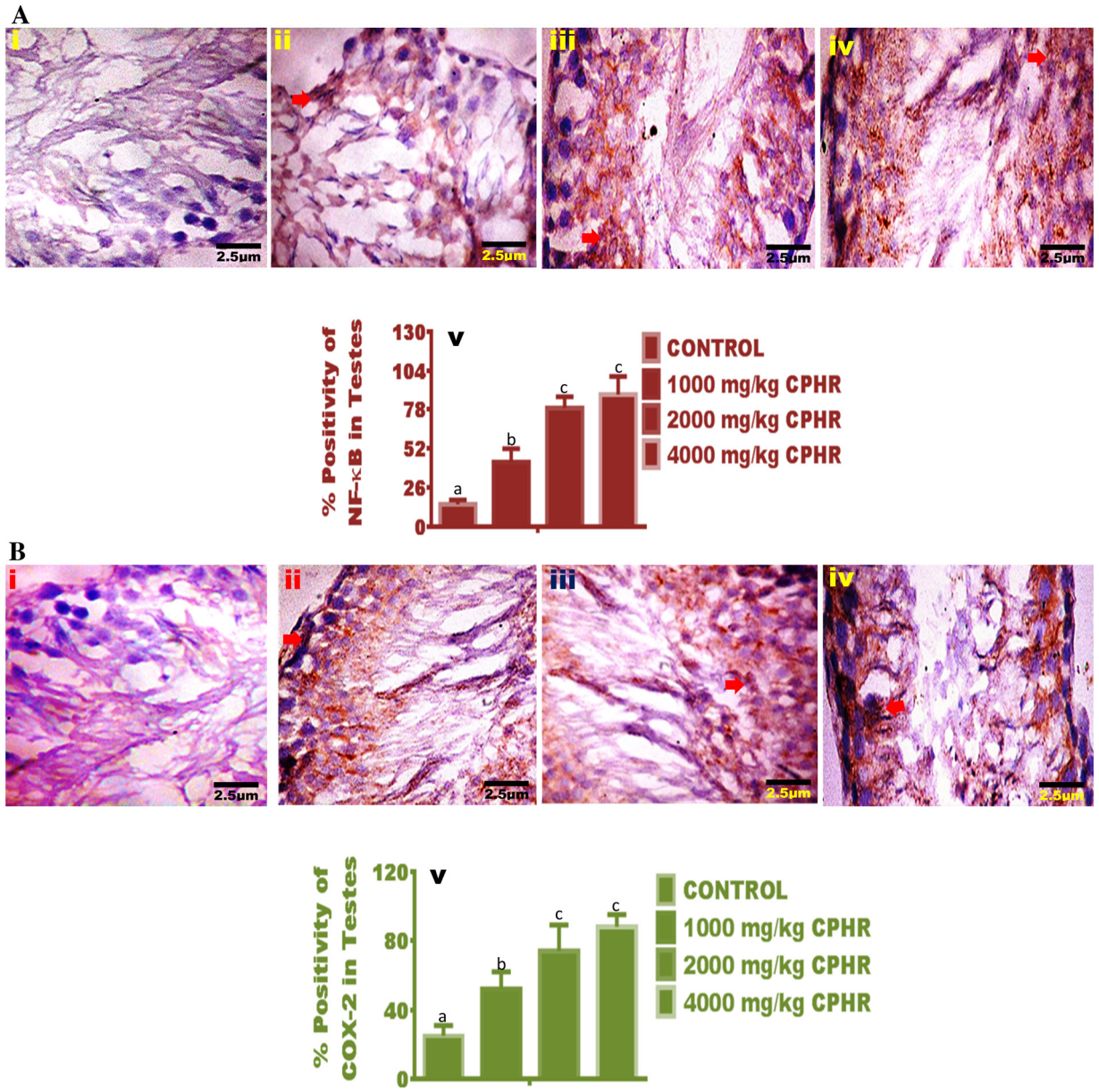

Fig. 7. A. Immunohistochemical expressions of testicular NFkB in camphor administered rats (Red arrow). i = Section from control rat not administered camphor (IHC x 2.5µ); ii = Section from rat administered 1000 mg/kg camphor (IHC x 2.5µ); iii = Section from rat administered 2000 mg/kg camphor (IHC x 2.5µ); iv = Section from rat administered 4000 mg/kg camphor (IHC x 2.5µ); v = graph showing percentage positive NFkB cells. Each bar represents mean ± SEM. Bars labeled with different superscript are statistically significant ($p < 0.05$). CPHR = camphor. **B.** Immunohistochemical expressions of testicular COX-2 in camphor administered rats (Red arrow). i = Section from control rat not administered camphor (IHC x 2.5µ); ii = Section from rat administered 1000 mg/kg camphor (IHC x 2.5µ); iii = Section from rat administered 2000 mg/kg camphor (IHC x 2.5µ); iv = Section from rat administered 4000 mg/kg camphor (IHC x 2.5µ); v = graph showing percentage positive COX-2 cells. Each bar represents mean ± SEM. Bars labeled with different superscript are statistically significant ($p < 0.05$). CPHR = camphor. **C.** Immunohistochemical expressions of testicular RANTES in camphor administered rats (Red arrow). i = Section from control rat not administered camphor (IHC x 2.5µ); ii = Section from rat administered 1000 mg/kg camphor (IHC x 2.5µ); iii = Section from rat administered 2000 mg/kg camphor (IHC x 2.5µ); iv = Section from rat administered 4000 mg/kg camphor (IHC x 2.5µ); v = graph showing percentage positive RANTES cells. Each bar represents mean ± SEM. Bars labeled with different superscript are statistically significant ($p < 0.05$). CPHR = camphor. **D.