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EFFECTS OF CO-ADMINISTRATION OF MYRISTICA FRAGRANS HOUTT (NUT MEG) AND VITAMIN E ON OSMOLITY FRAGILITY 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 osmolity fragility 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. There was significant difference in osmotic fragility of rats exposed to 30 mg bromate group, and those treated with 500 mg nutmeg, 300 mg vitamin E and the combination in week 1, 2 and 3.There was significant difference in osmotic fragility of rats exposed to 10 mg bromate group, and those treated with 100 mg vitamin E alone and in combination with 500 mg nutmeg in week 1, 2 and 3. 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 moreprotection against these toxicity. The protective effects of both nutmeg and vitamin E are linked with their anti-oxidant properties and as such can provide protection against toxicants whose mode of action involves generation of reactive oxygen species and oxidative stress.

KEYWORDS: *myristica fragrans houtt (nut meg), vitamin E, osmolity fragility, 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).

Potassium bromate was found to be unhealthy for human consumption when a research showed that it caused renal cancer in rats after consuming Ozonated water (Achukwu *et al.*, 2009). And bromate is formed as byproduct of Ozonation of water from bromide which occurs naturally in water (WHO, 1993).

However, several researches have been carried out in different parts of the world to prove that potassium bromate is dangerous to health if consumed in food or water (Uchida et al., 2006).

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 osmolity fragility 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.

Determination of Red Blood Cells Osmotic Fragility

The method used by Adenkule and Olurenmi, (2014) was adopted. Two hundred milliliter of sodium chloride solution of pH 7.4 was prepared for each sample in concentrations ranging from 0.1-0.85%. A set of 9 test tubes, each containing 5ml of sodium chloride solution of concentration ranging from 0.1 to 0.85% were serially arranged in a test tube rack. The 10^{th} test tube was labeled 0% and contained same volume of distilled water. One set of test tubes was used to analyze each sample. A drop of the freshly collected blood was placed into each of the ten test tubes using a dropper pipette and each was mixed by gently inverting the test tubes about three times. The test tubes were then allowed to stand at room temperature for 30 minutes and then centrifuged at 3000 rpm for 10 minutes before reading the absorbance of the supernatant in each test tube on a spectrophotometer at 540nm. The same procedure was repeated for each sample collected. Percentage haemolysis was calculated using the expression:

$$\text{Percentage Haemolysis} = \frac{\text{Absorbance of test x100}}{\text{Absorbance of control}}$$

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 osmotic fragility scores of potassium bromate induced toxicity.

	0					treatment	weeks	
Treatment	**Week 1**	**Week 2**	**Week 3**	**p-value**	**p-value**	**p-value**	**p-value**	**f-value**
10mg Bromate	100.00±0.00	100.00±0.00	100.00±0.00	1.000	1.000	1.000	1.000	0.000
10mg Bromate+ 500mg Nutmeg	100.00±0.00	100.00±0.00	100.00±0.00	1.000	1.000	1.000	1.000	0.000
10mg Bromate +100mg Vit E	100.00±0.00	100.00±0.00	100.00±0.00	1.000	1.000	1.000	1.000	0.000
10mg Bromate+ 500mg Nutmeg+100mg Vit E	100.00±0.00	100.00±0.00	100.00±0.00	1.000	1.000	1.000	1.000	0.000
30mg Bromate	100.00±0.00	100.00±0.00	100.00±0.00	1.000	1.000	1.000	1.000	0.000
30mg Bromate+ 500mg Nutmeg	100.00±0.00	100.00±0.00	100.00±0.00	1.000	1.000	1.000	1.000	0.000
30mg Bromate +300mg Vit E	100.00±0.00	100.00±0.00	100.00±0.00	1.000	1.000	1.000	1.000	0.000
30mg Bromate+ 500mg Nutmeg+300mg Vit E	100.00±0.00	100.00±0.00	100.00±0.00	1.000	1.000	1.000	1.000	0.000
50mg Bromate	100.00±0.00	100.00±0.00	100.00±0.00	1.000	1.000	1.000	1.000	0.000
50mg Bromate+ 500mg Nutmeg	100.00±0.00	100.00±0.00	100.00±0.00	1.000	1.000	1.000	1.000	0.000
50mg Bromate +500mg Vit E	100.00±0.00	100.00±0.00	100.00±0.00	1.000	1.000	1.000	1.000	0.000
50mg Bromate+ 500mg Nutmeg+500mg Vit E	100.00±0.00	100.00±0.00	100.00±0.00	1.000	1.000	1.000	1.000	0.000
Control	100.00±0.00	100.00±0.00	100.00±0.00				1.000	0.000

