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The Effects of Orally Administered Cinnamomum Camphora on Histoarchitecture, Antioxidant Defense, and Function Test of Kidneys in Wistar Rats

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ABSTRACT

Background: Camphor is an herbal medicine that has been reported to have various physiological effects. It has been reported to affect the respiratory system, circulatory system, skin, reproductive system, liver, and kidneys.

Objectives: This study is to determine the effect of orally administered Cinnamomum camphora on the histoarchitecture, antioxidant defense and renal function tests using Wistar rats as the experimental models.

Methods: A total of 20 Wistar rats were used and randomly divided into 4 groups of 5 animals, group A served as Control, received 1 ml of Distilled water; group B served as the Vehicle, received 1 ml of olive oil, Groups C and D received 200 and 400 mg/kg of Camphora (dissolved in olive oil) respectively for 4 weeks. Blood samples were collected for kidney function tests (urea, creatinine and albumin levels) and kidneys were harvested for histological (H&E) and oxidative stress analyses.

Results: There was a significant increase in urea, creatinine and albumin levels in groups B, C and D when compared to the control. There was a significant decrease in SOD and CAT when treatment groups were compared to control, however, significant increase was noted when treatment groups were compared to control in MDA levels. Histopathological studies showed moderate vascular congestion in the treatment group.

Conclusion: In conclusion, this study revealed that camphora had toxic effects on the anti-oxidative defense, renal functions and kidney histomorphology.

Keywords: Camphora, kidney, Olive oil, oxidative stress, renal functions.

Introduction

Camphora is a waxy, flammable, transparent solid with a strong aroma. It is a terpenoid found in the wood of the camphor laurel, *Cinnamomum camphora* [1]. It is naturally occurring in Asian countries including Japan, Taiwan and China, but has also been naturalized in other parts of the world; and also, of the unrelated kapur tree, a tall timber tree from the same region [2]. Basically, there are two types of camphors; one is chemically and synthetically manufactured camphor is known as karpura which is burnt during puja ceremonies, religious rituals in temples in India due to aromatic smell. Camphoric fumes are non-irritating to eyes. The second camphor is natural herbal camphor extracted from the camphor tree *Cinnamomum camphora* and *Cinnamomum agasthyamalayanum* used as an herbal medicine for many diseases [3].

Camphor is associated with a long-valued history for it being used diversely and extensively in the East: it is being used as a circulatory stimulant and analeptic in China, whilst the Japanese used it to add small quantities to fireworks to make them brighter, and also as a component of torch-light material [4]. Camphor is familiar to many people as a principal ingredient in topical home remedies for a wide range of symptoms, and its use is well consolidated among the population of the whole world, having a long tradition of use as antiseptic, antipruritic, rubefacient, abortifacient, aphrodisiac, contraceptive and lactation suppressant [5].

Camphora can be administered through inhalation, ingestion or dermal routes. Following exposure, metabolism of camphora is mediated by cytochrome P450, a class of heme-containing mono-oxygenases widely distributed in humans and animals' cells. The resulting hydroxylated metabolites of camphora following cytochrome P450 action are conjugated with glucuronic acid and excreted in the urine [6]. It is an herbal medicine that has many various physiological effects. It affects the respiratory system, circulatory system, skin, reproductive system, liver, and kidney. 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 [7]. Camphor is very toxic in nature and its toxicity has been reviewed and well documented. Camphor occurs in nature in its dextrorotatory form (D-camphor), while the levorotatory form (L-camphor) exists only as a synthetic form. The oral consumption of higher concentrations of 3.5 g of camphor can cause death. According to Ravindra et al., Further consumption of 2.0 g of camphor causes toxic effects in adults leading to congestion of the gastrointestinal tract, kidney and brain. In humans, the characteristic symptoms of camphor poisoning after ingestion are nausea, vomiting, headache, dizziness, muscular excitability causing tremor and twitching, convulsions and delirium depending on the dosage. In a severe overdose for several hours, causing coma and death by asphyxia or exhaustion. Camphor can also cause skin and eye irritation on contact. In 2007, according to Enaibe, et al. [8], kidney of rabbits administered various doses of camphora revealed mild edema with glomerulonephritis, tubular necrosis, glomerular lobulations, and congestion of the blood cells, suggesting a cytotoxic effect on the organ. The kidney is an essential organ required by the body to perform several important

functions including the maintenance of homeostasis, regulation of the extracellular environment, such as detoxification, and excretion of toxic metabolites and drugs. Therefore, the kidney can be described as the target organ for exogenous toxicants.

