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Cytotoxic and genotoxic activities of the aqueous extract of refined camphor

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ABSTRACT

Background: Medicinally, refined camphor commonly known as "kafura pelebe" in south western Nigeria is used in various parts of the world for managing various ailments. It is used as circulatory stimulant, analeptic and for managing gastrointestinal disturbances. A survey of colorectal carcinoma patients in a Nigerian teaching hospital revealed that half of the patients had a history of ingesting refined camphor. The study was carried out to determine the cytotoxic and genotoxic potential of the commonly used preparation, aqueous extract of refined camphor (AERC).

Methods: This was done using brine shrimp lethality test (at 5, 50, 500 µg/ml of AERC) and Allium cepa chromosomal aberration assays (at 2, 100, 200, 1000 and 2000 µg/ml of AERC) as well as micronucleus and comet assays (at 1.77, 8.83 and 44.13 mg/kg of AERC) in mice. An LC50 of 1017.65 µg/ml was estimated for the brine shrimp lethality assay using probit analysis

Results: Significant ($p<0.0001$) reduction of A. cepa root length and root tip cells' mitotic index as well as various forms of chromosomal aberrations including vagrant, bridged, and laggard types were observed at 200 - 2000 µg/ml of AERC. In the same vein, micronuclei polychromatic erythrocytes increased in the micronucleus assay.

Conclusion: The results of this study indicate that the aqueous extract of refined camphor is cytotoxic and genotoxic; its risk to benefit ratio needs to be properly evaluated before its use is encouraged going forward.

***Key words*— A. cepa, camphor, cytotoxicity, genotoxicity, micronucleus mitotic index,**

1. INTRODUCTION

Medicinal refined camphor is believed to be derived from the wood of camphor laurel tree (Cinnamomum camphora) or camphor tree through steam distillation and purification by sublimation [1]. However, synthetic forms made from turpentine oil are available nowadays and other aromatic plants have been reported to produce similar substance [2]. It may be helpful to identify the accurate source of the various camphor used in traditional medicine. Generally, in most places where they are used medicinally (including Nigeria), refined camphor occurs as colourless crystals or in transparent fibrous blocks with characteristic scent and pungent aromatic taste usually followed by a cold sensation. These crystals may volatilize at room temperature forming encrustations on the walls of the vessel in which they are stored [3]. Refined camphor belongs to the terpenoids group of phytochemicals and are reported to be extremely useful in traditional medicine. It is commonly used in cream, ointment, and lotion formulations [3]. It has been used as antiseptic, aphrodisiac, culinary spice, component of incense and cold remedy. Camphor is known to modulate sexual activity, contraception, induce abortion, and reduce milk production in lactating women according to Sikka and Bartolome [1]. They also reported that camphor-containing compounds have uterotrophic, antitussive, anti-convulsant, antiestrogenic activities and nicotinic receptor blocking activities [4]. It is also used topically to relieve pain, irritation and itching as well as to relieve chest congestion and inflammatory conditions [5]. Regarding its potential to induce adverse effects, camphor reduces human sperm motility and viability resulting in a contraceptive effect [6]. It crosses the placental barrier and could negatively impact embryo development [1] and is contraindicated in pregnant women. A previous study revealed sister chromatid exchange in mice exposed to 80 mg/kg of camphor, indicating its potential to induce DNA damage [7]. Yu et al. [8] reported association between recent (three years before) use of camphor and observable clinical symptoms of nasopharyngeal carcinoma. Application of a Chinese nasal oil,

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which contains camphor and menthol, has been indicated as a risk factor for nasopharyngeal carcinoma development [9]. In Nigeria, refined fibrous blocks of medicinal camphor is soaked in water and the extract ingested for various disorders especially in the South-western region. These fibrous blocks are also usually added to aqueous herbal preparations to serve preservative functions. Several health benefits of this aqueous extract have been reported by its users. However, given the association of camphor itself with genotoxicity, neoplastic disorders and its unregulated dosing regimen among its users, it is necessary to determine the potential for health risks to users of the aqueous extract preparation. To the best of our knowledge, there is yet to be any evidence-based report on its genotoxic or cytotoxic potential, hence this study to determine the cytotoxic and genotoxic potential of the aqueous extract of refined camphor (AERC).