In week one and two and three, there was no significant difference in all the treatment groups compared with the control group.

Table 2: Effects of *Nutmeg* extract and Vitamin E on osmotic fragility scores of potassium bromate induced toxicity.

	0.1			treatment			Weeks	
Treatment	**Week 1**	**Week 2**	**Week 3**	**p-value**	**p-value**	**p-value**	**p-value**	**f-value**
10mg Bromate	83.19±0.02 ^{c}	82.17±0.02 ^{c}	83.10±0.07 ^{c}	.000	.000	.000	.318	1.395
10mg Bromate+ 500mg Nutmeg	72.88±13.33 ^{b}	74.80±10.33 ^{b}	72.84±12.93 ^{b}	.000	.000	.000	.027	6.967
10mg Bromate +100mg Vit E	89.18±0.05 ^{fgh}	88.08±0.35 ^{fgh}	89.33±0.45 ^{fg}	.000	.000	.000	.249	1.770
10mg Bromate+ 500mg Nutmeg+100mg Vit E	86.31±0.01 ^{de}	85.30±0.61 ^{de}	86.29±0.21 ^{de}	.000	.000	.000	.327	1.353
30mg Bromate	87.12±0.01 ^{def}	86.02±0.31 ^{def}	87.39±0.21 ^{ef}	.000	.000	.000	.204	2.095
30mg Bromate+ 500mg Nutmeg	97.19±0.05 ^{i}	95.12±0.15 ^{j}	97.20±0.15 ^{h}	.000	.000	.000	.061	4.623
30mg Bromate +300mg Vit E	90.38±0.24 ^{gh}	91.68±0.14 ^{i}	90.19±0.14 ^{g}	.000	.000	.000	.172	2.396
30mg Bromate+ 500mg Nutmeg+300mg Vit E	88.12±0.02 ^{efg}	87.32±0.22 ^{efg}	89.11±0.32 ^{fg}	.000	.000	.000	.119	3.102
50mg Bromate	85.51±0.04 ^{cd}	84.01±0.64 ^{cd}	84.51±0.14 ^{cd}	.000	.000	.000	.168	2.441
50mg Bromate+ 500mg Nutmeg	96.65±1.69 ^{i}	95.55±1.59 ^{j}	95.65±1.59 ^{h}	.000	.000	.000	.363	1.206
50mg Bromate +500mg Vit E	91.19±0.02 ^{h}	90.29±0.32 ^{hi}	90.19±0.22 ^{g}	.000	.000	.000	.389	1.110
50mg Bromate+ 500mg Nutmeg+500mg Vit E	89.31±0.10 ^{fgh}	88.91±0.30 ^{gh}	87.31±0.10 ^{ef}	.000	.000	.000	.070	4.289
Control	45.60±1.74 ^{a}	45.90±0.84 ^{a}	44.60±1.84 ^{a}					6.753

In week one, two and three, there was significant increase in osmotic fragility in all the treatment groups compared with control except group 4 in week one and three. Osmotic fragility however did not vary significantly from week one to three in all the treatment groups.

There was significant difference in osmotic fragility of rats exposed to 50 mg bromate group, and those treated with 500 mg nutmeg, 100 mg vitamin E and the combination in week 1, 2 and 3.

In week one and two, there was significant difference in osmotic fragility of rats exposed to 30 mg bromate group, and those treated with 500 mg nutmeg alone and 300 mg vitamin E alone. In week three there was significant difference in osmotic fragility of rats exposed to 30 mg bromate group, and those treated with 500 mg nutmeg and 100 mg vitamin E.