** Immunohistochemical expressions of testicular MCP-1 in camphor administered rats (Red arrow). i = Section from control rat not administered camphor (IHC x 2.5µ); ii = Section from rat administered 1000 mg/kg camphor (IHC x 2.5µ); iii = Section from rat administered 2000 mg/kg camphor (IHC x 2.5µ); iv = Section from rat administered 4000 mg/kg camphor (IHC x 2.5µ); v = graph showing percentage positive MCP-1 cells. Each bar represents mean ± SEM. Bars labeled with different superscript are statistically significant ($p < 0.05$). CPHR = camphor.

torsion caused an increase in the expression of pro-inflammatory cytokines. This up-regulation in expression shifts the balance in favor of immune and inflammatory responses. Immediately immune cells are either recruited to the testis or activated in the testis germ cell, it can result into apoptosis and spermatogenesis is affected [42]. This could also explain the significant decrease in testes relative weight recorded in this study. In addition to camphor, previous studies have reported the involvement of chemical and biological substances in eliciting inflammatory responses. In a study by Yacoub et al. [43], *Montivipera bornmuelleri* venom administered to mice caused immunomodulatory effects by up-regulating the levels of TNF-α, IL-1β, IL-17, and interferon gamma (IFN-γ) in the spleen. Also, Chlorpyrifos was reported to elicit pro-inflammatory responses by increasing the rat levels of liver and kidney TNF-α and IL-1β [44], while in addition to TNF-α and IL-1β,