2. MATERIALS AND METHODS

2.1 Materials

2.1.1 Chemicals and reagents

Dimethylsulfoxide (DMSO), camphor (Asia camphor manufacturing company, China), cyclophosphamide, disodium EDTA, ethidium bromide, histopaque, phosphate buffered saline (PBS) (Ca++, Mg++ free), sodium chloride (NaCl), cyclophosphamide, sodium hydroxide (NaOH), triton X-100, trizma base, normal melting agarose, cytochalasin B, acridine orange, hydrochloride hydrate, potassium chloride, acetic acid, phosphate buffered saline, low melting point agarose, methanol. All the reagents used were of analytical grade.

2.1.2 Camphor procurement and extract preparation

Packs of refined ingestible camphor (Asia camphor manufacturing company, China) used in traditional medicine were purchased from the traditional medicine dealers in Ojuwoye market in Mushin, Lagos State, Nigeria. The extract was prepared in line with its mode of preparation among local users. Thirty (30) g of the refined camphor blocks was soaked in 750 ml of distilled water for 7 days, after which the mixture was decanted leaving the insoluble residue. The difference between the initial and final weight of camphor (insoluble residue) was used to determine the % yield of the extract, which was then refrigerated and used for the study. The % yield of the extract was found to be 4.3%.

2.1.3 Biological Materials

2.1.3.1 Experimental plant

The purple variety of Onion (Allium cepa) Linn. bulbs of average size (15-22 mm diameter) were purchased from Mushin market in Lagos Nigeria. They were then air-dried for a week after which the dried outer scales were carefully removed leaving the ring of the root primordial intact.

2.1.3.2 Experimental animals

Artemia salina (brine shrimps) eggs were graciously provided by Dr. A.A. Sowemimo of the Faculty of Pharmacy University of Lagos. They were stored in the freezer at and collected when needed. For the study, the eggs were allowed to hatch into nauplii in brine water for 24 hours, the nauplii were then exposed to the test substances for the study. Adult male and female albino mice of average weight 20-36 g were also used for the study. They were obtained from the Laboratory Animal Centre of the College of Medicine, University of Lagos, Nigeria. They were acclimatized for 7 days and housed in polycarbonate cages lined with wood chips bedding placed in rooms maintained under standard environmental conditions. The animals were allowed free access to feed (Raaf farm Limited Ogun state, Nigeria) and water. The experimental procedures were carried out in accordance with the United States National Institute of Health's Guide for the care and use of laboratory animals [10].

2.2 Methods

2.2.1 Brine shrimp lethality assay

Artemia salina (brine shrimp) lethality test was carried out using a modification of the procedure described by [11], [12]. The extract (5, 50, and 500 µg/ml respectively) or brine water (for 0 µg/ml of extract or as blank) was put into separate test tubes (3 test tubes per concentration). Ten A. salina nauplii were then added (using a Pasteur pipette) to each of the test tubes. After 24 hours, the number of surviving shrimps were counted with the aid of a magnifying lens and recorded. All experimental assays were done in triplicates. The concentration-response data obtained was transformed into a straight line plot by probit transformation. The median lethal concentration, LC50, was determined via the line of best fit obtained by linear regression analysis.

2.2.2 A. cepa cytotoxicity assay

Assessment AERC effect on exposed A. cepa root

Air dried A.cepa bulbs with their dried outer scales removed leaving the ring of root primodial intact were used for this assay following modifications of methods described by Fiskesjo [13]. The bulbs were first made to germinate in common potable water for 2-3 days by which time their roots reached at least 3 cm in length. Onion bulbs with

at least 3 cm root length were then allowed to grow in samples bottles containing water at various concentrations of AERC (2, 100, 200, 1000 or 2000 µg/ml), 500 µg/ml of cyclophosphamide (as positive control) or distilled water (as negative control) for 3 days in the dark. In the course of 3 days, length and macroscopic features of the roots were determined. After 3 days of exposure to the various treatments as described, the root strands were harvested and their length as well as macroscopic and microscopic features evaluated.

2.2.3 Microscopic analysis of root tip cells of A. cepa exposed to AERC

Root tips from bulbs in each treatment group (3 per group) were cut and fixed in a mixture of acetic acid and alchohol (1:3) for a week after which they were hydrolyzed in 1N hydrochloric acid for 5 min and excess acid blotted using a filter paper. The root tips were then carefully squashed on microscope slides and stained with 2 drops of Orcein for 20 min. The cover slip was then lowered on the stained area carefully to avoid air bubbles and excess stain removed with filter paper before the cover slips were sealed with 0.1 ml nail varnish. The slides were examined under a microscope to assess cytogenic parameters, chromosomal aberration and mitotic index, which was scored relative to number of dividing cells in 1000 cells [13].

