AMERICAN JOURNAL OF PHYSIOLOGY, BIOCHEMISTRY AND PHARMACOLOGY, 2020
VOL 10, NO. 1, PAGE 7–17
10.5455/ajpbp.20191004045314

ORIGINAL RESEARCH

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Effects of rutin in *Myristica fragrans* Houtt. against cyclophosphamide-induced genotoxicity in sperm cells, on liver and kidney tissues, in Swiss albino mice

Akeem Akinboro[1*], Kamaruzaman Mohammad[2], Zaini Asmawi[3]

[1]Pure and Applied Biology Department, Ladoke Akintola University of Technology, Ogbomoso, Nigeria.

[2]School of Biological Sciences, Universiti Sains Malaysia, Pulau Pinang, Malaysia.

[3]School of Pharmaceutical Sciences, Universiti Sains Malaysia, Pulau Pinang, Malaysia.

ABSTRACT

Scientific investigations on phytochemicals are done to reveal the safety or/and pharmacological potency of medicinal plant extracts. In this study, an aqueous fraction of *Myristica fragrans* (AFMF) was administered into Swiss albino male mice for genotoxic and antigenotoxic evaluations following the sperm morphology assay. Male mice were orally administered with AFMF alone for genotoxicity test, while antigenotoxicity involved the administration of cyclophosphamide (CP) at 50 mg/kg 5 minutes before oral administration of AFMF at 500, 1,000, 2,000, and 4,000 mg/kg. Distilled water and CP were the negative and positive controls, respectively. Histopathology of the liver and kidney of mice administered with AFMF was determined. Elucidation and quantification of phytochemical in AFMF were carried out using the ultra-performance liquid chromatography separation technique. The percentage of abnormal sperm induced by AFMF ranged between 7.25% and 13.75% at 500 and 1,000 mg/kg, respectively. Suppression of the genotoxic effect of CP was 61.75% and 38.99% at 500 and 2,000 mg/kg, respectively. AFMF caused acute and chronic inflammations in the liver tissue, whereas there was no adverse effect on the kidney tissue of the mice. Rutin was detected at 12 mg per gram of AFMF. These results suggest that rutin in AFMF was not significantly toxic to the kidney and liver cells, as well as to the chromosomes in the sperm cells of Swiss albino mice. However, it showed a significant antigenotoxic activity in the Swiss albino male mice.

ARTICLE HISTORY

Received October 04, 2019
Accepted December 18, 2019
Published January 16, 2020

KEYWORDS

Phytochemicals; *Myristica fragrans*; sperm cell; genotoxicity; antigenotoxicity.

Introduction

Myristica fragrans Houtt. belongs to the family Myristicaceae, and it is a popular tropical evergreen plant that has both the condimental and medicinal uses. The therapeutic and seasoning values are due to the presence of phytochemicals in plants [1,2]. Phytochemicals comprise primary and secondary metabolites that are synthesized and stored in plants [3]. Volatile substances (alkylbenzene derivatives, such as myristicin, elemicin, safrole, eugenol, and phenolics), terpenoids, lignin compounds, proteins, starch, and mucilage are the major phytochemical constituents of *M. fragrans* [4,5]. The phytochemicals in *M. fragrans* are known for their antioxidant potency which is effected through free radical scavenging, metal chelating, lipid peroxidation inhibition, and singlet oxygen quenching mechanisms [6]. The long-time usage history of commonly used medicinal plants without reported adverse effects has been the reason for the misunderstanding and prejudice toward the safety of herbal medicines, leading to an erroneous conception that their extracts are free of toxicity [7]. Conduction of a genotoxicity test on plant extracts reveals whether the extract could interact with DNA molecule, thereby causing damage and resulting in loss of its biological functions. Scientific studies have implicated some phytochemicals present in some widely used plants possessing mutagenic and genotoxic effects in *in vitro* and *in vivo* genetic models [8,9]. On the other hand, some plant

Contact Akeem Akinboro ✉ aakinboro@lautech.edu.ng Pure and Applied Biology Department, Ladoke Akintola University of Technology, Ogbomoso, Nigeria.

extracts contain antimutagenic or antigenotoxic phytochemicals that can suppress DNA or chromosomal damaging effects of some known mutagenic and genotoxic agents [8,10]. Water and methanol leaf extracts, as well as fruit juice of *M*. *fragrans*, were observed to suppress the mutagenicity of cyclophosphamide (CP) in *Allium cepa* and Swiss albino mice cells and benzo[a]pyrene in *Salmonella typhimurium* due to their antioxidant phytochemicals [5,11,12]. Antioxidant phytochemicals in the plant extracts of *Phellinus nigricans*, *Phellinus rimosus*, *Phellinus wahlbergii*, and several other plants were found to be responsible for antimutagenicity/antigenotoxicity [13]. Assessment of genotoxicity and antigenotoxicity of plant extracts in the germ cells is necessary to avoid the transfer of mutations to the next generation of organisms. Several studies on genotoxicity/antigenotoxicity screening of single and combined (polyherbal extract) plant extracts in the sperm of Swiss albino mice have been reported [14–17]. Previously, the findings from the investigations on the mutagenic and antimutagenic potentials of the fruit juice and aqueous and methanol leaf extracts of *M. fragrans* following the *in vitro* and *in vivo* genetic assays, namely: the Ames test, *Allium cepa* assay, and micronucleus assay, suggested nonmutagenic/nongenotoxic but with strong antimutagenic/antigenotoxic activities [5,11,12]. Screening of the aqueous fraction of *M*. *fragrans* was first carried out in the somatic cells of Swiss albino mice [1]. On further, the aqueous fraction of *M*. *fragrans* was presently evaluated for its possible genotoxic effects, antigenotoxic effects on the reproductive cells, and histopathological effects on the liver and kidney tissues of Swiss albino mice.