When the kidney becomes damaged, it loses its ability to function and over time may not filter blood the way they should. If kidneys do not work well, toxic waste and fluid accumulate in the body, which may lead to high blood pressure, heart disease, stroke and eventually death [9]. In most species, death occurs within a week after total cessation of renal function. Partial loss of renal function results in variable deviations from normal, depending on the quantity of functional tissue remaining. The term "azotemia" refers to accumulation of nitrogenous wastes in the blood. Blood concentrations of creatinine and urea are measured as indices of azotemia, although neither imparts significant toxicity because of its accumulation [10]. Nephrotoxicity is the adverse effect of substances on the renal function [11]. These substances can include molds and fungi, cancer therapeutics, metals and drugs of abuse. One of the indications of nephrotoxicity is a change in renal function as assessed by the glomerular filtration rate (GFR) blood urea nitrogen (BUN), serum creatinine (sCr), or urine output; however, nephrotoxicity can induce kidney damage without changing any established clinical marker of renal function [12]. Approximately 20% of nephrotoxicity is induced by drugs, but medicated of the elderly increases the incidence of nephrotoxicity up to 66% as the average life span increases. Chemotherapy or anticancer medicine has been of limited use due to nephrotoxicity [13].

Materials and Methods

Materials Used

The materials and reagent used for this project research includes: Plastic industrial cages, beakers, orogastric tube (cannula), syringes, specimen bottles, sensitive weighing balance, dissecting sets, dissecting board, olive oil, 10% formal-saline, edible camphora tablets, cotton wool, disposable gloves, methylated spirit, food plates, water bowl and wood shavings.

Experimental Design

Animal Care

A total of twenty healthy female Wistar rats weighing between 150±20g were used for this study. They were housed in a standard well-ventilated wire mesh plastic cage in the animal house of the Department of Anatomy, College of Health Sciences of Bowen university, Iwo under standard room temperature. The animals were exposed to twelve hours light and twelve hours dark cycle and were left to acclimatize for a period of two weeks before the commencement of the research project. The rats were fed with standard rat feed and water, with their cages being cleared and cleaned regularly. Wood shavings were used as beddings for the animals to help absorb any moist and also for comfort.

Ethical Approval

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85- 23, revised 1985) were followed, as well as specific national laws were applicable. All experiments

have been examined and approved by the appropriate ethics committee in Bowen University (BEC-02/24-11).

Animal Grouping

The twenty Wistar rats were grouped into four labeled Group A, B, C and D. Group A served as Control, received 1 ml of Distilled water (DW), group B served as Vehicle, received 1 ml of Olive oil, Groups C (low dose) and D (high dose) received 200 and 400 mg/kg of Camphora (dissolved in olive oil) respectively.

Duration of Experiment

The rats were acclimatized for two weeks (14 days), after which administration of camphora to the animals for 4 weeks was carried out.

Animal Sacrifice

After the completion of the study, the rats were sacrificed 24 hours after the last day of treatment. The kidneys were then harvested for both histological (preserved in 10% formal-saline) and Oxidative stress analyses, 2 mls of blood was collected via the ocular sinus, centrifuged and the sera were collected and kept in a -80°C freezer for kidney function test.

Statistical Analysis

Data were analyzed with GraphPad prism 9.5 software using one-way analysis of variance for multiple comparison between groups at $p < 0.05$. Data were expressed as Mean \pm SEM. Significance difference between the control and the experimental groups were determined by the 'T' test and values of $p < 0.05$.

Biochemical Analysis

Blood samples from the rats were analyzed for the following enzymes and reaction products: Urea, Creatinine and Albumin tests.

Determination of Creatinine concentration

The creatinine concentration in serum was measured using Creatinine K commercial kits (Labtest Diagnostica SA, Lagoa Santa, Brazil), which uses a two-point optimized kinetic procedure based on the modified-Jaffe reaction. For disposing purposes, 50 μ L of the serum sample was added to 50 μ L of alkaline picrate, mixed and aspirated into the automatic analyser bucket set to zero at 510 nm, and then measured the absorbance at 30 seconds. The results were expressed in μ mol/L.

