

Full Length Research Paper

Effects of ethanolic extract of *Myristica fragrans* Houtt. (nutmeg) on some haematological indices in albino rats

Bamidele O.^{1*}, Akinnuga A. M.², Alagbonsi I. A.³, Ojo O. A.¹, Olorunfemi J. O.⁴ and Akuyoma M. A.⁴

¹Department of Physiology, Bowen University, Iwo, Osun State, Nigeria.

²Department of Nursing Science, Lead City University, Ibadan, Oyo State, Nigeria.

³Department of Physiology, University of Ibadan, Ibadan, Oyo State, Nigeria.

⁴Department of Physiology, Madonna University, Okija, Anambra State, Nigeria.

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In spite of wide spread biological uses of *Myristica fragrans* (nutmeg), there is a dearth of information on its effects on haematological indices. This work was therefore conducted to evaluate the effects of ethanolic extract of *M. fragrans* on some haematological indices using albino rat as a model. Twenty four (24) Wistar strain albino rats weighing 140 to 160 g were randomly distributed into four (4) groups of six (6) animals per group. Group I consists of rats which received 10 ml/kg normal saline (orally) and served as the control while those in Groups II, III and IV received 50% ethanolic seed extract of *M. fragrans* (orally) at doses of 100, 250 and 500 mg/kg, respectively. In all groups, the blood samples were obtained by cardiac puncture for analysis of haematological indices after feeding regimens lasted for 14 days. The results showed significant decreases ($p < 0.05$) in red blood cell (RBC) count, packed cell volume (PCV), haemoglobin concentration (HbC) and platelet count especially at high doses. There was significant increase ($p < 0.05$) in total white blood cell (WBC) count. This study therefore seems to confirm the anti-inflammatory properties of seed of *M. fragrans* and also suggests that it may have deleterious effects on haemopoiesis at high doses.

Key words: *Myristica fragrans*, nutmeg, haematological indices, saponin, glycosides, albino rats.

INTRODUCTION

Myristica fragrans Houtt. (nutmeg) of the family Myristicaceae is a spice seed from the fruit of an evergreen tree called *M. fragrans* Houtt. tree (Gills and Cox, 1994). Both *M. fragrans* (nutmeg) and mace (its sister spice) are native to tropical Asia and Australia. Nutmeg is the actual seed of the tree, while mace is the dried "lacy" reddish covering on the seed. It is the species used for culinary and medicinal purposes and grew naturally only on a small group of island called the Bandas.

Nutmeg and mace taste similar though nutmeg is sweeter in flavour and mace more delicate. Many countries use nutmeg as a seasoning. In India, it is used in sweet dishes. In the Middle East, nutmeg spices savoury dishes. Europeans use it in most dishes to season

potatoes, eggs, meats and even spinach with it along soups, sauces and baked goods.

Nutmeg had been reported to have aphrodisiac (Tajuddin, 2005), stomachic, carminative (Green, 1959; Khory and Katrak, 1985), tonic (Burkill, 1935), nervous stimulant (Ainslie, 1979), aromatic, narcotic, astringent, hypolipidemic, antithrombotic, antifungal, antidiarrhoeic and anti-inflammatory (Tajuddin, 2005) properties.

Nutmeg is used by Arabs of Israel and people of its Jewish communities, especially Yemennites, as a drug of their folk medicine, as well as a spice and as an important ingredient in love-portions. It is used against vomiting and to regulate the movements of the bowels; it is good for liver and for the spleen. It is used in the treatment of tuberculosis, against colds, fever, and in general for respiratory ailments. It is said to be antihelminthic and also used against skin diseases like eczema and scabies (Zaitschek, 1964).

Phytochemical studies indicate that nutmeg contain a

*Corresponding author. E-mail: femi_bayo_is@yahoo.co.uk Tel: +2348067463528.

Table 1. Haematological indices in the different experimental groups of rats (n = 5).