Materials and Methods

Animal ethics

Approval to use animals, Swiss albino male mice (*Mus musculus*), for this study was contained in a letter with Reference number: PPSG/07 (A)/044/(2009) (51) issued by the Animal Ethics Committee of the Universiti Sains Malaysia (USM).

Mouse sperm morphology test

Genotoxicity

In this study, in general, the animal genetic model of systematic experimental design was adopted. Mature Swiss male albino mice aged 10–12 weeks were supplied by the animal house of the USM. The mice were transferred to the transit room, School of Pharmaceutical Sciences, USM, and acclimatized for 1 week. Twenty-four male mice were divided into six groups of four mice per group. They were weighed on an electric weighing balance, and the corresponding quantity of aqueous fraction of *Myristica fragrans* (AFMF) to prepare 500, 1,000, 2,000, and 4,000 mg/kg doses was weighed and completely dissolved in distilled water for oral gavage. AFMF was orally administered into the mice for 5 consecutive days and left for 5 weeks of spermatogenesis in mice [18]. Distilled water and CP served as negative and positive controls, respectively. After 5 weeks, each of the dosed mice was sacrificed by anesthetizing with a small quantity of diethyl ether immersed in a cotton wool. The scrotum sac was cut opened and the caudal epididymis on each testis was removed and placed inside normal saline in a Petri dish.

The epididymis was excised and left for 10 minutes in order to allow sperm cells to swim out of the epididymal tubules into the Petri dish containing normal saline. Sperm suspension was mixed with two drops of 1% eosin-yellow stain and left for 30 minutes [19]. A smear of the sperm suspension was prepared on precleaned slides and air-dried overnight at the room temperature [20]. Thereafter, 1,000 sperm cells per animal were observed (under immersion oil objective lens in a Nikon E400 eclipse microscope, Japan) and classified into normal and different types of abnormal sperm cells as described for mice [21,22].

Antigenotoxicity

To evaluate an inhibitory effect of AFMF against the genotoxicity of CP on sperm cells, CP at 50 mg/kg (w/v) was prepared in distilled water. Doses of AFMF were prepared in distilled water at 500, 1,000, 2,000, and 4,000 mg/kg (w/v). Sixteen male Swiss albino mice were weighed and orally administered with a solution of CP at 0.1 ml/10 g (w/v) and left for 5 minutes before they were also orally administered with AFMF at 500, 1,000, 2,000, and 4,000 mg/kg for 5 consecutive days. They were left for 5 weeks for spermatogenesis to take place in the mice [19]. Mice in the positive and negative control groups were administered with CP and distilled water, respectively.

The dosed mice were Sacrificed after 5 weeks by inhaling diethyl ether until they became unconscious.

Slides were prepared as described for the genotoxicity test in this study. Thereafter, 1,000 sperm cells per animal were observed (under immersion oil objective lens in a Nikon E400 eclipse microscope, Japan) and classified into normal and different types of abnormal sperm cells as described for mice [21,22]. The reduction percentage of abnormal sperm cells induced by CP was calculated using the following formula [23]:

$$\%\ \text{reduction of CP - induced genotoxicity} = \frac{A-B}{A-C}\times 100$$

where "A" is the average number of abnormal sperm cells induced by CP (positive control), "B" is the average number of abnormal sperm cells induced by CP + AFMF, and "C" is the average number of abnormal sperm cells induced by distilled water (negative control).

Histopathological effects

Twenty-four male Swiss albino mice were divided into four mice per group. They were orally administered with AFMF at 500, 1,000, 2,000, and 4,000 mg/kg for 3 consecutive days. The negative and positive controls were distilled water and CP, respectively. The mice were sacrificed by enclosing for 1 minute in a glass jar containing diethyl ether-soaked cotton wool. The liver and kidneys from each mouse were surgically removed and quickly preserved inside a specimen bottle containing 10% formalin (fixative) for further processing.

Sections (5-mm thick) from each of the liver and kidneys were cut and left in the fixative overnight to prevent them from postmortem changes. This was followed by the preparation of slides for histological examination as previously described [24,25]. The morphology of the glomerulus, tubules, interstitium, and blood vessels in the kidney tissue was observed and scored, while the parenchymal cell and portal tracts in the liver were observed and scored under 40× objective lens in a light compound microscope.