Determination of Urea concentration

The urea concentration in serum was measured using Liquiform Urea UV test (Labtest Diagnostica SA, Lagoa Santa, Brazil) which uses an enzymatic system by UV photometry and two-point Kinetics. For disposing purposes, 10 μ L of the serum were aspirated into the photometer reservoir adjusted to 340 nm, and then measured the absorbance at 30 seconds. The results were calculated and expressed in mmol/L.

Determination of Albumin concentration

The albumin concentration was measured by spectrophotometry

(412 nm) using DTNB (5,5'-dithiobis 2-nitrobenzoic acid) and the contents were calculated using the molar extinction coefficient (ϵ) of $1.36 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$.

Histological Processing

The histological tissue processing procedures were followed for processing the kidney specimen obtained from the rats. The specimens were processed into slides showing the structure of the kidney. The steps include: Fixation, Dehydration, Clearing, Infiltration, Embedding, Sectioning, Mounting, and Staining. Stock solutions of Hematoxylin and Eosin were purchased. The hematoxylin stain used was the Mayer's hematoxylin solution, which is composed of: Certified hematoxylin (1.0g/l), Sodium iodate (0.2g/l), Aluminium ammonium sulphate. 12h20 (50g/l), Chloral hydrate (50g/l), Citric acid. The eosin stain contained: Eosin (0.1mg), Phloxine B (0.01mg), 95% alcohol (78ml), Glacial acetic acid (0.4ml).

The following steps were carried out: Tissues were dewaxed in Xylene for 15 minutes and rehydrated sections were stained in hematoxylin solution for 20-40 minutes. Excess stain was removed with the running tap water, then tissue was blued (bluing) with ammonium hydroxide, tissue was then washed for 1-5 minutes in tap water. Differentiation of the tissues was done with acid alcohol (1% concentrated HCL in 70% ethanol) and tissues were blued under running tap water for 10 minutes. Staining in eosin solution for 10 minutes was done and tissues were washed for 1-5 minutes in tap water, dehydration was then carried out in ascending grades of alcohol and finally a cover slip was applied on the slide. Using this staining procedure, nuclear chromatin was stained blue and nucleoli were visible. Cytoplasm displayed various shades of pink to pinkish orange. Photographs were obtained and properly recorded using a photomicrograph.

Results

Running nose and were seldom undergoing seizures after administration. Death of two rats was recorded in the High-dose group; that occurred after a prolonged rigorous seizure.

Body Weight Analysis

Effects of Administration of Camphora on Body Weight (g) of Wistar Rats

There was a significant decrease in body weights of low and high dose groups when pre- and post-administration body weights were compared, but significant increase was seen in body weights of vehicle group and this is similar to what was observed for control animals. The percentage weight difference increased in low dose and high dose when compared to control group. Percentage weight difference of vehicle animals decreased when compared to control group. Treatment groups when compared to control group showed significant increase, there was significant increase in body weight when low and high dose group was compared to vehicle group. High dose showed significant increase in body weight when compared to low dose (Table 1).

Table 1: Effects of Administration of Cinnamomum camphora on Body Weight (g) of Wistar Rats.

Groups	Before (Pre) Administration	After (Post) Administration	% Weight Difference
Control	144.70 ± 2.51	174.30 ± 2.42*	20.46%
Vehicle	158.20 ± 2.01	184.80 ± 5.53*	16.81%
Low Dose	279.20 ± 2.20	185.80 ± 5.99*	33.45%
High Dose	298.40 ± 5.49	186.30 ± 3.97*	37.58%

Values are mean ± standard error of mean; n=5, *p<0.05 compared to control.

Oxidative Stress Analysis
Effects of Administration of Camphora on Renal Oxidative Stress Markers

There was a significant reduction in the level of SOD when low dose, high dose and vehicle groups were compared to the Control group. A significant reduction was seen when low dose and high dose were compared to vehicle group. High dose when compared to low dose shows a significant decrease. A significant increase in the level of MDA was recorded when low dose and high dose groups were compared to Control group, with vehicle group showing no significant difference in MDA level when compared to control group. When low and high dose groups were compared to Vehicle group a significant increase was observed. High dose when compared to low dose shows a significant increase in MDA levels. Vehicle group showed decrease with no significance in the level of catalase CAT when compared to control group, but low dose and high dose showed significant decrease when compared to control group. CAT level in low and high dose group showed significant decrease when compared to vehicle group, while CAT level in high dose group reported a significant decrease when compared to low dose (Table 2).