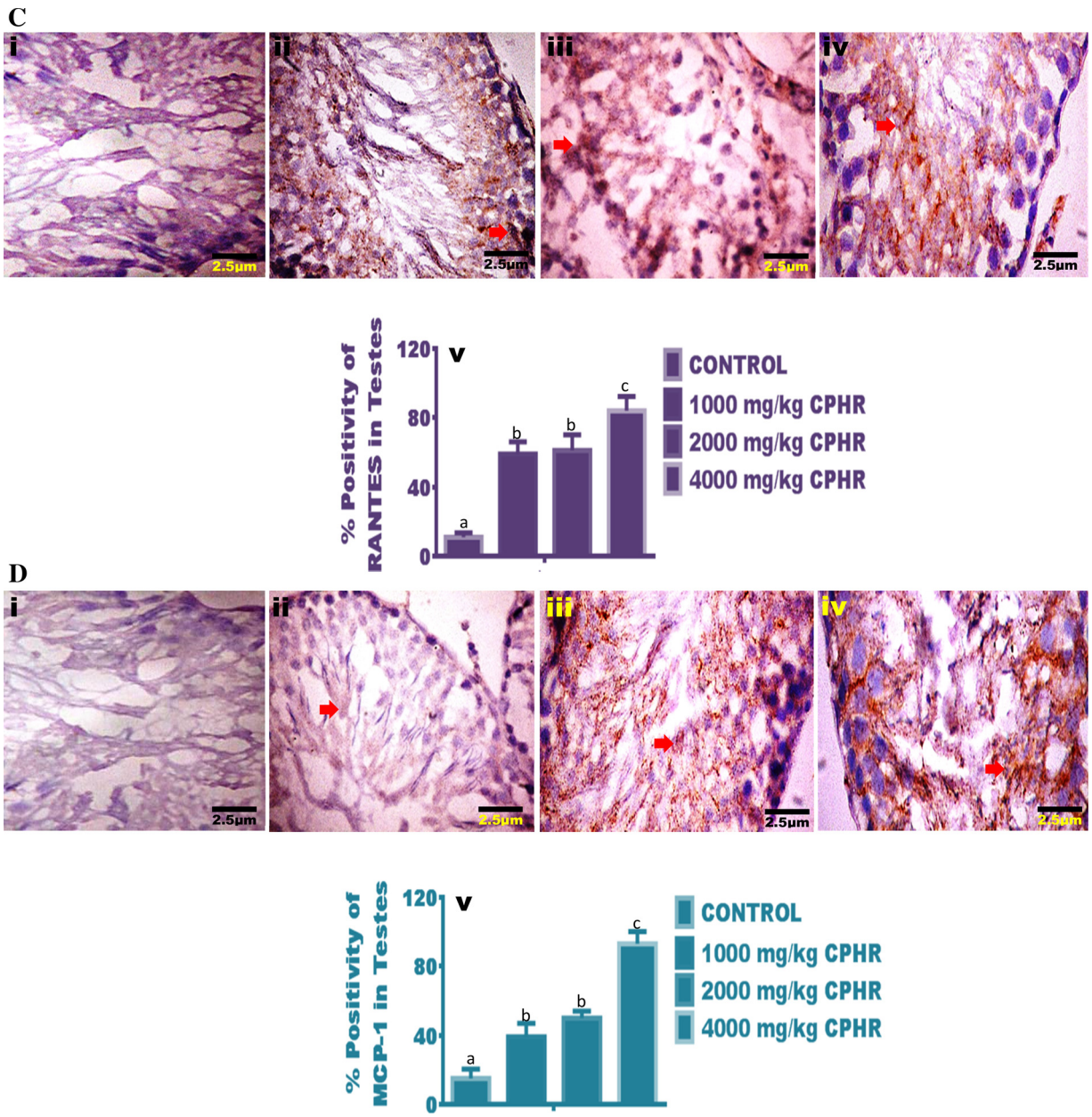

Fig. 7. (*continued*)

arsenic also led to the up-regulation of IFN-γ in hippocampal lysate of rats [45]. Furthermore, levels of TNF-α and IL-6 were significantly increased following combined administration of carboplatin and thalidomide in kidney and brain of rats [46].

NF-kB is crucial in coordinating cellular responses because it is one of the members of swift-acting primary transcription factors. These transcription factors are already localized in cells in an inactive state and will not require new protein synthesis to become active. This therefore, enables NF-kB to respond first to harmful cellular stimuli. Established activators of NF-kB activity include TNF-α, IL-1β, bacterial lipopolysaccharides (LPS), reactive oxygen species, ionizing radiation, cocaine, and isoproterenol [47]. A major function of NF-kB is seen in the regulation of inflammatory responses. Apart from mediating the induction of various pro-inflammatory genes in innate immune cells, NF-kB coordinates the activation, differentiation and effector function of inflammatory T cells [48,49]. It has been recently suggested that NF-kB also play a role in regulating the activation of inflammasomes [50]. Therefore, deregulated NF-kB activation is a hallmark of chronic inflammatory diseases [51]. The significant increase in the expressions of renal and testicular NF-kB following administration may be attributed to camphor-induced toxicity. The resulting injury or infection led to the recruitment of immune cells to the affected site. At the site of infection or injury, there is generation of pro-inflammatory cytokines including TNF-α, IL-1β, IL-6 that mediates the cytoplasmic NF-kB activation and its subsequent translocation to the nucleus where it further stimulates the transcription and expression of pro-inflammatory cytokines and other markers like COX-2, thereby exacerbating the inflammatory response.