Chromatographic separation of phytochemicals

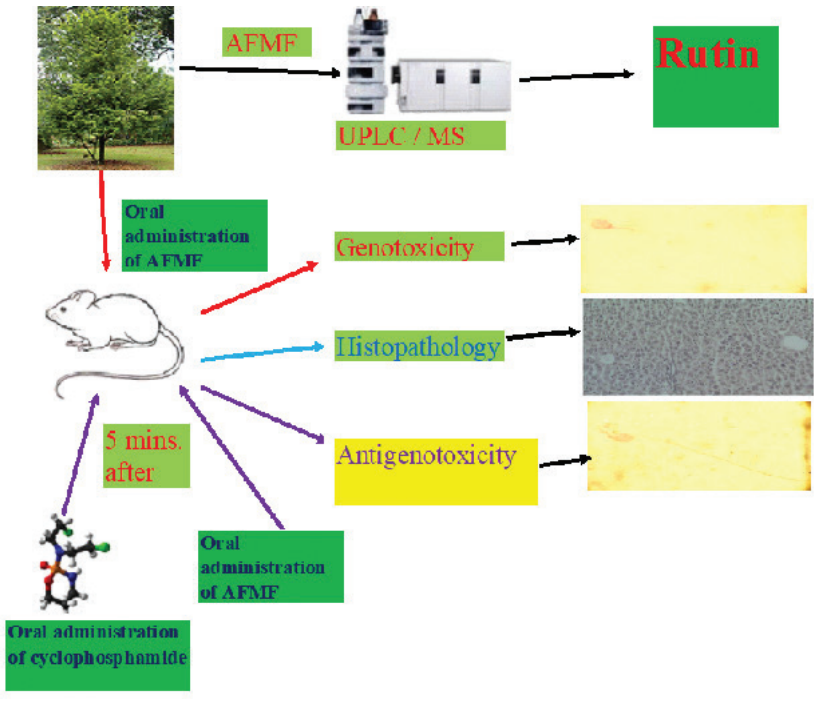
Ultra-performance liquid chromatography (UPLC) technique was adopted for separating and elucidating phytochemicals responsible for the observed effects of AFMF in this study. Pure catechin, quercetin, and rutin were the selected standards. A solution of the mixture of the three standards was prepared by weighing 0.1 mg each and dissolved in 1 ml of High Performance Liquid Chromatography (HPLC) grade methanol. Further, each standard was separately prepared in HPLC grade methanol at 0.1/ml. AFMF at 1 mg/ml was prepared in HPLC methanol. The complete dissolution of the sample and standards was achieved using a vortex mixer. The solutions of the sample and standards were separately passed through 0.22-µm pore syringe filters into a 2.0-ml vial and covered.

Chromatography was carried out with the Acquity-UPLC™ system (Waters, MA, USA). The chromatographic separation of AFMF and standards was performed by reversed-phase chromatography, using the Ethylene Bridged Hybrid (BEH) Column C18 column (Waters) with a particle size of 100 mm × 2.1 × 1.7 µm. The mobile phases consisted of 0.1% formic acid in deionized water (A) and 0.1% formic acid in acetonitrile (B). The proportion of the mobile phases was 60% (A)–40% (B). The injected volumes for the AFMF and standards were 3 µl and the flow rate was 0.1 ml/minute. This analysis was held for 10 minutes while the compounds were detected by a photodiode array detector at 280 nm [26]. The chromatographic separation of AFMF and standards was repeated three times. The retention times of the peaks that appeared with the separation of the standards and AFMF were compared.

Based on the similarity of the retention times of AFMF to that of rutin, the quantity of rutin in AFMF was determined by preparing 0.1 mg/ml of rutin. The solution was diluted in two folds with methanol to obtain lower concentrations; each solution was filtered through a syringe filter (0.22 µm) into 2 ml vials for chromatographic separation. The chromatographic analysis of different concentrations of rutin and AFMF was done following the same conditions used for the phytochemical elucidation. A graph of areas under the peaks was plotted against different concentrations of rutin, and the quantity of rutin in 1 mg of AFMF was extrapolated from the graph.

Statistical analysis

The obtained data were summarized as means, standard deviation, and percentage of the measured parameters. These were analyzed at 95% confidence interval using Duncan's multiple range comparisons in one-way analysis of variance of the SPSS software (15.0 Version). The differences between the treatments and controls' data were considered to be significant when $p \leq 0.05$.



Flowchart of the methodology adopted in this investigation.

Results

Genotoxicity

The genotoxic activity of the aqueous extract fraction of *Myristica fragrance* (AFMF) showed a non dose-dependent induction of abnormal sperm morphology (head and tail). The percentages of abnormal sperm cells induced at 500 and 4,000 mg/kg were 7.25% and 8.00%, respectively, and they were not significantly different from that of the negative control ($p \geq 0.05$). However, the percentage of abnormal sperm cells induced at 1,000 and 2,000 mg/kg was more than twice that of the negative control and significantly different from it ($p \leq 0.05$) (Table 1).

