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Anti-mutagenic Potential of Nutmeg (*Myristica fragrans*) in Wistar Rats

U.B. Ekaluo, Y.B. Ibiang, E.V. Ikpeme and P.B. Ekpo

This study was designed to evaluate the anti-mutagenic potential of *Myristica fragans* (Nutmeg) in male rats. Twenty male wistar rats were randomly distributed into four groups. The treatment groups were administered doses corresponding to 0, 200, 400 and 600 mg kg^{-1} b.wt. of nutmeg aqueous extract for a period of eight weeks. Data were collected on bone marrow micronucleus index, sperm head abnormality and mutation index. Analysis of variance revealed significant ($p<0.05$) reductions in sperm head abnormality in the nutmeg treated animals, as compared with the control, in a dose dependent manner. The control had the highest value for sperm head abnormality (6.18±0.55), while 600 mg kg^{-1} b.wt. group had the lowest value (3.80±0.09). Micronucleus index values in the 600 mg kg^{-1} b.wt. group (1.40±0.25) was significantly reduced, as compared with the control (3.60±0.51). It was also observed that the mutation indices decreased in a dose dependent manner, from 0.00 in the 0 mg kg^{-1} b.wt. group to -0.384 in the 600 mg kg^{-1} b.wt. group. Nutmeg extract showed anti-mutagenic properties, which means the plant contains phyto-antimutagens that could be exploited further in drug development, or as standardized phyto-preparations.

Key words: Anti-mutagen, nutmeg, mutation Index, sperm head abnormality

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For further information about this article or if you need reprints, please contact:

Y.B. Ibiang,
Department of Genetics and Biotechnology, University of Calabar, PMB 1115,
Calabar, Nigeria

ANSInet
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Department of Genetics and Biotechnology, University of Calabar, PMB 1115, Calabar, Nigeria

INTRODUCTION

Mutations refer to changes in genomic sequence or chromosome structure of an organism caused by mutagens (radiation, viruses, chemicals) as well as errors that occur during DNA replication (Klug and Cummings, 2000). There is evidence that mutations in somatic cells are involved in carcinogenesis and can also cause disorders like atherosclerosis, heart disease and several other degenerative disorders (De Flora *et al*., 1996). Desmutagens are substances which inactivate mutagens partially or fully by enzymatic or chemical interaction, while bio-antimutagens suppress the development of mutations after genes are damaged by mutagens. They also act on the repair and replication processes of mutagen damaged DNA, resulting in a decline in mutation frequency (Bhattacharya, 2011). Plants have been relied on in the healthcare needs of man and have been put to therapeutic applications as antimicrobials, antivirals, antioxidants, anticarcinogens, etc. (Olaleye *et al*., 2006; Helen *et al*., 2012). One group of plants that have been utilized much in this regard is spices (Mezzoug *et al*., 2006), even though some of them are gradually becoming endangered in certain parts of the globe (Ikpeme *et al*., 2012). Aside their culinary applications, exploring alternative uses for these plants, for example as anti-mutagens and anti-carcinogens, can lend impetus for their germplasm preservation and environmental conservation (Ibiang *et al*., 2012; Nta *et al*., 2013). Given the continued presence of mutagens in the ecosystem and the health related effects they could induce in living organisms, there has been extensive research in the last few decades on the detection and characterization of anti-mutagenic compounds from plants. Research suggests that natural anti-mutagens may belong to any of the following major classes of phyto-compounds, flavonoids, phenolics, carotenoids, coumarins, anthraquinones, tannins, terpenoids, saponins and several others, all of which are plant secondary metabolites (Shankel *et al*., 2000). Considering the therapeutic properties, phytochemical constitution and culinary popularity of nutmeg (*Myristica fragrans*) (Olaleye *et al*., 2006; Okukpe *et al*., 2012), this study was conducted to evaluate its anti-mutagenic potentials in albino rat experimental model.