Chemokines are produced in response to signals such as pro-inflammatory cytokines where they play a major role in selectively recruiting neutrophils, lymphocytes, and monocytes. RANTES (CCL5) belongs to the class of pro-inflammatory cytokines referred to as the C-C chemokine family [52]. RANTES protein is a chemoattractant for monocytes/macrophages [53], T lymphocytes, as well as eosinophils [54] and is secreted by monocytes, T lymphocytes, fibroblasts, and endothelial cells [55]. CCL2 (also referred to as MCP-1) on the other hand, is formed by diverse cell types, either constitutively or induction by cytokines, growth factors or oxidative stress [56]. It is produced by

numerous cell types, including epithelial, smooth muscle, mesangial, astrocytic, monocytic, endothelial, fibroblasts, and microglial cells [57–60]. These cells are necessary for antiviral immune responses in the peripheral circulation as well as in tissues. Macrophages/monocytes are the major source of CCL2 [61,62]. CCL2 regulates the migration and infiltration of memory T lymphocytes, natural killer (NK) cells, and monocytes [56]. Renal and testicular expressions of RANTES and CCL2 in this present study following camphor administration may be as a result of renal and testicular damage as well as NFkB activation which may have led to the expressions and recruitment of pro-inflammatory cytokines (TNF-α, IL-1β and IL-6) that in turn, coordinate the release of these pro-inflammatory chemokines (RANTES and CCL2) that selectively recruit immune cells to the site of tissue damage.

Expression of COX-2 has recently emerged as a key determinant of cytotoxicity associated with inflammation. COX, also referred to as prostaglandin H_2 synthase, is a regulatory enzyme for prostanoid biosynthesis, present in at least two isoforms, which are COX-1 and COX-2 [63]. COX-1 is constitutively expressed in numerous cell types producing prostanoids that sub-serve normal physiological functions [63]. Although COX-2 is normally not present in most cells, its expression is induced by cytokines and endotoxins [63]. COX-2 is greatly induced in inflamed tissues, and its products of reaction are responsible for the various cytotoxic effects of inflammation [64]. Increased in the renal and testicular COX-2 expressions recorded in this study can be attributed to camphor-induced inflammation as evident by increased pro-inflammatory cytokines reported in this study. This may be responsible for the increased expressions of COX-2 needed to synthesize prostaglandins from arachidonic acid, at the site of tissue damage or infection.

Camphor is seen in various non-prescription products, either in combination with other ingredients or alone. It can also be acquired, especially in shops that provide alternative medications, as solid blocks of pure camphor used in this study [65]. According to Food and Drug Administration (FDA), camphor-containing products cannot exceed 11% camphor [66]. However, incidences of chronic toxicity and convulsions are still reported to happen even with the lower concentration products that remain in the market. In addition to pharmaceutical products containing camphor, there are imported, non-FDA approved, ethnic remedies sold in the US that contain camphor in amounts greater than the 11% limit set by the FDA [66]. Camphor being a ketone has been traced in the brain, kidney, blood and other tissues, where it bioaccumulates [67]. Backed with the findings of this study, the use and consumption of camphor should be with caution as it could trigger renal and testicular inflammation through activation of NF-kB and up-regulation of pro-inflammatory markers.

Declaration of Competing Interest

None to declare.