Different forms of sperm cell abnormalities induced by the AFMF alone and in combination with CP (AFMF + CP) in mice are presented in Figure 1. They were banana shape head (BSH), hookless (HL), microcephalis (MC), amorphous head (AMH), coiled tail over head (CTOH), hook at wrong angle (HAWA), and two-tailed (TT) (Plate A, II–VII). None of the selected doses induced all the forms of sperm cell abnormalities as reported in this study. However, the amorphous type of sperm cell abnormality was most induced than any other form of abnormality (Fig. 1).

Antigenotoxicity

The antigenotoxic activity of the aqueous extract fraction of *M. fragrance* against the genotoxicity of CP was not dose-dependent. The genotoxicity of CP was most suppressed by 61.75% at 500 mg/kg, and the least genotoxicity inhibition was 38.99% at 2,000 mg/kg. At 1,000 and 4,000 mg/kg, the suppression was 40.92% and 50.48%, respectively (Table 2).

Histopathology

Histopathological examination of the liver and kidney of the mice orally gavaged with AFMF showed the development of few foci of acute and chronic inflammatory cells around central veins, hepatic parenchyma, and portal tracts (Plate B, I–II). The glomerulus tubules, interstitium, and blood vessels of the kidney tissues of these mice were not affected (Plate B, III).

3.4 Phytochemical elucidation and quantification

The phytochemical that was responsible for the observed activities of the aqueous extract fraction of *M. fragrans* was rutin. This was because AFMF had similar retention times with that of the standard rutin (Table 3). The quantity of rutin contained in 1 g of AFMF was calculated to be 12 mg as extrapolated from the graph of a regression equation, $y = 37{,}329x - 41{,}970$, and a correlation coefficient R^2 of 0.866 (Fig. 2).

Discussion

The assessment of phytochemical potency as well as their toxicity to the reproductive cells is not

Table 1. Genotoxic activity of aqueous extract fraction of *M. fragrans* in inducing abnormal sperm cells in Swiss albino male mice.

Dose (mg/kg)	No of Normal sperm cells ± standard deviation (SD)	No of abnormal sperm cells ± SD	Percentage of normal sperm cells (%)	Percentage of abnormal sperm cells (%)
Distilled water	956.93 ± 8.39	43.08 ± 54.41	95.69	4.31
500	927.5 ± 12.58	72.5 ± 62.67*	92.75	7.25
1,000	862.5 ± 27.54*	137.5 ± 92.30*	86.25	13.75
2,000	896.0 ± 23.02*	104.0 ± 81.48*	89.60	10.40
4,000	920.0 ± 26.46	80.0 ± 76.06*	92.00	8.00

Asterisked values are significantly different from the control ($p \leq 0.05$).