2.2.4 Study design for in-vivo studies in mice

The extract (1.77, 8.83 and 44.13 mg/kg), distilled water (10 ml/kg) and cyclophosphamide (10 mg/kg) were administered to five different groups of mice respectively for 28 days. Their weekly body weight of the mice were determined as they were closely monitored. On the 29th day, peripheral blood samples were collected from the animals for comet assay before they were humanely sacrificed and hind limb bones harvested for micronucleus assays.

2.2.4.1 Comet assay

This assay was carried out following modifications of the methods described by Osipov [14]. Briefly, 10 µl of blood cell suspensions (from buffy coat of blood sample re-suspended in phosphate buffer solution) were mixed with 180 µl of low melting point agarose (0.5%) and spread onto microscope slides precoated with normal melting point 1.5% agarose. These were covered with coverslips and kept at 4 °C for 10 min after which coverslips were removed and slides immersed in freshly prepared lysis solution consisting of 2.5 M NaCl, 100 mM ethylenediaminetetraacetic acid, 10% dimethylsulfoxide, 1% Triton X-100, and 10 mM Tris maintained at pH 10, for 22 h at 4 °C. The slides were then placed in an electrophoresis unit containing 300 mM NaOH and 1 mM EDTA at pH > 13 and left for 20 min for DNA denaturation. Electrophoresis was run for 20 min at an electric field strength of 1 V cm-1 (25 V and 300 mA) followed by rinsing of slides in a neutralization buffer (0.4 M Tris–HCl, pH 7.5). The slides were then air dried and fixed in 70% ethanol for 10 min. They were then stained for 30 min with a working solution of Giemsa stain prepared from a commercially available stock solution (AppliChem, GmbH, Darmstadt, Germany) following manufacturer's instruction. The slides were then washed in Sorensen's phosphate buffer (pH 6.8) and air dried before being subjected to imaging analysis using the open-source software OPEN COMET as a plug-in to IMAGE J image-editing software.

2.2.4.2 Micronucleus assay

Hind limb bones (tibia) of mice from each treatment group were excised for marrow extraction. The marrow content was flushed from the tibia into centrifuge tubes containing 3 to 5 ml phosphate buffer saline (one tube per animal). Following centrifugation of the marrow into pellets, the supernatant was removed by aspiration and portions of the pellet spread on slides and air-dried. The slides were fixed in methanol, stained in May-Grunwald Giemsa stain before being covered with slips. Microscopical examination of the slides was then performed. One thousand polychromatic erythrocytes (PCEs) per animal were scored for incidence of micronucleated PCEs (MPCEs). The PCE:MPCE ratio was determined by scoring the number of PCEs and MPCEs observed while scoring at least 500 erythrocytes for each mouse.

2.3 Statistical Analysis

Values of data obtained are expressed as mean ± standard error of mean (S.E.M). For statistical comparisons among positive and negative controls as well as the various concentrations and doses of AERC, data analysis was performed using one way analysis of variance followed by Tukey's multiple comparison post hoc test using Graphpad Prism software (GraphPad Prism Software, La Jolla, CA, USA). Using the same software, Student's t test analysis was used for comparison between groups in some cases. Results were considered significant at $p < 0.05$. The LC50 of AERC in brine shrimp assay was determined via probit transformation of data obtained followed by linear regression analysis to obtain line of best fit using the same statistical analysis software.3**.**

RESULTS

3.1 Effect of AERC on brine shrimp lethality assay

In the brine shrimp lethality assay, AERC resulted in the mortality of brine shrimp in a concentration-dependent manner. The median lethal concentration, LC50, was estimated at 1017.65 µg/ml (Figure 1).