References

[1] M. Azadbakht, Classification of Medicinal Plants, Taimourzadeh, Tehran, 1999.
[2] J. Guilbert, C. Flamant, F. Hallalel, D. Doummar, A. Frata, S. Renolleau, Anti-flatulence treatment and status epilepticus: a case of camphor intoxication, Emerg. Med. J. 24 (2007) 859–860.
[3] H. Osawa, I. Endo, Y. Hara, Y. Matsushima, T. Tange, Transient proliferation of proanthocyanidin-accumulating cells on the epidermal apex contributes to highly aluminum-resistant root elongation in camphor tree, Plant Physiol. 155 (2011) 433–446.
[4] R.C. Baselt, R.H. Cravey, Disposition of Toxic Drug and Chemicals and Drugs, 3rd edition, Year Book Medical Publishers Inc, 1990.
[5] D.D. Ho, C.P. Lau, K.H. Ng, Y.C. Kong, K.F. Cheng, K.P. Chan, Antiimplantation activity of S(-) and (+) camphor-yuehchukene in rats, Eur. J. Pharmacol. 205 (1991) 209–212.
[6] P.C. Ng, D.D. Ho, K.H. Ng, Y.C. Kong, K.F. Cheng, G. Stone, Mixed estrogenic and antiestrogenic activities of yuehchukene a bis-indole alkaloid, Eur. J. Pharmacol. 264 (1994) 1–2.
[7] N. Chatterjie, G.J. Alexander, Anticonvulsant properties of spirohydantoins derived from optical isomers of camphor, Neurochem. Res. 11 (1986) 1669–1676.
[8] E.A. Laude, A.H. Morice, T.J. Grattan, The antitussive effects of menthol, camphor and cineole in conscious guinea pig, Pulm. Pharmacol. Ther. 7 (1994) 179–184.
[9] H. Tinwell, P.A. Lefevre, G.J. Moffat, A. Burns, J. Odum, T.D. Spurway, G. Orphanides, J. Ashby, Confirmation of uterotrophic activity of 9-(4-methylbenzylidine) camphor in the immature rat, Environ. Health Prospect. 110 (2002) 533–536.
[10] T.J. Park, H.K. Seo, B.J. Kang, K.T. Kim, Noncompetitive inhibition by camphor of nicotinic acetylcholine receptors, Biochem. Pharmacol. 61 (2000) 787–793.
[11] M. Schlumpf, B. Cotton, M. Conscience, V. Haller, B. Steinmann, W. Litchtensteiger, *In vitro* and *in vivo* estrogenicity of UV screens, Environ. Health Prospect. 109 (2001) 239–244.
[12] H. Holbech, U. Norum, B. Korsgaard, B. Poul, The chemical UV filter 3- benzylidene camphor causes an estrogenic effect in an in vivo assay, Pharmacol. Toxicol. 91 (2002) 204–208.
[13] S.O. Mueller, M. Kling, F.P. Arifin, A. Mecky, E. Duranti, J. Shields-Botella, R. Delansorne, T. Broschard, P.J. Kramer, Activation of estrogen receptor alpha and Erbeta by 4-methylbenzylidene-camphor in human and rat cells: comparison with phyto- and xenoestrogens, Toxicol. Lett. 142 (2003) 89–101.
[14] S. Shahabi, S.G.A. Jorsaraei, A.A. Moghadamnia, E. Zabihi, S.M. Aghajanpour, S.N.M. Kani, R. Pourbagher, S.A. Hosseini, M. Esmaili, A.A. Yoonesi, A. Zarghami, F. Alinezhad, Central effects of camphor on GnRH and sexual hormones in male rat, Int. J. Mol. Cell. Med. 1 (2012) 191–196.
[15] J.B. Leikin, F. Paloucek, Poisoning and Toxicology Hand Book, 3rd edition, Lexi-Comp, INC, Hudson, Ahio, 2002, pp. 316–317.
[16] J.R. Collins, G.H. Loew, Theoretical study of the product specificity in the hydroxylation of camphor, norcamphor, 5,5-difluorocamphor, and pericyclocamphanone by cytochrome P450, J. Biol. Chem. 263 (1988) 3164–3170.
[17] H. Boxenbaum, Interspecies pharmacokinetic scaling and the evolutionary-comparative paradigm, Drug Metab. Rev. 15 (1984) 1071–1121.
[18] S.C. Sweetman, Martindale: the Complete Drug Reference, 34th edition, The Pharmaceutical Press, London, 2005.
[19] J.A. Ruszkiewicz, A. Pinkas, B. Ferrer, T.V. Peres, A. Tsatsakis, M. Aschner, Neurotoxic effect of active ingredients in sunscreen products, a contemporary review, Toxicol. Rep. 4 (2017) 245–259.