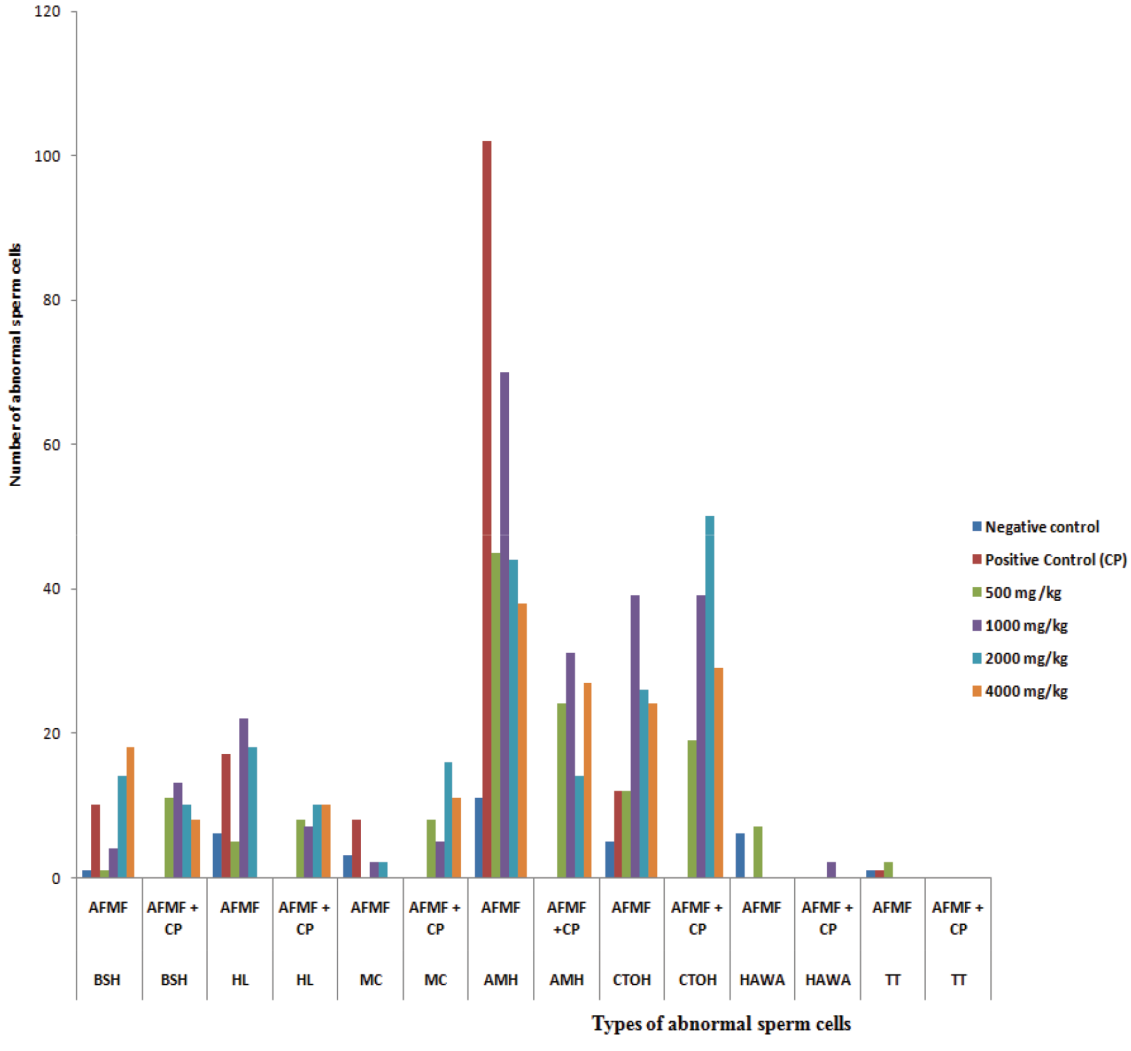


Figure 1. Types of abnormal sperm cells induced in albino mice orally administered with the aqueous extract fraction of *Myristica fragrans.*

only revealing probable effect on the present subjects but can also serve as a source of information about the effect on future generation's offspring formed from affected germ cells if such effect does not result into infertility. In this study, the effect of AFMF in causing abnormal sperm morphology in the treated Swiss albino mice in a non dose-related manner suggests weak genotoxicity. A strong genotoxic agent is expected to cause an ascending degree of genetic damage(s) in the targeted cells according to the tested doses. In our previous investigations, similar insignificant mutagenic effects of methanol leaf extract of *M. fragrans* and its aqueous fraction inducing chromosomal aberration in *Allium cepa*

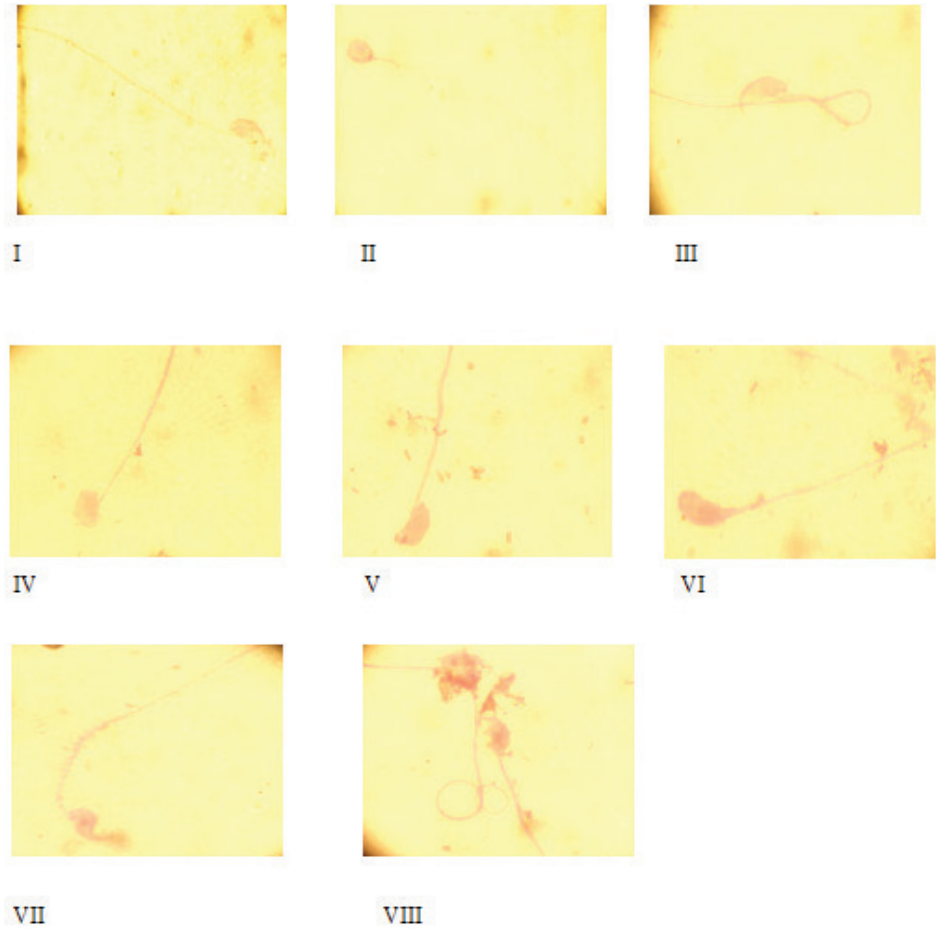


Plate A. Various sperm morphologies induced by the aqueous extract fraction of *Myristica fragrans* (AFMF) alone and AFMF + CP; I = normal sperm cell; II = Microcephalis; III = Coiled tail over head; IV = Hookless; V = Banana shape head; VI = Amorphous head; VII = Hook at wrong angle; VIII = two-tailed sperm cell.