3.2 Effect of AERC on A. cepa assay

Figure 2 shows the effect of AERC on the root length of A. cepa. In the control group, maximum root growth was observed; the roots were whitish in colour, straight and unbroken. The extract on the other hand significantly reduced root length, albeit in a non-concentration dependent manner on days 2 and 3. The maximum root length reduction by AERC was observed on day 2 at 100 µg/ml ($p < 0.0001$). Table 1 shows the effect of AERC on cytogenic parameters of A. cepa root tip cells. The cells were observed to be in various stages of cell division and different forms of chromosomal aberrations were noted. At 2000 µg/ml of AERC up to 3 counts of abnormal metaphase was noted on day 1. On days 2 and 3, 1 abnormal metaphase, anaphase bridge and lagging chromosome each along with 2 vagrant chromosomes were noted. Cyclophosphamide exposure resulted in the lowest number of dividing cells, while AERC reduced number of dividing cells relative to control in a manner that was concentration-dependent on days 2 and 3. Figure 2 shows the reducing effect of AERC on the mitotic index of A. cepa root tip cells. This effect was concentration-dependent especially on days 2 and 3, with a significance level of $p < 0.0001$ at all the concentrations of AERC by day 3. Depicted in Figure 7 are the various forms of chromosomal aberrations observed in the root tip cells of exposed A. cepa. Most of the aberrations were observed at the metaphase and anaphase stage of mitosis. Some of the chromosomes are linked together instead of separating to the poles forming bridges and fragments; lags were also observed in the chromosomal migration to the poles. Several chromosomal fragments forming micronuclei were also noted at various concentrations of AERC.

3.3 Effect of sub-acute exposure to AERC on mice body weight

Table 3 shows the mean body weights of mice on days 0, 7, 14 and 21. There was no significant change in the body weight of treated mice relative to control. Only slight dose-dependent reductions in body weight (from what it was on day 0) were noted in both AERC and cyclophosphamide treated mice compared to mice in the control group.

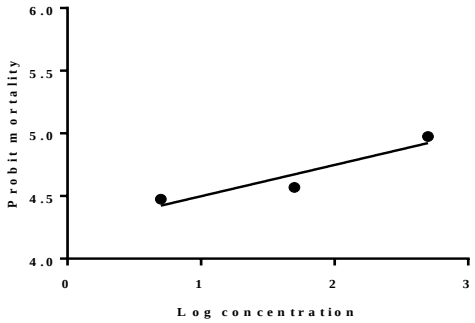

Figure 1: The Log concentration vs probit graph for mortality of brine shrimp induced by AERC. LC50 = 1017.65 µg/ml

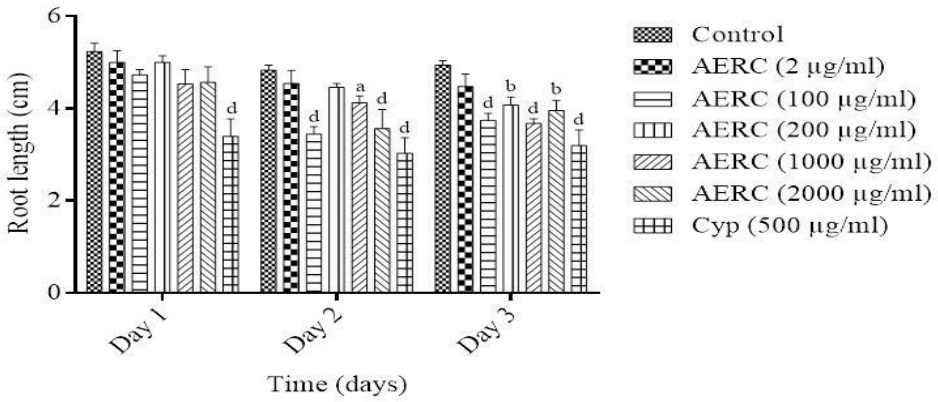

Figure 2: Effect of AERC on root length of A. cepa. Bars represent mean ± S.E.M (n = 9), ap < 0.05, bp<0.01, dp<0.0001 vs control (One way analysis of variance followed by Tukey's multiple comparison test). AERC- aqueous extract of refined camphor, Cyp – cyclophosphamide.

Table 1: Effect of AERC on cytogenic parameters of A. cepa root tip cells.