[20] I. Jiménez-Díaz, J.M. Molina-Molina, A. Zafra-Gómez, O. Ballesteros, A. Navalón, M. Real, J.M. Sáenz, M.F. Fernández, N. Olea, Simultaneous determination of the UV-filters benzyl salicylate, phenyl salicylate, octyl salicylate, homosalate, 3-(4-methylbenzylidene) camphor and 3-benzylidene camphor in human placental tissue by LC-MS/MS. Assessment of their in vitro endocrine activity, J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 936 (2013) 80–87.
[21] M. Schlumpf, B. Cotton, M. Conscience, V. Haller, B. Steinmann, W. Lichtensteiger, In vitro and in vivo estrogenicity of UV screens, Environ. Health Perspect. 109 (2001) 239–244.
[22] M. Schlumpf, P. Schmid, S. Durrer, M. Conscience, K. Maerkel, M. Henseler, M. Gruetter, I. Herzog, S. Reolon, R. Ceccatelli, O. Faass, E. Stutz, H. Jarry, W. Wuttke, W. Lichtensteiger, Endocrine activity and developmental toxicity of cosmetic UV filters-an update, Toxicology 205 (2004) 113–122.
[23] R. Schreurs, P. Lanser, W. Seinen, B. Van Der Burg, Estrogenic activity of UV filters determined by an in vitro reporter gene assay and an in vivo transgenic zebrafish assay, Arch. Toxicol. 76 (2002) 257–261.
[24] C. Schmutzler, I. Hamann, P.J. Hofmann, G. Kovacs, L. Stemmler, B. Mentrup, L. Schomburg, P. Ambrugger, A. Grüters, D. Seidlova-Wuttke, H. Jarry, W. Wuttke, J. Köhrle, Endocrine active compounds affect thyrotropin and thyroid hormone levels in serum as well as endpoints of thyroid hormone action in liver heart and kidney, Toxicology 205 (2004) 95–102.
[25] O. Faass, M. Schlumpf, S. Reolon, M. Henseler, K. Maerkel, S. Durrer, W. Lichtensteiger, Female sexual behavior: estrous cycle and gene expression in sexually dimorphic brain regions after pre- and postnatal exposure to endocrine active UV filters, Neurotoxicology 30 (2009) 249–260.
[26] K. Maerkel, S. Durrer, M. Henseler, M. Schlumpf, W. Lichtensteiger, Sexually dimorphic gene regulation in brain as a target for endocrine disrupters: developmental exposure of rats to 4-methylbenzylidene camphor, Toxicol. Appl. Pharmacol. 218 (2007) 152–165.
[27] K. Maerkel, W. Lichtensteiger, S. Durrer, M. Conscience, M. Schlumpf, Sex- and region-specific alterations of progesterone receptor mRNA levels and estrogen sensitivity in rat brain following developmental exposure to the estrogenic UV filter 4-methylbenzylidene camphor, Environ. Toxicol. Pharmacol. 19 (2005) 761–765.
[28] M. Krause, A. Klit, M. Blomberg Jensen, T. Søeborg, H. Frederiksen, M. Schlumpf, W. Lichtensteiger, N.E. Skakkebaek, K.T. Drzewiecki, Sunscreens: are they beneficial for health? An overview of endocrine disrupting properties of UV-filters, Int. J. Androl. 35 (2012) 424–436.
[29] T. Søeborg, N.C. Ganderup, J.H. Kristensen, P. Bjerregaard, K.L. Pedersen, P. Bollen, S.H. Hansen, B. Halling-Sørensen, Distribution of the UV filter 3-benzylidene camphor in rat following topical application, J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 834 (2006) 117–121.
[30] M. Schlumpf, S. Durrer, O. Faass, C. Ehnes, M. Fuetsch, C. Gaille, M. Henseler, L. Hofkamp, K. Maerkel, S. Reolon, B. Timms, J.A. Tresguerres, W. Lichtensteiger, Developmental toxicity of UV filters and environmental exposure: a review, Int. J. Androl. 31 (2008) 144–151.
[31] O.T. Somade, K.D. Adeniji, A.A. Adesina, O.J. Olurinde, Oral acute toxicity study as well as tissues oxidative stress and histopathological disorders in edible camphor administered rats, Exp. Toxicol. Pathol. 69 (2017) 99–108.
[32] C.A. Feghali, T.M. Wright, Cytokines in acute and chronic inflammation, Front. Biosci. 2 (1997) 12–26.
[33] NRC, Guide for the Care and Use of Laboratory Animals, National Academy Press, Washington, DC, 1996.