Table 2. Antigenotoxic activity of aqueous extract fraction of *M. fragrans* against CP-induced abnormal sperm morphology in Swiss albino mice.

Dose (mg/kg)	No of normal sperm cells ± SD	No of abnormal sperm cells ± SD	Percentage of normal sperm cells (%)	Percentage of abnormal sperm cells (%)	Reduction percentage of CP-induced abnormal sperm cells (%)
Distilled water	979.00 ± 14.28	21.00± 38.54	97.90	2.10	–
CP	849.52 ± 82.87*	150.48 ± 118.01*	84.95	15.05	–
500 + CP	929.48 ± 28.82*	70.52 ± 64.19*	92.95	7.05	61.75
1,000 + CP	902.50 ± 26.30*	97.50 ± 57.89*	90.25	9.75	40.92
2,000 + CP	900.00 ± 24.50*	100.00 ± 114.91*	90.00	10.00	38.99
4,000 + CP	914.88 ± 25.36*	85.12 ± 121.68*	91.49	8.51	50.48

Asterisked values are significantly different from the control ($p \leq 0.05$).

cells, base pair substitution mutation in *Salmonella typhi*, and micronucleated cells in the bone marrow of Swiss albino mice were observed [1,5]. An induction of abnormal sperm cells by spermatotoxic agents occurs when there is point mutation(s) in the early spermatocytes and spermatogonia at the premeiotic stages of spermatogenesis. Phenolics and polyphenolics from plants are antioxidants but sometimes become prooxidant to induce DNA damage. The prooxidant effect of plant-derived polyphenols, such as flavonoid, tannins, curcumin, vitamins, and phloroglucinol, causes an oxidative damage to

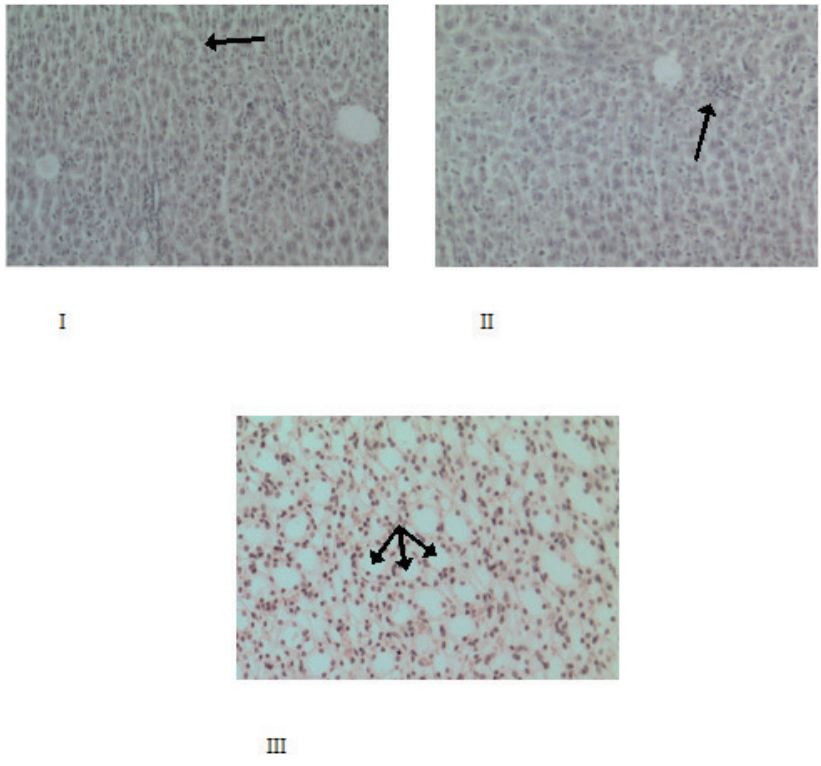


Plate B. Effects of the aqueous extract fraction of *Myristica fragrans* (AFMF) on the liver and kidney tissues; I = normal liver tissue of a mouse (arrow); II = A focus of acute and chronic inflammatory cells in the mouse parenchyma (arrow); III = normal tubules in the mouse kidney tissue (arrows).

Table 3. Chromatographic characteristics of the aqueous extract fraction of *M. fragrans* and mixture of three antioxidant standards (catechin, quercetin, and rutin) separated by the UPLC.