Haematological Indices	Group I (Control)	Group II (Low dose)	Group III (Medium dose)	Group IV (High dose)
RBC ($\times 10^6/\text{mm}^3$)	8.15 \pm 0.02	8.13 \pm 0.18	8.10 \pm 0.53	7.16 \pm 2.51*
WBC ($\times 10^3/\text{mm}^3$)	6.51 \pm 1.30	6.65 \pm 0.02	6.72 \pm 0.07*	7.16 \pm 0.07*
PCV (%)	38.19 \pm 0.27	38.11 \pm 0.33	37.98 \pm 0.29	36.00 \pm 0.21*
HbC (g/dl)	12.34 \pm 0.25	12.32 \pm 0.76	11.86 \pm 0.43	11.44 \pm 0.10*
Platelet ($\times 10^3/\text{mm}^3$)	338.00 \pm 7.33	143.17 \pm 16.17*	03.33 \pm 2.14*	88.33 \pm 2.30*

* Signifies $P < 0.05$ vs. control. Values are mean \pm SEM.

volatile oil, a fixed oil, proteins, fats, starch and mucilage. The fixed oil contains myristin and myristic acid. Nutmeg yield 5 to 15% of volatile oil, which contain pine, sabincene, camphene, myristin, elemicin, isoelemicin, eugenol, isoeugenol, methoxyeugenol, safrole, diametric phenylpropanoids, lignas and neolignas (Isogai et al., 1973; Janssen and Laeckman, 1990).

The nutmeg is reported to be useful in paralysis (Antaki, 1930) and increases blood circulation (Lindley, 1981). It is demonstrated to have antioxidant property (Murcia et al., 2004; Olaleye et al., 2006). The petroleum ether extracts of *M. fragrans* fruits possess antidiarrheal property, and its n-hexane extract has been reported to have memory enhancing effect in mice (Parle, 2004).

In view of wide spread biological uses of nutmeg and with the fact that there is little or no investigation has been conducted so far as we are aware on its haematological effects, therefore this study was undergone to demonstrate the effect of *M. fragrans* Houtt. (nutmeg) on haematological indices in albino rats.

MATERIALS AND METHODS

Animal model

Wistar strain albino rats weighing between 140 to 160 g were used. The rats were purchased at the College of Medical Sciences' animal house of the University of Nigeria, Nsukka. The rats were housed in wire mesh cages under standard conditions (temperature, 25 \pm 2 $^{\circ}$ C, 12 h light and 12 h dark cycle) and fed with standard rat pelleted diet and water given *ad libitum*.

Plant materials

Large quantity of dried *M. fragrans* seeds were bought from Benin City in Edo State, Nigeria. The plant materials were identified and authenticated in the Department of Pharmacognosy, Faculty of Pharmacy Madonna University, Elele Campus. The seed were shade-dried after which they were reduced into fine powder by grinding and soaked for 72 h in ethanol (50% v/v, BDH) at room temperature. It was then filtered with Whatman No. 1 filter paper to separate the filtrate from the residue. The filtrate was then concentrated using a rotary vacuum evaporator to obtain the solid mass. The ethanolic extract of *M. fragrans* seeds obtained weighed about 58.1 g. A stock solution of 1 mg/ml was then prepared and kept in capped sample bottles in refrigerator until used.

Experimental design

Twenty four (24) wistar strain albino rats were randomly distributed into four (4) groups of six (6) animals per group. Group I consists of rats which received 10 ml/kg normal saline and served as the control. Groups II, III and IV received 50% ethanolic extract of *M. fragrans* seeds at doses of 100, 250 and 500 mg/kg, respectively. Administration of the extract was done through gastric intubation once a day for a period of 14 days. Blood samples were collected from the animal through cardiac puncture into EDTA bottles after anaesthetizing the animals with chloroform at the 14th day of the experiment for haematological studies.

Determination of haematological indices

Red blood cell (RBC) count was done using the conventional method of Dacie and Lewis (2001). Blood was diluted to 1:200 with Hayem's fluid which preserved the corpuscles and then counted with a Neubauer counting chamber under a light microscope. The counting of total white blood cells was done using a diluting fluid (Turk's fluid) in a ratio of 1:20. The conventional method (Sahli's haemoglobinometer) was employed for estimation of haemoglobin (Hb) content of the blood, and packed cell volume (PCV) was done using the macrohaematocrit method (Dacie and Lewis, 2001).

Statistical analysis

All data were presented as mean \pm SEM. The one way ANOVA was used to analyze the data, followed by a post-hoc test (LSD). The results were considered significant at p values of less than 0.05.

