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EFFECTS OF CO-ADMINISTRATION OF MYRISTICA FRAGRANS HOUTT (NUT MEG) AND VITAMIN E ON PLATELETS, PT AND APTT OF POTASSIUM BROMATE INTOXICATED WISTAR RATS

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ABSTRACT
The present study was designed to evaluate the effects of co-administration of Myristica Fragrans Houtt (nut meg) and vitamin E on platelets, PT and APTT of potassium bromate intoxicated wistar rats. Dried nuts of nut meg were purchased from Ubani market in Umuahia North Local Government Area, Abia State, Nigeria and were identified by a botanist in the Department of Forestry and Environmental Management, Michael Okpara University of Agriculture, Umudike. In week two and three, there was significant decrease in platelet in all the treatment groups compared with control. There was no significant change in platelet after one week. In week one there was no significant difference in platelet of rats exposed to 10, 30 and 50 mg bromate and those treated with corresponding doses of nutmeg and vitamin E alone. However the combination of doses of nutmeg and vitamin E increased platelet count. In week 2 and 3, there was significant difference in platelet of rats exposed to 10, 30, and 50 mg bromate group, and those treated with corresponding doses of nutmeg and vitamin E alone and in combination compared with rats exposed to bromate alone. In week one, two and three, there was significant increase in average PT in all the treatment groups compared with control. Average PT varied significantly from week one to three in all the treatment groups. In week one two and three, there was significant difference in Average PT of rats exposed to 50 mg bromate and those treated with 500 mg nutmeg and 100 mg vitamin E alone and in combination compared with rats exposed to 50g bromated alone. The trend was similar in groups exposed to 10 and 30mg bromate compared with the respective treatment groups. In week one, two and three, there was significant increase in average APTT in all the treatment groups compared with control. Average APTT varied significantly from week one to three in all the treatment groups. In week one two and three, there was significant difference in Average APTT of rats exposed to 50 mg bromate and those treated with 500 mg nutmeg and 100 mg vitamin E alone and in combination compared with rats exposed to 50mg bromate alone. The trend was similar in groups exposed to 10 and 30mg of bromate compared with the respective treatment groups.

KEYWORDS: Myristica fragrans houtt (nut meg), vitamin E, platelets, PT, APTT, potassium bromate intoxicated wistar rats.

INTRODUCTION
Potassium bromate as an additive had been in use in so many countries including Nigeria to strengthen the dough and allow higher rising added to fish paste as a conditioner; to beer or cheese and also, as a constituent in cold wave hair solution (Ueno, 2000; Oseni *et al.*, 2015).

Alpha-Tocopherol is absorbed via the lymphatic pathway and transported in association with chylomicrons. In plasma alpha-tocopherol is found in all lipoprotein fractions, but mostly associated with apo B-containing lipoproteins in man. In rats approximately 50% of alpha-tocopherol is bound to high density lipoproteins (HDL). After intestinal absorption and transport with chylomicrons, alpha-tocopherol is mostly transferred to parenchymal cells of the liver where most of the fat-soluble vitaminsare stored. Little vitamin E is stored in the non-parenchymal cells (endothelial, stellate and Kupffer cells). Alpha-Tocopherol is secreted in association with very low density lipoprotein (VLDL) from the liver. In the rat, about 90% of total body mass of alpha-tocopherol is recovered in the liver, skeletal muscle and adipose tissue. Most alpha-tocopherol is located in the mitochondrial fractions and in the endoplasmic reticulum, whereas little is found in cytosol and peroxisomes.

Vitamin E is a natural cancer fighter found in the germ of wheat and other grains, nuts, and beans (Traber *et al.*,

2007). It is a fat-soluble vitamin that is thought to protect the body from a plethora of diseases, such as arthritis; heart disease; diabetes; bowel, lung, and renal disease; and also cancer. Its major function in the body is to act as an antioxidant.