Days of exposure	Treatment	Concentration (µg/ml)	Abnormal metaphases	Anaphase bridges	Lagging chromosomes	Vagrant chromosomes	No of dividing cells
Day 1	Control	0	0	0	0	0	291 [P177 M44 A36 T34]
	AERC	2	0	0	0	0	258 [P180 M35 A26 T17]
	AERC	100	1	1	1	1	226 [P193 M16 A7 T10]
	AERC	200	2	1	1	0	270 [P193 M36 A20 T21]
	AERC	1000	0	0	1	0	226 [P193 M16 A7 T10]
	AERC	2000	3	0	0	0	177 [P150 M9 A11 T7]
	Cyp	500	1	0	0	0	24 [P22 M1 A1 T0]
Day 2	Control	0	0	0	0	0	299 [P208 M39 A28 T24]
	AERC	2	1	1	0	0	282 [P196 M42 A17 T27]
	AERC	100	0	0	0	0	279 [P_{195} M_{31} A_{18} T_{17}]
	AERC	200	1	1	0	0	271 [P187 M29 A19 T17]
	AERC	1000	1	1	0	0	212 [P180 M11 A10 T11]
	AERC	2000	1	1	1	2	173 [P150 M 12 A6 T5]
	Cyp	500	0	0	0	0	24 [P20 M1 A3 T0]
Day 3	Control	0	0	0	0	0	318 [P232 M38 A31 T17]
	AERC	2	0	0	0	0	237 [P185 M18 A17 T17]
	AERC	100	0	0	1	0	226 [P193 M16 A7 T10]
	AERC	200	3	0	0	0	216 [P173 M13 A15 T15]
	AERC	1000	1	1	1	0	185 [P171 M3 A5 T6]
	AERC	2000	1	1	1	2	159 [P136 M10 A9 T4]
	Cyp	500	0	0	0	0	12 [P11 M1 A0 T0]

P, M, A, and T represents prophase, metaphase, anaphase, and telophase respectively with the subscript representing the number of cells at this stages. AERC- aqueous extract of refined camphor, Cyp- Cyclophosphamide

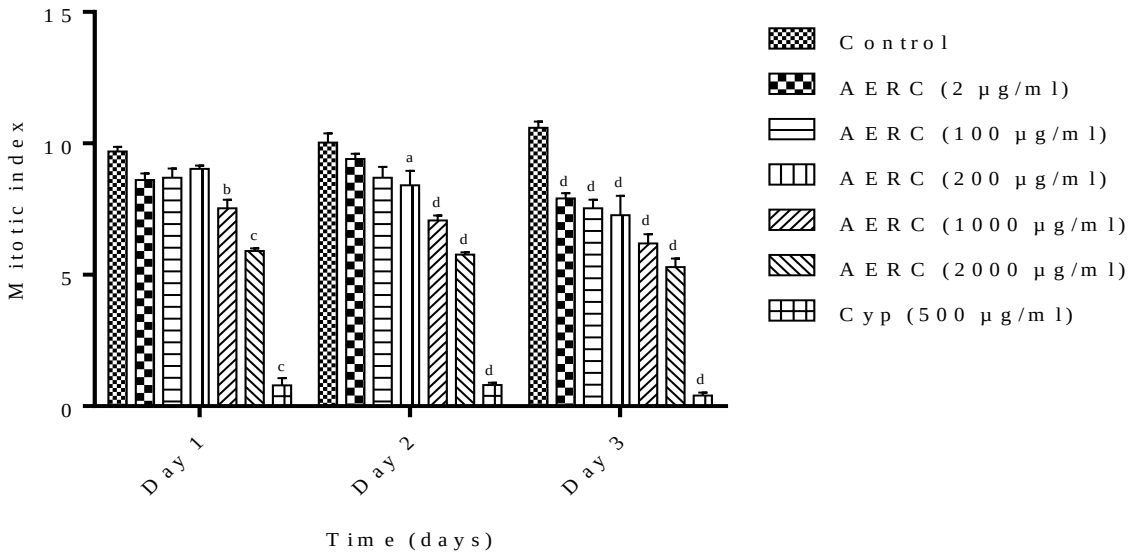

Figure 3: The effect of AERC on the mitotic index of exposed A. cepa. Bars represent mean ±S.E.M, AERC- aqueous extract of refined camphor, Cyp- Cyclophosphamide ap < 0.05, bp<0.01, cp<0.001, dp<0.0001 vs control.