MATERIALS AND METHODS

Experimental animals: Twenty male albino rats of approximately two months of age were obtained from the Animal House of Department of Genetics and Biotechnology, University of Calabar, Calabar. The animals were housed in standard wire mesh cages, with light period of 12 h day^{-1}, temperature of 27±2°C and allowed free access to feed and water. In a completely randomized design, the animals were divided into four equal groups of five rats each. Group A served as the control while groups B, C and D were administered aqueous extracts of nutmeg prepared at doses of 200, 400 and 600 mg kg^{-1} b.wt. The test substance was administered orally using gavage. The body weight of each animal were determined every week during the treatment period and this was used to determine the amount of test substance which was given according to Eq. 1:

$$\text{X mg of nutmeg} = \text{Group dose} \times \text{kg body weight of animals} \quad (1)$$

One week of acclimatization and quarantining was allowed before commencement of treatment (Uzunhisarcikli *et al*., 2007). All rats were handled in accordance with the standard guide for the care and use of laboratory animals, as laid down in the EU directive 2010/63/EU for animal experiments and as mandated by the Animal genetics research committee of the Department.

Preparation of test sample: The dried kernel of nutmeg was obtained locally from Watt market, Calabar, Cross River State, Nigeria. The nutmeg was pulverized and respective amounts were soaked in 10 mL of hot distilled water and left to stand for 15 h. This was then filtered and the aqueous extract was obtained and refrigerated. One milliliter of the respective extract preparations was administered to the rats daily for ten weeks, after which the animals were sacrificed via cervical dislocation.

Determination of study parameters

Micronucleus test: After sacrifice, the femurs of the rats were surgically removed. The bone marrow was flushed from the femur with foetal bovine serum. The cells were centrifuged for 5 min at 2000 rpm and the slide preparations were made using May-Gruenwald and Giemsa stains respectively. The smears were air dried and viewed under the light microscope (HM-LUX, Leitz Wetzler, Germany). Erythrocytes in bone marrow were analysed per 1000 cells, according to the method of Schmidt (1975) and the result was expressed as percentage.

Sperm head abnormality test: The epididymes were dissected from the male rats and sperm suspension was obtained by mincing the epididymes with fine scissors in 10 mL aliquots of physiological saline. The sperm suspensions were mixed with 1% eosin Y solution (10:1) for 30 min and air-dried smears were prepared on glass slides for the sperm head abnormality test. The slides were examined for percentage abnormalities (Nahas *et al*., 1989; Mori *et al*., 1991; Ekaluo, 2003) in every 200 spermatozoa observed on each slide.

Mutation index: Mutation index was calculated using the method of Ekaluo *et al*. (2009) as shown in Eq. 2:

$$\text{Mutation Index (MI)} = \frac{\text{Frequency of abnormal sperm heads (treated-control)}}{\text{Frequency of abnormal sperm heads (control)}} \quad (2)$$

Statistical analysis: All data generated was subjected to Analysis of Variance (ANOVA) to check for significant differences between the treatment groups at 5% Probability level, using SPSS 15.0 statistical software.