Nutmeg has been reported to have aphrodisiac stomachic carminative tonic nervous stimulant aromatic, narcotic astringent hypolipidemic antithrombotic antifugal antidysentric and anti inflammatory properties (Tajuddin *et al*., 2005).

The present study was designed to evaluate the effects of co-administration of Myristica Fragrans Houtt (nut meg) and vitamin E on platelets, PT and APTT of potassium bromate intoxicated wistar rats

MATERIALS AND METHODS

Purchase of Nutmeg and Identification

Dried nuts of nut meg were purchased from Ubani market in Umuahia North Local Government Area, Abia State, Nigeria and were identified by a botanist in the Department of Forestry and Environmental Management, Michael Okpara University of Agriculture, Umudike. Voucher number MOUAU/VPP/18/013 was assigned to a sample specimen before being deposited in the departmental herbarium.

Extract preparation

Extract was prepared using the method described by Jensen, (2007). The dried nut meg fruits were ground into powder using a manual blender powered by a Honda petrol engine. Eighty grams of the powdered sample was transferred into the extraction chamber of the soxhlet extractor and extraction was carried out for 48 hours using ethanol as solvent. Temperature was maintained at 65^0C throughout the extraction period. At the end of the period, the extract in solution concentrated to dryness in a hot air oven at 40^oC to obtain a dry dark oily extract. The weight of the extract was taken and percentage yield was calculated using the relationship:

$$\% \text{ yield} = \frac{X}{Q} \times \frac{100}{1}$$

Where X = weight of dried extract and Q = weight of powdered plant material before extraction (100g).

Animals

One hundred and ninety-five mature wistar albino rats were used for the various experiments. Thirty were used for the acute toxicity study of the extract, 35 for acute toxicity study of $KBrO_3$ and 130 for experimental studies). The animals were kept in aluminum cages and allowed to acclimatize for two weeks to allow for proper adaptation to the environment and living conditions. They were allowed access to feed (Vital feed, Nigeria) and water *ad libitum* but were starved for 12 hours prior to commencement of any experiment. All animal experiments were carried out in accordance with the guidelines for Care and Use of Laboratory Animals as stipulated by OECD (OECD, 2001). All experiments were carried out in the Physiology Laboratory of the Department of Physiology and Pharmacology, College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike, Nigeria.

Experimental design

The rats (130 in number) were assigned to 13 groups of 10 rats each and were treated according to the order below:

Group I: 10 mg/kg K_2BrO_4
Group II: 10 mg/kg K_2BrO_4 + 500 mg/kg Extract
Group III: 10 mg/kg K_2BrO_4 + 100 mg/kg Vit.E
Group IV: 10 mg/kg K_2BrO_4 + 500mg/kg Extract +100 mg/kg Vit. E
Group VI: 30 mg/kg K_2BrO_4
Group VII: 30 mg/kg K_2BrO_4 + 500 mg/kg Extract
Group VIII: 30mg/kg K_2BrO_4 + 100 mg/kg Vit. E
Group IX: 50 mg/kg K_2BrO_4
Group X: 50 mg/kg K_2BrO_4 + 500 mg/kg Extract
Group XI: 50mg/kg K_2BrO_4 +500mg/kg Vit. E
Group XII: 50mg/kg K_2BrO_4 + 500mg/kg Extract +500 mg/kg Vit. E
Group XIII: Food and water only

All administrations were done orally and lasted for 21 days. However, three animals were sacrificed from each group and blood was collected by cardiac puncture into EDTA containers for 8-OHdG studies. Liver, kidney and duodenum samples were also collected and preserved in 10% formalin for histological examination.

Platelets studies

Platelets (PLT) count was determined at once for each blood sample in an Automated Haematology Analyser (Mindray company, China), following the procedures outlined by the producer.