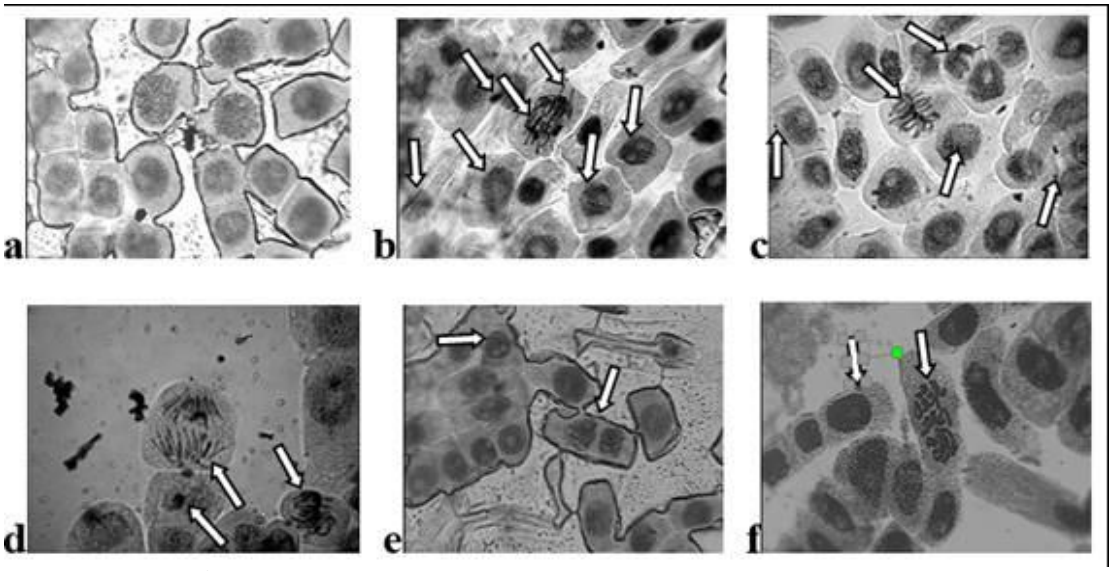

Figure 4: Some types of abnormal cells induced in A.cepa root tip cells by AERC. (a) normal cells mostly in prophase (control on day 3); (b) Vacuolated nucleus at prophase, anaphase bridge with lagging chromosomes, bi-nucleated cells, micronuclei and irregular prophase (2000 µg/ml at day 3); (c) Sticky prophase, lagging chromosomes at metaphase, micronuclei and vacuolated nucleus (1000 µg/ml at day 3); (d) Lagging chromosome with fragment at metaphase, micronuclei and sticky metaphase (200 µg/ml at day 1); (e) Bi-nucleated cells and unequally sized nuclei at interphase (100 µg/ml at day 1); (f) Abnormal metaphase and micronuclei (2 µg/ml at day 2**)**

Table 2: Effect of AERC on the weekly body weight of exposed mice

Group	Dose (mg/kg)	Body weight (g) Day 0	Day 7	Day 14	Day 21
Control	10 ml/kg	27.40 ± 2.70	27.80 ± 2.63	28.20 ± 2.80	28.40 ± 2.58
AERC	1.77	25.20 ± 1.02	24.60 ± 0.95	24.00 ± 1.00	23.00 ± 0.95
AERC	8.83	25.60 ± 1.99	24.20 ± 2.08	23.00 ± 2.00	21.80 ± 1.77
AERC	44.13	24.60 ± 1.17	23.60 ± 0.93	22.40 ± 0.75	21.60 ± 0.68
Cyp	10	29.20 ± 1.24	26.40 ± 1.54	26.30 ± 1.86	24.30 ± 1.45

Values are expressed as mean ± S.E.M (n=5). AERC –Aqueous extract of refined camphor, Cyp – Cyclophosphamide

3.4 Effect of AERC in micronucleus assay in mice

Polychromatic erythrocytes (PCE) and micronuclei in polychromatic erythrocytes (MPCE) were detected in the bone marrow content of AERC exposed mice. Relative to control group, significant reduction in PCE was noted in bone marrow samples of AERC treated mice at 1.77 mg/kg ($p < 0.001$) and 8.83 mg/kg ($p < 0.01$) in a non-dose dependent manner. The level of micronuleated PCE (MPCE) on the other hand was significantly ($p < 0.0001$) increased by AERC at 1.77 and 8.83 mg/kg as well as cyclophosphamide at 10 mg/kg. However at 44.13 mg/kg, AERC increased PCE significantly ($p < 0.05$) and MPCE non-significantly. The ratio of PCE to NCE reduced relative to control at 1.77 and 8.83 mg/kg of AERC (Table 3).

3.5 Effect of AERC in comet assay in mice

As shown in Figure 5, AERC increased the indices of DNA damage assessed in this study. Tail DNA percentage, tail moment, tail length and olive moment were all significantly ($p < 0.01$) increased albeit only at 1.77 mg/kg. Other parameters such as comet area, obtained from analysis also showed a similar pattern (data not shown). The effect of the extract at 1.77 mg/kg was comparable to that of the reference cytotoxic agent, cyclophosphamide.