Sample	Peak	Retention time (min)	Area	% Area	Height
Aqueous extract fraction	1	2.09	396,18,906	72.24	27,15,508
	2	2.837	152,24,158	27.76	23,83,789
Mixture of three standards (catechin, quercetin, and rutin)	1	2.153	162,45,542	22.31	910,195
	2	2.841	209,66,184	28.80	28,28,805
	3	4.325	355,92,891	48.89	27,70,847
Catechin alone	1	2.277	82,75,522	39.83	453,172
	2	2.813	124,99,439	60.17	19,15,178
Quercetin alone	1	4.321	313,08,527	100.00	25,87,250
Rutin alone	1	2.109	68,22,785	39.19	484,356
	2	2.841	105,85,689	60.81	18,31,980

cellular DNA in the presence of transition metals such as copper as well as lipid peroxidation in the cell membrane due to the generation of reactive oxygen species (ROS) [27]. ROS from peroxidized metabolites of fatty acids has been implicated in abnormal sperm morphology and function due to damage done to phosphatides of the cell membrane [15]. The induction of abnormal sperm cells by AFMF in this study might be due to rutin. Rutin is a polyphenolic bioflavonoid antioxidant and redox agent capable of becoming prooxidant to cause lipid peroxidation and DNA damage in the sperm cells of the treated mice. This was in accordance with the previous report [28].

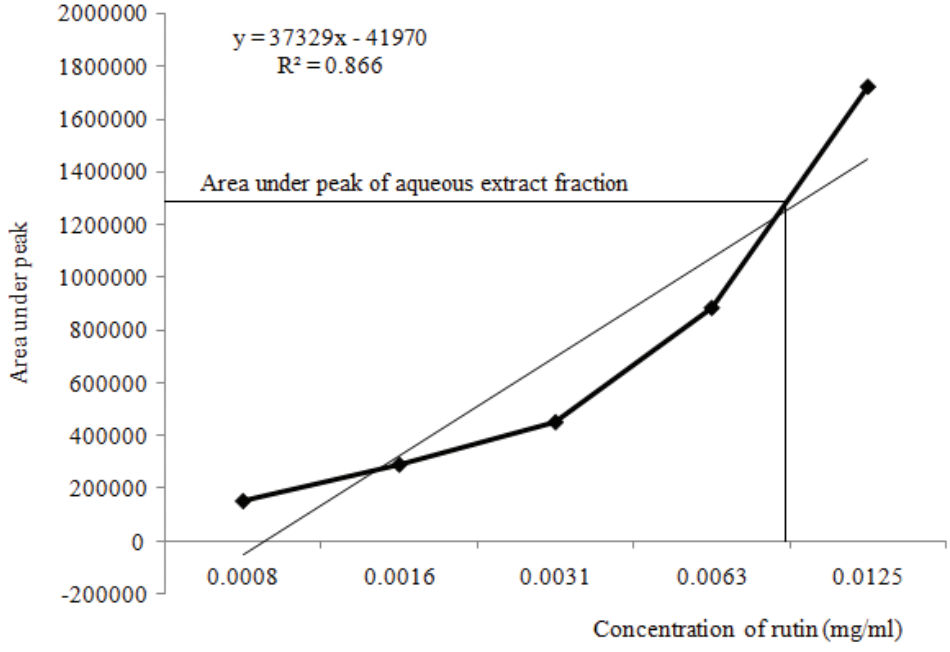


Figure 2. Quantification of rutin in the aqueous extract fraction of *M. fragrans*.

The reduction in the number of abnormal sperm cells induced by CP implies antigenotoxicity of the AFMF. This is in accordance with the antimutagenic activity of aqueous extract of nutmeg in male Wistar rats [29]. We reported that fruit juice and aqueous and methanol extracts of *M. fragrans* suppressed mutagenicity of benzo[a]pyrene and CP in the cells of *Salmonella typhi*, *Allium cepa*, and Swiss albino mice [1,5,11,12]. The antigenotoxic activity of AFMF in this study against the genotoxicity of CP was non dose-related as the least dose of 500 mg/kg reduced the genotoxicity of CP better than any of the higher doses. This is interesting because the possible toxicity of AFMF is reduced at a low effective dose. It is possible that at the tested higher doses, AFMF became more genotoxic resulting in the induction higher frequency of abnormal sperm cells. As expected, in the positive control, CP (50 mg/kg) induced the highest number of abnormal sperm cells. CP causes reproductive toxicity in human and experimental models [30,31]. It is an antineoplastic and immunosuppressive agent, also known to reduce fertility. As an alkylating agent, it is capable of inducing gene mutation (point mutation), chromosomal aberrations, sister chromatid exchanges, micronuclei, as well as other genotoxic effects through the generation of ROS to produce oxidative stress, which, in turn, causes oxidative damages on the macromolecules of the affected cells [16]. Our study, in the past, attributed an antimutagenic and antigenotoxic activity of fruit juices and plant extracts to the antioxidants properties [1,5,11,12]. Similarly, the antimutagenic activity of extracts from *Phellinus nigricans*, *Phellinus rimosus*, and *Phellinus wahlbergii* was due to antioxidants [13]. Rutin, an antioxidant detected in AFMF by the UPLC technique, might be responsible for the observed antigenotoxicity. Rutin is a natural antioxidant capable of ameliorating neurodegenerative diseases and protecting and improving the qualities of sperm cells in Wistar rats [28,32]. Sperm viability, motility, and count are dependent on sperm morphology. These qualities decline in the sperm cells with an abnormal morphology possibly caused by DNA damage of gene mutation type, denaturation of DNA, DNA base pair oxidation, as well as DNA fragmentation [33]. Abnormal sperm morphologies are induced through the generation of ROS both from intrinsic and extrinsic factors such as inflammation, infection, sertoli cells, cigarette smoking, exposure to radiation, mutagens, and carcinogens [34]. Adverse effects of ROS on sperm cells range from alteration of sperm parameters in the form of lipid peroxidation, protein modification, and DNA damage. The abnormal sperm morphologies such as AMH, BSH, HL, MC, CTOH, HAWA, and TT sperm cells induced by CP might be due to DNA mutations caused by the generation of ROS in the sperm cells of the treated mice. These effects of CP were suppressed by AFMF possibly because it contained rutin. This result corroborates the earlier reported antimutagenic potential of AFMF as