Determination of Activated Partial Thromboplastin Time (APTT)

Enough volume of reagent 1 was introduced into a clean and dry plastic tube maintained at 37^0C for immediate use. One hundred micro liters (100 µl) of test plasma was pipette into a test cuvette at 37^0C and 100 µl of pre-warmed reagent 2 was added to the content of the cuvette. The mixture was well mixed and incubated at 37^0C for 3 minutes before forcibly pipetting 100µl of pre-warmed reagent 1 into the test cuvette. A stop watch was started at the same time reagent 1 was added to note the time in seconds it took for blood to clot.This time in seconds was recorded as the APTT.

Determination of Prothrombin Time (PT)

Prothrombin reagent was dispensed into a thoroughly clean and dry plastic tube. The content of the test tube was pre-warmed at 37^0C for 10 minutes and kept for immediate use. One hundred micro liters of test plasma was introduced into a test cuvette at 37^0C and incubated for 3 minutes before adding forcibly 200µl of the pre-warmed prothrombin reagent. The stop watch was started

at the same time the prothrombin reagent was added and the time in seconds it took for blood to clot was noted.This time in seconds was recorded as the clotting time.

Statistical Analysis

Results were expressed as means ± standard error of mean (SEM). Statistical analysis was done using one-way analysis of variance (ANOVA). Significant differences were assessed at 95% level of significance between control and test groups using Duncan and LSD (Post Hoc) tests. P values less than 0.05 were considered significant. Computer software package, SPSS version 21 was employed.

RESULTS

Table 1: Effects of *Nutmeg* extract and Vitamin E on Platelet of potassium bromate induced toxicity rats.

Treatment	Platelet			Treatment			weeks	
	Week 1	Week 2	Week 3	p-value	p-value	p-value	p-value	f-value
10mg Bromate	874±5.77 ^{a}	800±11.55 ^{c}	730±1.15 ^{c}	.165	.005	.000	.000	37.595
10mg Bromate+ 500mg Nutmeg	878±9.24 ^{ab}	819±2.31 ^{d}	792±2.31 ^{d}	.200	.009	.000	.000	60.448
10mg Bromate +100mg Vit E	881±6.93 ^{ab}	820±2.31 ^{d}	794±0.58 ^{d}	.230	.003	.000	.000	111.484
10mg Bromate+ 500mg Nutmeg+100mg Vit E	888±5.77 ^{b}	822±3.46 ^{d}	797±1.73 ^{d}	.313	.005	.000	.000	137.193
30mg Bromate	821±15.59 ^{a}	720±2.89 ^{b}	719±2.31 ^{b}	.007	.001	.000	.000	40.142
30mg Bromate+ 500mg Nutmeg	826±5.77 ^{a}	723±9.81 ^{c}	721±10.97 ^{c}	.010	.000	.000	.000	43.276
30mg Bromate +300mg Vit E	830±5.77 ^{a}	738±5.77 ^{c}	728±1.15 ^{c}	.013	.000	.000	.000	139.471
30mg Bromate+ 500mg Nutmeg+300mg Vit E	859±10.97 ^{b}	756±7.51 ^{c}	738±4.62 ^{c}	.075	.000	.000	.000	64.581
50mg Bromate	774±63.51 ^{a}	715±8.66 ^{a}	551±4.04 ^{a}	.000	.000	.000	.013	9.711
50mg Bromate+ 500mg Nutmeg	780±11.55 ^{a}	717±4.04 ^{b}	591±5.2 ^{b}	.000	.003	.000	.000	157.262
50mg Bromate +500mg Vit E	800±11.55 ^{a}	719.33±2.67 ^{b}	596±1.73 ^{b}	.002	.007	.000	.000	1.373
50mg Bromate+ 500mg Nutmeg+500mg Vit E	815±66.4 ^{b}	720±8.66 ^{b}	600±11.55 ^{b}	.005	.773	.000	.023	7.543
Control	990±5.77 ^{b}	950±26.56 ^{b}	970±8.66 ^{e}				.091	7.846

In week two and three, there was significant decrease in platelet in all the treatment groups compared with control. There was no significant change in platelet after one week.