Table 3: Effect of AERC on cytotoxicity index in the micronucleus assay

Treatment	Dose (mg/kg)	PCE	MPCE	NCE	PCE:NCE
Control	10 mg/ml	592.70 ± 14.03	3.70 ± 0.50	500	1.18
AERC	1.77	480.50 ± 27.09^{c}	36.25 ± 0.88^{d}	500	0.96
AERC	8.83	520.50 ± 5.12^{b}	30.50 ± 1.04^{d}	500	1.04
AERC	44.13	635.20 ± 15.09^{a}	9.00 ± 1.07	500	1.27
Cyp	10	222.10 ±050.37^{d}	49.89 ± 4.55^{d}	500	0.44

Values are mean ± SEM (n = 5), ap < 0.05, bp<0.01, cp<0.001, dp<0.0001 vs control (Student's t test) AERC –Aqueous extract of refined camphor, Cyp – Cyclophosphamide, PCE - Polychromatic erythrocyte, MPCE - Micronuclei in polychromatic erythrocyte.

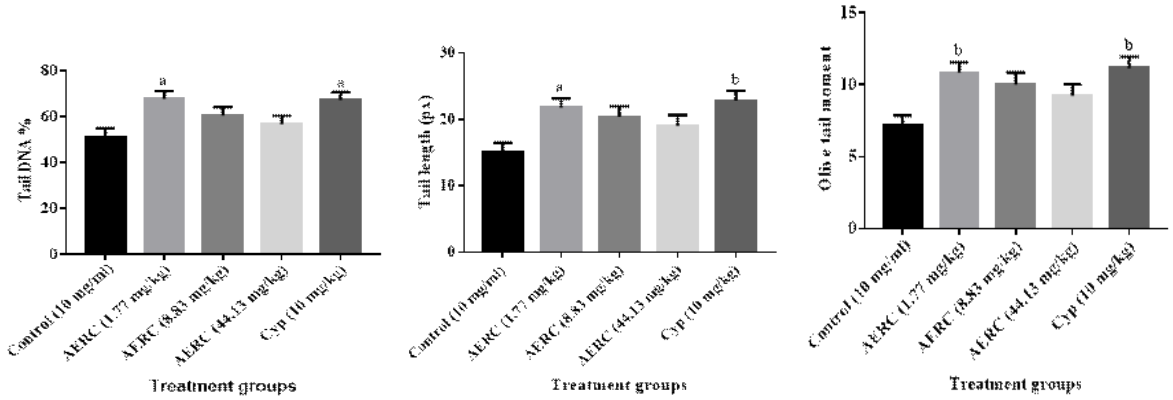

Figure 5: The effect of AERC on DNA damage parameters (a) tail DNA percentage, (b) tail length (c) Olive tail moment in comet assay. Bars represent mean ± SEM (n = 122). ap < 0.05, bp<0.01 vs control (One way analysis of variance followed by Tukey's multiple comparison test). AERC- aqueous extract of refined camphor, Cyp – cyclophosphamide.