[34] B.O. Ajayi, I.A. Adedara, E.O. Farombi, Benzo(a)pyrene induces oxidative stress, pro-inflammatory cytokines, expression of nuclear factor-kappa B and deregulation of wnt/beta-catenin signaling in colons of BALB/c mice, Food Chem. Toxicol. 95 (2016) 42–51.
[35] K. Gyoubu, M. Miyazawa, In vitro metabolism of (-)-camphor using human liver microsomes and CYP2A6, Biol. Pharm. Bull. 30 (2) (2007) 230–233.
[36] J.M. Zhang, J. An, Cytokines, inflammation and pain, Int. Anesthesiol. Clin. 45 (2) (2007) 27–37.
[37] O.T. Somade, R.N. Ugbaja, A.A. Adebayo, Effect of edible camphor administrations on levels of steroid and thyroid hormones in male wistar rats, Am. J. Res. Med. Sci. 1 (1) (2017) 27–34.
[38] G. Ramesh, W.B. Reeves, TNF-α mediates chemokine and cytokine expression and renal injury in cisplatin nephrotoxicity, J. Clin. Invest. 110 (2002) 835–842.
[39] A.C. Santana, S. Degaspari, S. Catanozi, H. Delle, L. de Sa Lima, C. Silva, P. Blanco, K. Solez, C. Scavone, I.L. Noronha, Thalidomide suppresses inflammation in adenine-induced CKD with uraemia in mice, Nephrol. Dial. Transplant. (2013) 1–9.
[40] J.J. Lysiak, Q.A.T. Nguyen, J.L. Kirby, T.T. Turner, Ischemia-reperfusion of the murine testis stimulates the expression of proinflammatory cytokines and activation of c-jun N-terminal kinase in a pathway to E-selectin expression, Biol. Reprod. 69 (2003) 202–210.
[41] K. Cseh, B. Beutler, Alternative cleavage of the cachetin/tumor necrosis factor propeptide results in a larger inactive form of secreted protein, J. Biol. Chem. 264 (1989) 16256–16260.
[42] J.J. Lysiak, The role of tumor necrosis factor-alpha and interleukin-1 in the mammalian testis and their involvement in testicular torsion and autoimmune orchitis, Reprod. Biol. Endocrinol. 2 (2004) 9.
[43] T. Yacoub, M. Rima, R. Sadek, W. Hleihel, Z. Fajloun, M. Karam, *Montivipera bornmuelleri* venom has immunomodulatory effects mainly up-regulating pro-inflammatory cytokines in the spleens of mice, Toxicol. Rep. 5 (2018) 318–323.
[44] S.E. Owumi, U.J. Dim, Manganese suppresses oxidative stress, inflammation and caspase-3 activation in rats exposed to chlorpyrifos, Toxicol. Rep. 6 (2019) 202–209.
[45] F. Firdaus, M.F. Zafeer, E. Anis, M. Ahmad, M. Afzal, Ellagic acid attenuates arsenic induced neuro-inflammation and mitochondrial dysfunction associated apoptosis, Toxicol. Rep. 5 (2018) 411–417.
[46] M.I. Yousef, D.K.A.M. Khalil, H.M. Abdou, Neuro- and nephroprotective effect of grape proanthocyanidin extract against carboplatin and thalidomide through modulation of inflammation, tumor suppressor protein p53, neurotransmitters, oxidative stress and histology, Toxicol. Rep. 5 (2018) 568–578.
[47] N.S. Chandel, W.C. Trzyna, D.S. McClintock, P.T. Schumacker, Role of oxidants in NF-kappa B activation and TNF-alpha gene transcription induced by hypoxia and endotoxin, J. Immunol. 165 (2) (2000) 1013–1021.
[48] T. Lawrence, The nuclear factor NF-kappaB pathway in inflammation, cold spring Harb, Perspect. Biol. Med. 1 (2009) a001651.
[49] P.P. Tak, G.S. Firestein, NF-kappaB: a key role in inflammatory diseases, J. Clin. Invest. 107 (2001) 7–11.
[50] F.S. Sutterwala, S. Haasken, S.L. Cassel, Mechanism of NLRP3 inflammasome activation, Ann. N. Y. Acad. Sci. 1319 (2014) 82–95.
[51] T. Liu, L. Zhang, D. Joo, S.C. Sun, NF-κB signaling in inflammation, Signal Transduct. Target Ther. 2 (2017) e17023, , https://doi.org/10.1038/sigtrans.2017.23.