In week one there was no significant difference in platelet of rats exposed to 10, 30 and 50 mg bromate and those treated with corresponding doses of nutmeg and vitamin E alone. However the combination of doses of nutmeg and vitamin E increased platelet count. In week 2 and 3, there was significant difference in platelet of rats exposed to 10, 30, and 50 mg bromate group, and those treated with corresponding doses of nutmeg and vitamin E alone and in combination compared with rats exposed to bromate alone.

Table 2: Effects of *Nutmeg* extract and Vitamin E on average PT (s) of potassium bromate induced toxicity rats.

	AVERAGE PT (s)			treatment		Weeks		
Treatment	Week 1	Week 2	Week 3	p-value	p-value	p-value	p-value	f-value
10mg Bromate	16.8±0.12 ^{de}	17.8±0.12 ^{b}	18.9±0.12 ^{b}	.000	.000	.000	.000	82.750
10mg Bromate+ 500mg Nutmeg	15.9±0.06 ^{b}	18.5±0.17 ^{c}	19.8±0.17 ^{c}	.000	.000	.000	.000	99.947
10mg Bromate +100mg Vit E	15±0.29 ^{b}	18.3±0.17 ^{c}	19.7±0.23 ^{c}	.000	.000	.000	.000	62.820
10mg Bromate+ 500mg Nutmeg+100mg Vit E	14.4±0.17 ^{b}	18.1±0.17 ^{c}	19.6±0.23 ^{c}	.000	.000	.000	.000	119.912
30mg BromTate	18.1±0.17 ^{fg}	20±0.17 ^{e}	22.6±0.29 ^{f}	.000	.000	.000	.000	106.814
30mg Bromate+ 500mg Nutmeg	17.3±0.12 ^{de}	19.2±0.12 ^{d}	22.3±0.17 ^{de}	.000	.000	.000	.000	337.235
30mg Bromate +300mg Vit E	17±0.17 ^{de}	19.1±0.17 ^{d}	22±0.23 ^{d}	.000	.000	.000	.000	166.853
30mg Bromate+ 500mg Nutmeg+300mg Vit E	16.7±0.12 ^{cd}	19±0.06 ^{d}	21.8±0.35 ^{d}	.000	.000	.000	.000	143.195
50mg Bromate	19±0.06 ^{h}	21±0.23 ^{f}	23.7±0.29 ^{e}	.000	.000	.000	.000	119.214
50mg Bromate+ 500mg Nutmeg	18.92±0.24^{g}	20.7±0.06 ^{e}	21.6±0.29 ^{f}	.000	.000	.000	.000	102.562
50mg Bromate +500mg Vit E	18.6±0.29 ^{gh}	20.6±0.06 ^{e}	21.4±0.23 ^{f}	.000	.000	.000	.000	124.571
50mg Bromate+ 500mg	17.6±0.17 ^{ef}	20.2±0.12 ^{e}	21±0.29 ^{f}	.000	.000	.000	.000	172.737

Nutmeg+500mg Vit E								
Control	13±0.06 [a]	13.6±0.23 [a]	13.4±0.17 [a]				.112	3.231

In week one, two and three, there was significant increase in average PT in all the treatment groups compared with control. Average PT varied significantly from week one to three in all the treatment groups.

In week one two and three, there was significant difference in Average PT of rats exposed to 50 mg bromate and those treated with 500 mg nutmeg and 100 mg vitamin E alone and in combination compared with rats exposed to 50g bromated alone. The trend was similar in groups exposed to 10 and 30mg bromate compared with the respective treatment groups.

Table 3: Effects of *Nutmeg* extract and Vitamin E on Average APTT (s) of potassium bromate induced toxicity.