There was significant difference in osmotic fragility of rats exposed to 10 mg bromate group, and those treated with 500 mg nutmeg, 100 mg vitamin E and the combination in week 1, 2 and 3.

Table 3: Effects of *Nutmeg* extract and Vitamin E on osmotic fragility scores of potassium bromate induced toxicity.

	0.2			treatment			weeks	
Treatment	**Week 1**	**Week 2**	**Week 3**	**p-value**	**p-value**	**p-value**	**p-value**	**f-value**
10mg Bromate	80.24±0.09a	81.04±0.59 bc	80.24±0.09 a	.000	.138	.000	.426	.987
10mg Bromate+ 500mg Nutmeg	90.20±0.03 d	89.30±0.73 f	90.20±0.03 d	.000	.000	.000	.421	1.002
10mg Bromate +100mg Vit E	84.74±1.64 bc	83.94±1.54 de	84.74±1.64 bc	.999	.997	.999	.456	.897
10mg Bromate+ 500mg Nutmeg+100mg Vit E	83.32±0.02 bc	82.42±0.32 cd	83.32±0.02 bc	.992	.992	.992	.371	1.175
30mg Bromate	84.14±0.02 bc	85.10±0.22 e	84.14±0.02 bc	1.000	.312	1.000	.342	1.292
30mg Bromate+ 500mg Nutmeg	97.47±0.67 e	98.47±0.67 h	97.47±0.67 e	.000	.000	.000	.408	1.045
30mg Bromate +300mg Vit E	83.12±0.02 a	82.02±0.12 cd	83.12±0.02 b	.957	.860	.957	.249	1.767
30mg Bromate+ 500mg Nutmeg+300mg Vit E	85.79±1.64 c	84.39±1.64 de	85.79±1.64 c	.492	.890	.492	.146	2.692
50mg Bromate	79.52±0.02 a	78.52±0.02 ab	79.52±0.02 a	.000	.000	.000	.278	1.595
50mg Bromate+ 500mg Nutmeg	96.33±0.02 e	95.73±0.10 g	96.33±0.02 e	.000	.000	.000	.693	.390
50mg Bromate +500mg Vit E	78.43±0.23 a	77.03±0.63 a	78.43±0.23 a	.000	.000	.000	.112	3.224
50mg Bromate+ 500mg Nutmeg+500mg Vit E	85.29±0.14 bc	83.20±0.94 cde	85.29±0.14 bc	.895	1.000	.895	.036	6.103
Control	84.13±0.02 bc	83.23±0.72 cde	84.13±0.02 bc				.377	1.153

In week one, two and three, there was significant increase in osmotic fragility in all the treatment groups compared with control except group 4 in week one and three. Osmotic fragility however did not vary significantly from week one to three in all the treatment groups.

There was significant difference in osmotic fragility of rats exposed to 50 mg bromate group, and those treated with 500 mg nutmeg, 500 mg vitamin E and the combination in week 1, 2 and 3.

In week one and two, there was significant difference in osmotic fragility of rats exposed to 30 mg bromate group, and those treated with 500 mg nutmeg alone and 300 mg vitamin E alone. In week three there was significant difference in osmotic fragility of rats exposed to 30 mg bromate group, and those treated with 500 mg nutmeg and 300 mg vitamin E.

There was significant difference in osmotic fragility of rats exposed to 10 mg bromate group, and those treated with 500 mg nutmeg, 100 mg vitamin E and the combination in week 1, 2 and 3.

Table 4.