4. DISCUSSION

Refined camphor is commonly used for various purposes in traditional medicine practice. In Nigeria its aqueous extract prepared by allowing it to seep in water and stored as such for days is consumed daily or on as needed basis for various ailments. As expected, the poor water solubility of refined camphor makes it such that the concentration of the resulting extract is relatively low. Nevertheless the extract did show various non-negligible effects in this study despite its relatively high LC50 value of 1017.65 µg/ml in the brine shrimp lethality assay. The results of this study demonstrate the toxic potential of AERC vis-à-vis cytotoxicity and genotoxicity. According to Cabaravdic [15], various substances can be screened for their genotoxic potential using the A. cepa assay. With this test, the mutagenic, genotoxic and sometimes carcinogenic potential of compounds may be analyzed. This is done by monitoring macroscopic parameters, such as the appearance and growth of A. cepa roots and microscopic parameters such as type and frequency of chromosome aberrations and abnormal cell division [16], [17], [18]. In our study, AERC significantly reduced A. cepa root length to varying degrees; this could be due to a number of mechanisms including interference with mitotic processes in the root tip cells. A corresponding reduction in mitotic index was also evident, indicating the mitodepressive, cytotoxic and genotoxic potential of AERC. Possible mechanisms include alteration of processes involved in DNA or protein synthesis [19] and disruption of cells entry into mitotic phase of cell cycle [20]. Regarding chromosomal aberration, nuclear abnormalities and formation of micronuclei, several relevant observations indicative of the genotoxic effect of AERC were made. Identification of chromosome fragments and bridges in anaphase stage of mitosis as was observed in this study are important pointers to the clastogenic activity of test compounds [20]. According to Nefic [18], dicentric chromosomes and unequally exchanged chromatids undergoing translocation contribute to the formation of chromosomal bridges at anaphase. In addition, chromosomal stickiness as also observed in this study, is usually due to inter-chromosomal linkages of sub-chromatid strands, excessive formation of nucleoproteins and inappropriate protein-protein interaction. Various abnormalities in nuclear and cellular morphology were also observed. These include nuclear buds, anucleate cells known as ghost cells, fragmented nuclei, binucleated cells and micronucleus formation as well as alterations in shape and size of cells (extended and gigantic cells). These induced anomalies in cell morphology indicate cytotoxic and genotoxic potential of AERC. With respect to the aspect of the study carried out in mice, although the extract did not significantly alter the body weight of treated mice relative to control, it significantly altered parameters assayed for in micronucleus and comet assays. Evaluation of bone marrow samples of AERC exposed mice revealed the presence of micronuclei formed from chromosome breaks (as by clastogenic agents) or whole chromosomes that fail to be incorporated into the main nucleus during cell division cycle (as by aneugenic agents). Although this effect was not dose-dependent, the significant reduction in PCE and increase in MPCE at 1.77 and 8.83 mg/kg of AERC relative to control demonstrate that its potential in this respect is non-negligible. According to an OECD report [21], a dose-dependent increase in the micronucleus frequency is necessary to show the genotoxic effect of a substance. However, clear increase in the micronucleus frequency in even a single dose group relative to control group is also acceptable as proof of the same. Micronuclei formation may also be due to chromosomal or mitotic spindle apparatus damage [22]. More detailed study would be beneficial to properly identify the precise mechanism utilized by AERC in this study. Damage to DNA characterized by DNA strand breakage in alkaline comet assay is measured by endpoints such as % tail DNA, tail length and olive tail moment. They are important indicators of genotoxic properties of test substances in the assay. The % tail DNA is a measure of the intensity of DNA fragments in the tail of comet-shaped figure resulting from migrating damaged DNA material during

electrophoresis. Tail length refers to the distance covered by migrating DNA fragment from nuclear core body and is also used to evaluate the extent of DNA damage. [23], [24]. Olive tail moment, the product of tail DNA and mean migration distance in tail [25] describes the heterogeneity within a cell population, accounting for DNA distribution variations within the tail. The significant increase in these parameters as noted with cyclophosphamide in this study further demonstrates the DNA damaging and genotoxic activity of AERC.

The observations in this study therefore reveal that though poorly water soluble, medicinally used refined camphor does contain bioactive water soluble components capable of inducing health effects such as have been attributed to camphor itself [7]. Genotoxic and cytotoxic activities such as were observed with AERC in this study contribute to the development of various diseases including cancer. Further studies to establish whether or not the findings made in this study may translate or otherwise to mutagenicity and carcinogenicity among users of AERC would be beneficial. Meanwhile, considering our current findings and its relatively widespread use, closer attention must also be paid to traditional medicine practice requiring the use of refined camphor. Extreme caution is advised where its use is unavoidable.

5. CONCLUSION

The findings of this study reveal that the aqueous extract of refined camphor is both genotoxic and cytotoxic. This could have far reaching effect on the health of its users. It is therefore necessary to carefully weigh the risk versus the benefit of its use. Even then, extreme caution is advised regarding its application.

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Conflict of Interest

The authors declare that there is no conflict of interest with respect to this study.

Contribution of the Authors

Flora R. Aigbe conceptualized, supervised project execution, analyzed and interpreted data and also developed the manuscript for publication. Aminat A. Ajasa executed aspects of the project, analyzed data and provided report on aspects of the study. Bukola T. Omowumi also executed aspects of the project, analyzed data and provided report on aspects of the study. Utom-Ubong U. Akpan executed, supervised project execution and also analyzed as well as interpreted data. Olufunmilayo O. Adeyemi revised manuscript.

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