[52] P.J. Nelson, H.T. Kim, W.C. Manning, T.J. Goralski, A.M. Krensky, Genomic organization and transcriptional regulation of the RANTES chemokine gene, J. Immunol. 151 (1993) 2601–2612.
[53] T.J. Schall, K. Bacon, K.J. Toy, D.V. Goedell, Selective attraction of monocytes and T lymphocytes of the memory phenotype by cytokine RANTES, Nature 347 (1990) 669–671.
[54] Y. Kameyoshi, A.I. Dorshner, A.I. Mallet, E. Chrisophers, J.M. Schroder, Cytokine RANTES released by thrombin-stimulated platelets is a potent chemoattractant for human eosinophils, J. Exp. Med. 176 (1992) 587–592.
[55] T.J. Schall, Biology of RANTES/SIS cytokine family, Cytokine 3 (1991) 165–183.
[56] S.L. Deshmane, S. Kremlev, S. Amini, B.E. Sawaya, Monocyte chemoattractant Protein-1 (MCP-1): an overview, J. Interferon Cytokine Res. 29 (6) (2009) 313–326.
[57] S.D. Cushing, J.A. Berliner, A.J. Valente, M.C. Territo, M. Navab, F. Parhami, R. Gerrity, C.J. Schwartz, A.M. Fogelman, Minimally modified low density lipoprotein induces monocyte chemotactic protein1 in human endothelial cells and smooth muscle cells, Proc. Natl. Acad. Sci. U. S. A. 87 (1990) 5134–5138.
[58] T.J. Standiford, S.L. Kunkel, S.H. Phan, B.J. Rollins, R.M. Strieter, Alveolar macrophage-derived cytokines induce monocyte chemoattractant protein-1 expression from human pulmonary type II-like epithelial cells, J. Biol. Chem. 266 (1991) 9912–9918.
[59] Z. Brown, R.M. Strieter, G.H. Neild, R.C. Thompson, S.L. Kunkel, J. Westwick, IL-1 receptor antagonist inhibits monocyte chemotactic peptide1 generation by human mesangial cells, Kidney Int. 42 (1992) 95–101.
[60] B.P. Barna, J. Pettay, G.H. Barnett, P. Zhou, K. Iwasaki, M.L. Estes, Regulation of monocyte chemoattractant protein-1 expression in adult human non-neoplastic astrocytes is sensitive to tumor necrosis factor (TNF) or antibody to the 55-kDa TNF receptor, J. Neuroimmunol. 50 (1994) 101–107.
[61] T. Yoshimura, E.A. Robinson, S. Tanaka, E. Appella, E.J. Leonard, Purification and amino acid analysis of two human monocyte chemoattractants produced by phytohemagglutinin-stimulated human blood mononuclear leukocytes, J. Immunol. 142 (1989) 1956–1962.
[62] T. Yoshimura, N. Yuhki, S.K. Moore, E. Appella, M.I. Lerman, E.J. Leonard, Human monocyte chemoattractant protein-1 (MCP-1). Full-length cDNA cloning, expression in mitogen-stimulated blood mononuclear leukocytes, and sequence similarity to mouse competence gene JE, FEBS Lett. 244 (1989) 487–493.
[63] W.L. Smith, D.L. DeWitt, Biochemistry of prostaglandin endoperoxide H synthase-1 and synthase-2 and their differential susceptibility to nonsteroidal anti-inflammatory drugs, Semin. Nephrol. 15 (1995) 179–1194.
[64] K. Seibert, J. Masferrer, Y. Zhang, S. Gregory, G. Olson, S. Hauser, K. Leahy, W. Perkins, P. Isakson, Mediation of inflammation by cyclooxygenase-2, Agents Actions Suppl. 46 (1995) 41–50.
[65] A.S. Manoguerra, A.R. Erdman, P.M. Wax, L.S. Nelson, E.M. Caravati, D.J. Cobaugh, P.A. Chyka, K.R. Olson, L.L. Booze, A.D. Woolf, D.C. Keyes, G. Christianson, E.J. Scharman, W.G. Troutman, Camphor poisoning: an evidenced-based practice guideline for out-of-hospital management, Clin. Toxicol. 44 (2006) 357–370.
[66] C. Verano, Camphor poisoning, drug intell, Clin. Pharm. 11 (1977) 110.
[67] O.T. Somade, D.M. Ogunberu, T.T. Fakayode, A.O. Animashaun, Edible camphor-induced histopathological changes in hippocampus and cerebral cortex following oral administration into rats, J. Interdisc. Histopath. 5 (1) (2017) 7–11.