	0.3					treatment	Weeks	
Treatment	**Week 1**	**Week 2**	**Week 3**	**p-value**	**p-value**	**p-value**	**p-value**	**f-value**
10mg Bromate	75.24±0.11 a	77.24±0.51 a	75.24±0.11 a	.000	.000	.000	.027	6.941
10mg Bromate+ 500mg Nutmeg	88.21±0.04 f	87.29±0.14 d	88.21±0.04 f	.000	.004	.000	.393	1.095
10mg Bromate +100mg Vit E	82.28±0.20 c	81.27±0.40 b	82.76±0.10 c	.984	.016	1.000	.156	2.574
10mg Bromate+ 500mg Nutmeg+100mg Vit E	84.36±0.01 cde	83.06±0.21 bc	84.26±0.11 cde	.854	.939	.910	.189	2.231
30mg Bromate	85.51±0.63 de	84.41±0.53 c	85.41±0.53 de	.079	1.000	.105	.290	1.532
30mg Bromate+ 500mg Nutmeg	92.18±0.03 g	91.16±0.13 e	91.08±0.03 g	.000	.000	.000	.328	1.349
30mg Bromate +300mg Vit E	84.30±0.18 cde	83.35±0.28 bc	83.30±0.18 cd	.890	.995	1.000	.325	1.361
30mg Bromate+ 500mg Nutmeg+300mg Vit E	79.15±0.03 b	78.35±0.13 a	79.12±0.13 b	.000	.000	.000	.425	.992
50mg Bromate	78.55±0.03 b	78.05±0.13 a	78.50±0.53 b	.000	.000	.000	.705	.370
50mg Bromate+ 500mg Nutmeg	93.31±0.01 g	92.41±0.21 e	93.31±0.11 g	.000	.000	.000	.443	.936
50mg Bromate +500mg Vit E	86.21±0.01 ef	87.11±0.71 d	86.21±0.21 ef	.008	.009	.007	.397	1.082
50mg Bromate+ 500mg Nutmeg+500mg Vit E	83.38±0.03cd	82.37±0.13 bc	83.38±0.23 cd	1.000	.401	1.000	.300	1.479
Control	83.15±0.01 cd	84.10±0.31 c	83.15±0.41 cd					1.295

In week one, two and three, there was significant increase in osmotic fragility in all the treatment groups compared with control except group 3, 4, 5, 7 and 12. Osmotic fragility however did not vary significantly from week one to three in all the treatment groups except group 1.

There was significant difference in osmotic fragility of rats exposed to 50 mg bromate group, and those treated with 500 mg nutmeg, 500 mg vitamin E and the combination in week 1, 2 and 3.

There was significant difference in osmotic fragility of rats exposed to 30 mg bromate group, and those treated with 500 mg nutmeg, 300 mg vitamin E and the combination in week 1, 2 and 3.

There was significant difference in osmotic fragility of rats exposed to 50 mg bromate group, and those treated with 500 mg nutmeg, 100 mg vitamin E and the combination in week 1, 2 and 3.

Table 5: Effects of *Nutmeg* extract and Vitamin E on osmotic fragility scores of potassium bromate induced toxicity.

	0.4			treatment			weeks	
Treatment	**Week 1**	**Week 2**	**Week 3**	**p-value**	**p-value**	**p-value**	**p-value**	**f-value**
10mg Bromate	68.12±0.04 ^{b}	69.12±0.04 ^{bc}	68.12±0.04 ^{ab}	.094	1.000	.940	.200	2.134
10mg Bromate+ 500mg Nutmeg	80.21±0.04 ^{g}	80.21±0.04 ^{g}	80.21±0.04 ^{e}	.000	.000	.000	1.000	0.000
10mg Bromate +100mg Vit E	72.09±0.04 ^{de}	71.09±0.04 ^{cd}	71.09±0.14 ^{c}	.105	.105	.069	.221	1.960
10mg Bromate+ 500mg Nutmeg+100mg Vit E	70.32±0.02 ^{cd}	71.32±0.02 ^{d}	68.32±0.02 ^{ab}	1.000	.046	.991	.005	14.287
30mg Bromate	68.50±0.30 ^{bc}	69.50±0.30 ^{cd}	67.50±2.30 ^{ab}	.305	1.000	.381	.033	6.393
30mg Bromate+ 500mg Nutmeg	83.16±0.03 ^{h}	81.16±0.03 ^{g}	82.16±1.03 ^{ef}	.000	.000	.000	.065	4.444
30mg Bromate +300mg Vit E	71.17±0.02 ^{de}	70.17±0.02 ^{cd}	69.17±0.12 ^{bc}	.855	.856	1.000	.036	6.092
30mg Bromate+ 500mg Nutmeg+300mg Vit E	68.13±0.02 ^{b}	67.13±0.02 ^{b}	68.03±0.22 ^{ab}	.098	.098	.891	.218	1.982
50mg Bromate	65.50±0.03 ^{bc}	64.50±0.03 ^{a}	66.40±0.23 ^{a}	.000	.000	.008	.033	6.322
50mg Bromate+ 500mg Nutmeg	85.30±1.03 ^{h}	86.30±1.03 ^{h}	84.20±1.13 ^{f}	.000	.000	.000	.063	4.552
50mg Bromate +500mg Vit E	73.22±0.02 ^{e}	77.22±0.02 ^{f}	73.52±0.22 ^{d}	.001	.000	.000	.001	26.702
50mg Bromate+ 500mg Nutmeg+500mg Vit E	75.40±0.02 ^{f}	74.70±0.04 ^{e}	75.20±0.22 ^{d}	.000	.000	.000	.537	.691
Control	70.12±0.02 ^{bcd}	69.12±0.03 ^{bc}	69.02±0.12 ^{bc}				.181	2.303