	AVERAGE APTT (s)			treatment			Weeks	
Treatment	**Week 1**	**Week 2**	**Week 3**	**p-value**	**p-value**	**p-value**	**p-value**	**f-value**
10mg Bromate	27±0.12 [a]	28.9±0.17 [b]	29.9±0.06 [c]	1.000	.008	.000	.000	220.500
10mg Bromate+ 500mg Nutmeg	26.9±0.12 [b]	27.7±0.12 [c]	28.6±0.23 [b]	.346	.041	.000	.000	67.875
10mg Bromate +100mg Vit E	26.5±0.06 [b]	27.6±0.23 [c]	28.5±0.17 [b]	.569	.085	.000	.000	62.654
10mg Bromate+ 500mg Nutmeg+100mg Vit E	26.2±0.23 [b]	27.1±0.12 [c]	28.2±0.17 [b]	1.000	.880	.000	.000	62.379
30mg Bromate	28.2±0.17 [d]	29.7±0.17 [e]	30.2±0.12 [d]	.000	.000	.000	.000	44.318
30mg Bromate+ 500mg Nutmeg	28±0.35 [c]	28.5±0.17 [d]	30±0.17 [c]	.000	.000	.000	.003	18.056
30mg Bromate +300mg Vit E	27.8±0.12 [bc]	28.3±0.17[d]	29.8±0.29 [c]	.000	.000	.000	.001	25.658
30mg Bromate+ 500mg Nutmeg+300mg Vit E	27.1±0.06 [abc]	28±0.23 [d]	29.7±0.17 [c]	.184	.004	.000	.000	60.346
50mg Bromate	31±0.06 [f]	32±0.12 [f]	33.5±0.29 [f]	.000	.000	.000	.000	47.500
50mg Bromate+ 500mg Nutmeg	30.8±0.17 [e]	31.7±0.17 [e]	33.2±0.17 [e]	.000	.000	.000	.000	49.000
50mg Bromate +500mg Vit E	30.3±0.35 [e]	31.5±0.29 [e]	33±0.06 [e]	.000	.000	.000	.001	32.661
50mg Bromate+ 500mg Nutmeg+500mg Vit E	30±0.17 [e]	31.1±0.32 [e]	32.8±0.35 [e]	.000	.000	.000	.002	19.303
Control	26.3±0.17 [a]	26.6±0.29 [a]	25.8±0.35 [a]				.204	2.100

In week one, two and three, there was significant increase in average APTT in all the treatment groups compared with control. Average APTT varied significantly from week one to three in all the treatment groups.

In week one two and three, there was significant difference in Average APTT of rats exposed to 50 mg bromate and those treated with 500 mg nutmeg and 100 mg vitamin E alone and in combination compared with rats exposed to 50mg bromate alone. The trend was similar in groups exposed to 10 and 30mg of bromate compared with the respective treatment groups.

DISCUSSION

The significant lowering in platelet values following treatment with potassium bromate suggests thrombocytopenia and fibrinolytic activity. These physiologic processes are of advantage in reducing the risk of developing clots within the blood vessels and its attendant cardiovascular problems. However, significant increase in platelet was observed in rats co-treated with nutmeg. This in effect suggests the ability of nutmeg to counter the toxicity of potassium bromate induced thrombocytopenia. Although the mechanism of nutmeg protection against thrombocytopenia is not clear, it is probable that nutmeg co-treatment might mitigate thrombocytopenia through the alleviation of the loss of bone marrow cells in potassium bromate -induced cytotoxicity.

The observed fall in platelets values may have accounted for the increased PT and APTT values in the bromate treated groups and the ameliorative effects offered by the extract and vitamin E may be due to the already discussed haemopoetic and antioxidant effects of these agents.

CONCLUSION

This study showed that while nutmeg and vitamin E ameliorates the toxic effects of potassium bromate, the co-administration of Vitamin E and nutmeg may offer more protection against this toxicity.

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