Table 2: Effects of Administration of Cinnamomum camphora on Renal Oxidative Stress Markers of Wistar Rats.

Groups	MDA (Mmol/g)	SOD (Mmol/g)	CAT (Mmol/g)
Control	432.10 ± 2.70	125.70± 1.41	319.70± 4.18
Vehicle	432.10± 4.95	119.70± 0.31a	309.10± 1.60
Low Dose	461.40± 1.59ab	106.80± 0.91ab	294.70± 0.83ab
High Dose	527.80± 0.78abc	97.92± 0.64abc	227.00± 2.89abc

Values are expressed as Mean ± Standard Error of Mean (SEM). ap<0.05 significant compared to control; bp<0.05 significant compared to vehicle and cp<0.05 significant compared to low dose.

Biochemical Analysis Result
Effects of Administration of Camphora on Biochemical Analysis

For Creatinine test, a statistically significant increase was seen in treatment groups when compared to control group, low dose and high dose increased significantly when compared to vehicle

group. Significant increase was also seen in high dose when it was compared to low dose.

For Urea test, significant increase was seen in treatment groups when they were compared to animals in control group. Low and high dose increased significantly when compared to vehicle groups and high increased significantly when compared to low dose.

For Albumin test, vehicle group showed significance increase when compared to animals in control group. High and low dose group showed significant increase when compared to control group. Low dose and high dose were increased significantly when compared to vehicle group and high dose also significantly increased when it was compared to animals in low dose group (Table 3).

Table 3: Effects of Administration of Camphora on Biochemical Analysis (Creatinine, Urea, and Albumin Test).

Groups	Creatinine	Urea	Albumin
Control	1.16± 0.09	50.69± 0.23	1.76± 0.03
Vehicle	1.86± 0.06a	52.69± 0.36a	2.08± 0.04a
Low Dose	3.14± 0.10ab	73.72± 0.33ab	8.65± 0.20ab
High Dose	7.27± 0.03abc	92.99± 0.40abc	15.71± 0.22abc

Values are expressed as Mean ± Standard Error of Mean (SEM). ap<0.05 significant compared to control; bp<0.05 significant compared to vehicle and cp<0.05 significant compared to low dose.

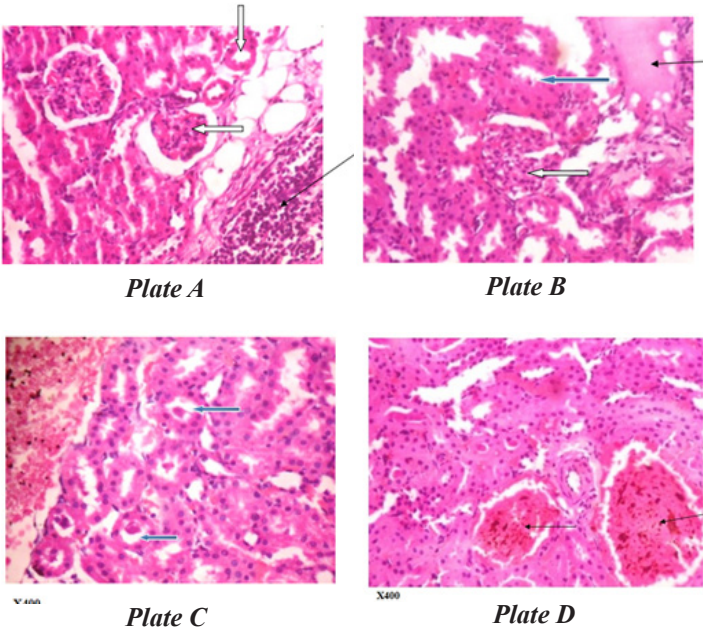


Plate A (control group): Section show normal architecture, normal glomeruli with normal mesangial cells and capsular space, the renal tubules appear normal.
Plate B (vehicle group): Section show normal glomeruli with normal mesangial cells and capsular spaces.
Plate C (Low dose): Section show poor architecture, and the renal tubules show presence of renal cast.
Plate D (High dose): Sections show that the interstitial spaces show moderate vascular congestion (slender arrow).