In week one, two and three, there was significant increase in osmotic fragility in all the treatment groups compared with control except group 1, 3, 4, 5, 7 and 8. Osmotic fragility however did not vary significantly from week one to three in all the treatment groups except group 4, 5. 7. 9, and 11.

There was significant difference in osmotic fragility of rats exposed to 50 mg bromate group, and those treated with 500 mg nutmeg, 500 mg vitamin E and the combination in week 1, 2 and 3.

In week one and two, there was significant difference in osmotic fragility of rats exposed to 30 mg bromate group, and those treated with 500 mg nutmeg and 300 mg vitamin E alone and the combination. In week three there was significant difference in osmotic fragility of rats exposed to 30 mg bromate group, and those treated with 500 mg nutmeg and 300 mg vitamin E.

There was significant difference in osmotic fragility of rats exposed to 10 mg bromate group, and those treated with 500 mg nutmeg, 100 mg vitamin E and the combination in week 1, except in week 2 and 3were only nutmeg alone and the combination with 100 mg vitamin E was different from rats 10 mg bromate alone.

Table 6.

	0.5			treatment			Weeks	
Treatment	**Week 1**	**Week 2**	**Week 3**	**p-value**	**p-value**	**p-value**	**p-value**	**f-value**
10mg Bromate	30.14±0.03 ^{ef}	32.14±0.13 ^{de}	32.14±0.13 ^{f}	.000	.000	.000	.000	40.345
10mg Bromate+ 500mg Nutmeg	42.21±0.03 ^{h}	32.21±0.23 ^{e}	42.21±0.23 ^{i}	.000	.000	.000	.000	652.053
10mg Bromate +100mg Vit E	35.09±0.04 ^{g}	33.09±0.14 ^{ef}	35.09±0.14 ^{h}	.000	.000	.000	.001	33.731
10mg Bromate+ 500mg Nutmeg+100mg Vit E	30.30±0.03 ^{e}	29.30±0.23 ^{e}	30.30±0.33 ^{e}	.000	.000	.000	.010	11.133
30mg Bromate	29.14±0.01 ^{cd}	29.74±0.51 ^{c}	29.14±0.21 ^{cd}	.000	.000	.000	.073	4.182
30mg Bromate+ 500mg Nutmeg	46.15±0.06 ^{h}	45.16±0.26 ^{h}	46.15±0.16 ^{j}	.000	.000	.000	.060	4.668
30mg Bromate +300mg Vit E	30.15±0.03 ^{de}	31.15±0.73 ^{d}	30.15±0.23 ^{de}	.000	.000	.000	.010	10.759
30mg Bromate+ 500mg Nutmeg+300mg Vit E	28.12±0.04 ^{c}	29.12±0.14 ^{c}	28.12±0.14 ^{c}	.000	.000	.000	.007	12.349
50mg Bromate	26.51±0.04 ^{b}	27.79±0.24 ^{b}	26.51±0.14 ^{b}	.000	.000	.000	.002	22.569