RESULTS AND DISCUSSION

Akinboro *et al*. (2011) reported mito-depressive and antimutagenic effects of *Myristica fragrans* leaf extract preparations, against cyclophoshamide induced chromosomal aberrations in *Allium cepa*. Anti-genotoxic efficacy of nutmeg was also demonstrated in Drosophila (up to 50% inhibition rate of induced spot mutation) (Mezzoug *et al*., 2006). These findings, though significant, were made in non-mammalian experimental models and so couldn't cover parameters like sperm head abnormality. As suggested by Akeem *et al*. (2011), we conducted this study on antimutagenic potential of *Myristica fragrans* in animal model. As abnormal sperms heads and aberrant micronuclei occur naturally in mammals due to spontaneous mutations caused by "latent" mutagens like viruses and errors in DNA replication (Klug and Cummings, 2000), we did not administer any mutation inducing substances to the experimental animals. No abnormal behavior was observed in the nutmeg treated animals, even in the highest dose group. Feed and water intake was also not affected. And as a result, no significant differences were observed in the body weight of the animals at the end of the treatment (data not shown). Table 1 shows the effect of aqueous preparations of nutmeg (*Myristica fragrans*) on sperm head abnormality, micronucleus index and mutation index in wistar rats. Analysis of variance revealed significant ($p<0.05$) reductions in sperm head abnormality in the nutmeg treated animals, as compared with the control. And this occurred in a dose dependent manner, with 600 mg kg^{-1} b.wt. group having the lowest value (Fig. 1). Micronucleus index value in the 600 mg kg^{-1} b.wt. group was significantly lower than the control. Values in the 200 and 400 mg kg^{-1} b.wt. groups were lower than control, although this was not significant. It was also observed that the mutation index decreased in a dose dependent manner, from the 0 mg kg^{-1} b.wt. group to 600 mg kg^{-1} b.wt. group. These observations reveal that aqueous preparations of nutmeg possess significant antimutagenic properties. Many spices are rich in antioxidants which are known to be good scavengers of active oxygen radicals (Kim and Lee, 2004). Dietary phytochemicals are also known to induce phase I and phase II detoxification enzymes and these have an overall effect of reducing frequency of mutations in cells (Ferguson, 1994). The rich phytochemical constitution of *Myristica fragrans* (Helen *et al*., 2012) can account for the observed antimutagenic effects in the experimental animals treated with aqueous preparations of the plant for ten weeks. Already, Akeem *et al*. (2011) showed that freeze dried fruit juice of *Myristica fragrans* exhibit high

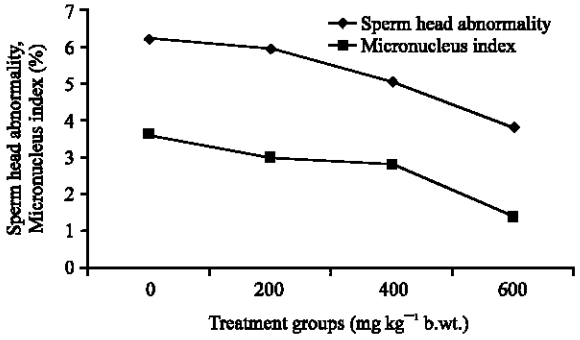

Fig. 1: Antimutagenic effect of nutmeg extract in wistar rats

Table 1: Effect of aqueous preparations of nutmeg on sperm head abnormality, micronucleus index and mutation index in wistar rats

Parameter	(A) 0 mg kg^{-1} b.wt.	(B) 200 mg kg^{-1} b.wt.	(C) 400 mg kg^{-1} b.wt.	(D) 600 mg kg^{-1} b.wt.
Sperm head abnormality (%)	6.18±0.55^{a}	5.95±0.19^{b}	5.03±0.18^{b}	3.80±0.09^{c}
Micro-nucleus index (%)	3.60±0.51^{a}	3.00±0.32^{a}	2.80±0.37^{a}	1.40±0.25^{b}
Mutation index	0.0	-0.0364	-0.186	-0.384

Values are presented as Mean±SEM, Values across the table with similar superscript are not significantly different at 5% based on ANOVA

free radical scavenging ability, almost comparable with BHA and BAT and has high phenols content. Phenolics in *Myristica fragrans* have been reported to exhibit DNA protecting ability (Chatterjee *et al*., 2007).

CONCLUSION

Mutations are involved in the initiation and promotion of several animal diseases. And as such, dietary intake of antimutagenic substances is desirable. Aqueous preparations of nutmeg at 600 mg kg^{-1} b.wt. significantly reduced sperm head abnormalities, aberrant micronuclei and mutation index in wistar rats. We conclude that this plant possess significant phyto-antimutagens, which could be exploited further in drug development, or as standardized phyto-preparations.

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