Discussion

This study is aimed at investigating the possible effects of camphora on the kidney, which focuses on the weight, oxidative stress marker, biochemical and also the histological study of camphora on the kidney. There was a significant decrease in body weights of low and high dose groups when pre and post-administration body weights were compared, but significant increase was seen in body weights of vehicle group and this is similar to what was observed for control animals. The percentage weight difference increased in low dose and high dose when compared to control group. Percentage weight difference of vehicle animals decreased when compared to control group. In pre-administration of camphora, treatment groups when compared to control group showed significant increase, there was significant increase in body weight when low and high dose group was compared to vehicle group. High dose showed significant increase in body weight when compared to low dose.

In the oxidative stress study, there was a significant reduction in the level of SOD when low dose, high dose and vehicle groups were compared to the Control group. A significant reduction was seen when low dose and high dose were compared to vehicle group. High dose when compared to low dose shows a significant decrease. A significant increase in the level of MDA was recorded when low dose and high dose groups were compared to Control group, with vehicle group showing no significance when compared to control group. When low and high dose groups were compared to Vehicle group a significant increase was seen. High dose when compared to low dose reports a significant increase. Vehicle group showed decrease with no significance in the level of catalase CAT when compared to control group, but low dose and high dose showed significant decrease when compared to control group. CAT level in low and high dose group showed significant decrease when compared to vehicle group, while CAT level in high dose group reported a significant decrease when compared to low dose. Fawzia et al. [14] gave a similar report of reduction in SOD level as a result of administration of Camphora.

In the biochemical study, there was a significant increase in the creatinine level in the animals in group D (High dose) compared to the animals in control group ($p < 0.05$). Animals in group C (Low dose) and group B (vehicle) showed significant increase when compared to control group ($p < 0.05$). Low dose and high dose group reported significant increase when compared to vehicle group and high dose increased significantly when it was compared to animals in high dose

There was a significant increase in the urea level in the animals in group D (High dose) compared to the animals in the control group ($p < 0.05$), animals in group B (vehicle) and group C (Low dose) also showed significant increase when compared to the control group ($p < 0.05$). Low dose and high dose were increased significantly when compared to vehicle group. High dose group increased significantly when compared to low dose group.

Albumin level in the animals in group D when compared to the

animals in control group ($p < 0.05$) was statistically significant, animals in group C (Low dose) showed significant increase when it was compared to control group ($p < 0.05$), albumin level in group B (Vehicle) when compared to control group showed significant increase. Low dose and high dose were compared to vehicle group gave a significant increase result. High dose increased significantly when compared to low dose.

In the histological study, using hematoxylin and eosin stain, group A (control group) shows normal glomeruli with normal mesengial cells and capsular spaces, the renal tubules appear normal; the interstitial spaces show area of inflammation. In group B (Vehicle), the renal cortex shows normal glomeruli with normal mesengial cells and capsular spaces, the renal tubules appear collapsed; the interstitial spaces show mild vascular congestion. Group C (Low dose) shows poor architecture, the renal cortex shows normal glomeruli with normal mesengial cells and capsular spaces, the renal tubules show presence of renal cast, the interstitial spaces show moderate vascular congestion. Group D (High dose) the renal cortex shows normal glomeruli with normal mesengial cells and capsular spaces, the renal tubules appear normal, the interstitial spaces show moderate vascular congestion.

This is in accordance with Enaibe et al., who worked on the toxicological effects of camphor administration on the histology of the kidney of Rabbit (*Oryctolagus cuniculus*), which revealed that administration of camphor solution caused varying degree of congestion of blood cells in the renal parenchyma and tubular necrosis.

Conclusion

In conclusion, this study revealed that camphora has adverse effects on the kidney, with degenerative changes seen also, in its histomorphological studies. However, there was no significant adverse effect observed in the kidneys of rats administered with olive oil, except for the slight changes observed in the renal tubules and interstitial spaces which appear collapsed and showed a mild vascular congestion.

Contribution of Authors

- » Adebajo AO was responsible for the statistical analysis and initial draft of the manuscript.
- » Ayoade OH was responsible for the conceptualization of the work.
- » Ojo JH was responsible for the benchwork and initial draft.
- » Oladipo JO and AHB were responsible for the analysis of the biochemical parameters.
- » Ajayi AJ was responsible for the final draft and benchwork
- » Adejumo F was responsible for the initial draft, benchwork and final draft.

Conflict of Interest

The authors declare that they have no financial or personal conflicts of interest to disclose.

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