50mg Bromate+ 500mg Nutmeg	49.29±0.03 [j]	49.29±0.33 [i]	49.29±0.33 [k]	.000	.000	.000	1.000	0.000
50mg Bromate +500mg Vit E	33.38±0.11 [f]	33.38±0.11 [f]	33.38±0.11 [g]	.000	.000	.000	1.000	0.000
50mg Bromate+ 500mg Nutmeg+500mg Vit E	35.39±0.03 [g]	35.39±0.03 [g]	35.39±0.13 [h]	.000	.000	.000	1.000	0.000
Control	23.13±0.01 [a]	23.13±0.01 [a]	23.13±0.21 [a]	.000	.000	.000	1.000	0.000

In week one, two and three, there was significant increase in osmotic fragility in all the treatment groups compared with control except group 1, 3, 4, 5, 7 and 8. Osmotic fragility however did not vary significantly from week one to three in all the treatment groups except group 4, 5. 7. 9, and 11.

There was significant difference in osmotic fragility of rats exposed to 50 mg bromate group, and those treated with 500 mg nutmeg, 500 mg vitamin E and the combination in week 1, 2 and 3.

In week one and two, there was significant difference in osmotic fragility of rats exposed to 30 mg bromate group, and those treated with 500 mg nutmeg alone and in combination 300 mg vitamin E alone. In week three there was significant difference in osmotic fragility of rats exposed to 30 mg bromate group, and those treated with 500 mg nutmeg, 300 mg vitamin E and the combination.

There was significant difference in osmotic fragility of rats exposed to 10 mg bromate group, and those treated with 500 mg nutmeg, 100 mg vitamin E and the combination in week 1, 2 and 3.

Table 7: Effects of *Nutmeg* extract and Vitamin E on osmotic fragility scores of potassium bromate induced toxicity.

		0.6		treatment	weeks			
Treatment	**Week 1**	**Week 2**	**Week 3**	**p-value**	**p-value**	**p-value**	**p-value**	**f-value**
10mg Bromate	18.18±0.05 [c]	18.18±0.05 [bc]	18.18±0.05 [c]	.000	.000	.000	1.000	0.000
10mg Bromate+ 500mg Nutmeg	23.35±0.14 [f]	23.35±0.14 [g]	23.35±0.14 [f]	.000	.000	.000	1.000	0.000
10mg Bromate +100mg Vit E	28.03±0.01 [g]	28.03±0.01 [h]	28.03±0.01 [g]	.000	.000	.000	1.000	0.000
10mg Bromate+ 500mg Nutmeg+100mg Vit E	20.20±0.02 [e]	20.20±0.02 [e]	20.20±0.02 [e]	.000	.000	.000	1.000	0.000
30mg Bromate	19.15±0.03 [d]	19.15±0.03 [d]	19.15±0.03 [d]	.000	.000	.000	1.000	0.000
30mg Bromate+ 500mg Nutmeg	28.13±0.02 [g]	28.13±0.02 [h]	28.13±0.02 [g]	.000	.000	.000	1.000	0.000
30mg Bromate +300mg Vit E	18.14±0.02 [c]	18.14±0.02 [bc]	18.14±0.02 [bc]	.000	.000	.000	1.000	0.000
30mg Bromate+ 500mg Nutmeg+300mg Vit E	20.15±0.02 [e]	20.15±0.02 [e]	20.15±0.02 [e]	.000	.000	.000	1.000	0.000
50mg Bromate	17.50±0.03 [b]	17.50±0.23 [b]	17.50±0.03 [b]	.000	.000	.000	1.000	0.000
50mg Bromate+ 500mg Nutmeg	32.12±0.02 [h]	32.12±0.02 [i]	32.12±0.02 [h]	.000	.000	.000	1.000	0.000
50mg Bromate +500mg Vit E	23.14±0.02 [f]	22.14±0.52 [f]	23.14±0.02 [f]	.000	.000	.000	.002	19.217
50mg Bromate+ 500mg Nutmeg+500mg Vit E	20.13±0.02e	20.13±0.02 [e]	20.13±0.02 [a]	.000	.000	.000	1.000	0.000
Control	14.12±0.02 [a]	13.12±0.72 [a]	14.12±0.02 [a]					52.550

In week one, two and three, there was significant increase in osmotic fragility in all the treatment groups compared with control. Osmotic fragility however did not vary significantly from week one to three in all the treatment groups except group 12.