confirmed in the somatic cells of plant and animal models. Interestingly, in this study, antigenotoxicity of AFMF has also been established in the germ cells of Swiss albino male mice treated with CP. In the same vein, septilin, a polyherbal herbal drug formulation consisting of extracts from different medicinal plants and minerals, suppressed CP-induced genotoxicity in both somatic and germ cells of Swiss albino mice due to its significant power of antioxidant [16]. Mechanisms of plant extracts exhibiting antimutagenicity include free radical scavenging and gene regulations. CP is metabolized to reactive molecules involved in its elicitation of genotoxicity in the sperm cells of the treated mice. Scavenging of these reactive molecules is suggested to be the mechanism of antigenotoxicity possessed by AFMF. AFMF contained rutin as a flavonol phytochemical and a hydrogen-donating antioxidant whose chemical structure confers the potency to directly scavenge free radicals of reactive oxygen and nitrogen species. It also forms chelates with metals and neutralizes ferric ion to form redox inactive iron, thereby protecting cells against oxidative damage [35,36]. A number of pharmacological uses of rutin are based on its antioxidant, anti-inflammatory, cardiovascular, neuroprotective, antidiabetic, and anticancer properties. Antioxidant activity of rutin involves upregulation of the gene coding for the synthesis of antioxidant enzymes such as catalase and superoxide dismutase and inhibition of xanthine oxidase involved in the generation of ROS [28].

Evaluation of AFMF for hepato-nephrotoxicity in the treated mice revealed the development of few foci of acute and chronic inflammation in the liver suggesting mild toxicity. This is usually seen due to randomly distributed aggregates of inflammatory cells that are found in the liver as a background lesion, and they are more common in aging mice and rats than in young animals. Adult mature male mice (12 weeks and above) are normally considered in the sperm morphology assay adopted in this study. The occurrence of foci, inflammatory cells in the liver tissue of the treated mice might be due to their age. Foci of inflammatory cells occurring spontaneously in the livers of rodents were determined through prechronic studies [37]. This is not unconnected with the elicitation of hypersensitivity reaction to AFMF by the immunological cells in the liver which might recognize AFMF as a foreign body to which there was an antigen-antibody challenge. The effect of different doses of AFMF on the kidney tissue of the treated mice which was not different from that of distilled water (the negative control) implies that AFMF was not toxic to the kidney tissue. This nonnephrotoxic effect of AFMF may be due to rutin as an antioxidant capable of scavenging free radicals. Rutin has been reported to protect kidney tissue from damages induced by ischemia and reperfusion in rats [38]. Rutin content of AFMF was higher than that of the methanol bark extract of *Lagerstroemia speciosa* but lower than those of the methanol bark extract of *Punica granatum*, leaves of *Melissa officinalis*, and flowers of *Lagerstroemia tomentosa* [39]. The quantity of phytochemicals present in plants of the same species and different species depends on the genetic makeup, nutrient availability in the soil for plant growth, extraction methods, and solvents.

Conclusion

This study revealed that the AFMF possessed a weak genotoxic effect on the sperm cells of Swiss albino mice as it induced abnormal sperm morphology in a non dose-dependent manner at the selected doses. However, the antigenotoxic effect of AFMF against CP-induced abnormal sperm cells was interesting as the highest activity was recorded at the least dose of 500 mg/kg. This was attributed to rutin phytochemical, in which its mechanism of action against CP was suggested to be due to free radical scavenging power of this antioxidant. Furthermore, AFMF was mildly toxic to the liver cells, whereas it remains non toxic to the kidney tissue. One gram of AFMF was found to contain 12 mg of rutin.

Study's limitation

This study did not include evaluation of AFMF for genotoxicity and antigenotoxicity using the bone marrow metaphase chromosome assay.

Conflict of interest

No conflict of interest is declared in this article.

Acknowledgment

The author would like to appreciate the financial and material support to carry out this investigation which was received through the jointly sponsored fellowship scheme of Universiti Sains Malaysia and the World Academy of Sciences. The author would also like to thank the management of LAUTECH, Ogbomoso, Oyo State, Nigeria, for granting study leave with pay.

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