RESULTS

The results in the Table 1 showed that normal saline has no effect on haematological indices.

The mean RBC counts of Group I (Control), Groups II, III and IV were summarized in the Table 1. There were decreases in the values of RBC counts in Groups II and III compared with the control group but not significant. There was only significant decrease in the value of RBC counts in Group IV compared with the control group at $P < 0.05$.

The mean total WBC count in control group was 6.51 \pm 1.30 $\times 10^3$ cells/mm³ while those of Groups II, III and IV were 6.65 \pm 0.02, 6.72 \pm 0.07 and 7.16 \pm 0.07 $\times 10^3$ cells/mm³ respectively. There were significant increases in total WBC count in Groups III and IV compared with

control group. The increases observed in the test groups were dose dependent.

The mean PCV in control group was $38.19 \pm 0.27\%$ while those of Groups II, III and IV were 38.11 ± 0.33 , 37.98 ± 0.29 and $36.00 \pm 0.21\%$ respectively. The mean PCV of the Group IV was significantly decreased compared to the control group ($P < 0.05$) while the Groups II and III were not significantly different. Also, the mean haemoglobin concentration (HbC) in Group II (12.32 ± 0.76 g/dl) and Group III (11.86 ± 0.43 g/dl) were statistically insignificant compared with control group (12.34 ± 0.25 g/dl) while that of Group IV (11.44 ± 0.10 g/dl) was significantly decreased.

The mean platelet of Group II ($143.17 \pm 16.17 \times 10^3$ cells/mm³), Group III ($103.33 \pm 2.14 \times 10^3$ cells/mm³) and Group IV ($88.33 \pm 2.30 \times 10^3$ cells/mm³) were significantly different compared with that of control group ($338.00 \pm 7.33 \times 10^3$ cells/mm³).

DISCUSSION

The effects of ethanolic extract of *M. fragrans* Houtt. on some haematological indices were examined in this study. The results showed that ethanolic seed extract of *M. fragrans* at high dose appears to suppress the haemopoietic system. There were significant decreases in red blood cell (RBC) count, packed cell volume (PCV), haemoglobin concentration (HbC) and platelet count. The decrease may be due to lysis of blood cells or probably due to suppression of blood cells synthesis by saponin which had been earlier reported to be present in the seed of *M. fragrans* (Olaleye et al., 2006). Saponin is known to be toxic to body system (Watt and Breyer-Brandwijk, 1962; Effraim, 2000). In addition, the decrease in red blood cell (RBC) count, packed cell volume (PCV), haemoglobin concentration (HbC) and platelet count following administration of ethanolic seed extract of *M. fragrans* for 14 days were dose dependent. This explains the effect of saponin on haemopoietic system. High dose contains more saponin than the medium and low doses and this may be the reason for non-significant effects observed at the medium and low dosage levels for red blood cell (RBC) count, packed cell volume (PCV), haemoglobin concentration (HbC).

The significant decreases observed in RBC, PCV, HbC and platelet count at high dose in this study contrast no significant differences reported after feeding atherogenic diet for 30 days and replacement with normal diet for 60 days as well as after administration of *M. fragrans* with normal diet for 60 days in rabbits by Ram et al. (1996). The differences might be due to the use of different animal models, different conditions in which the animals were subjected and different method of extraction of the seed. Ram et al. (1996) used rabbits in which hyperlipidemia was induced while we used normal fed rats. Also they used Soxhlet apparatus for their extraction

while we employed crude method of extraction. Moreover, the extract in the dosage range used significantly increased total WBC count in the treated animals compared to the control group. The increase may not be unconnected with the presence of flavonoids and glycosides in the seed extract of *M. fragrans* (Olaleye, 2006). Glycosides are implicated to cause increase in WBC count (Anthai et al., 2009) because they possess anti-inflammatory properties and have vital effect on inflammatory processes of some pathological states such as bacterial infections, malaria and liver diseases (Olajide et al., 1999; Ugochukwu, 2002; Balasundram et al., 2006).

In conclusion, this study seems to confirm the anti-inflammatory properties of the seed of *M. fragrans* and also suggests that it may have deleterious effects on haemopoiesis at high doses.

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