There was significant difference in osmotic fragility of rats exposed to 50 mg bromate group, and those treated with 500 mg nutmeg, 500 mg vitamin E and the combination in week 1, 2 and 3.

There was significant difference in osmotic fragility of rats exposed to 30 mg bromate group, and those treated with 500 mg nutmeg, 300 mg vitamin E and the combination in week 1, 2 and 3.

There was significant difference in osmotic fragility of rats exposed to 50 mg bromate group, and those treated with 500 mg nutmeg, 100 mg vitamin E and the combination in week 1, 2 and 3.

Table 8.

		0.7		treatment	weeks			
Treatment	**Week 1**	**Week 2**	**Week 3**	**p-value**	**p-value**	**p-value**	**p-value**	**f-value**
10mg Bromate	17.17±0.03 ^{e}	18.16±0.63 ^{h}	17.17±0.03 ^{e}	.000	.000	.000	.001	31.981
10mg Bromate+ 500mg Nutmeg	20.23±0.04 ^{f}	21.83±0.04 ^{j}	20.23±0.04 ^{f}	.000	.000	.000	.000	59.303
10mg Bromate +100mg Vit E	16.02±0.02 ^{d}	15.42±0.02 ^{d}	16.02±0.02 ^{d}	.000	.000	.000	.005	14.380
10mg Bromate+ 500mg Nutmeg+100mg Vit E	14.21±0.03 ^{a}	14.21±0.03 ^{c}	14.21±0.03 ^{c}	.000	.000	.000	1.000	0.000
30mg Bromate	17.16±0.03 ^{e}	16.16±0.13 ^{ef}	17.16±0.03 ^{e}	.000	.000	.000	.000	35.291
30mg Bromate+ 500mg Nutmeg	21.11±0.05 ^{g}	20.11±0.15 ^{i}	21.11±0.05 ^{g}	.000	.000	.000	.002	23.154
30mg Bromate +300mg Vit E	16.11±0.03 ^{d}	15.91±0.03 ^{de}	16.11±0.03 ^{d}	.000	.000	.000	.286	1.554
30mg Bromate+ 500mg Nutmeg+300mg Vit E	14.13±0.02 ^{c}	14.12±0.82 ^{c}	14.13±0.02 ^{c}	.000	.000	.000	.995	.005
50mg Bromate	13.46±0.03 ^{b}	12.46±0.09 ^{b}	13.46±0.03 ^{b}	.000	.000	.000	.000	57.960
50mg Bromate+ 500mg Nutmeg	23.14±0.03 ^{h}	22.24±0.23 ^{j}	23.14±0.03 ^{h}	.000	.000	.000	.004	15.522
50mg Bromate +500mg Vit E	16.23±0.02 ^{d}	16.83±0.22 ^{g}	16.23±0.02 ^{d}	.000	.000	.000	.006	13.332
50mg Bromate+ 500mg Nutmeg+500mg Vit E	17.30±0.20 ^{e}	16.60±0.40 ^{fg}	17.30±0.20 ^{e}	.000	.000	.000	.003	16.817
Control	10.20±0.06 ^{a}	11.20±0.16 ^{a}	10.20±0.06 ^{a}	.000	.000	.000	.000	89.950

In week one, two and three, there was significant increase in osmotic fragility in all the treatment groups compared with control. Osmotic fragility however did not vary significantly from week one to three in all the treatment groups except group 12.

There was significant difference in osmotic fragility of rats exposed to 50 mg bromate group, and those treated with 500 mg nutmeg, 500 mg vitamin E and the combination in week 1, 2 and 3.

There was significant difference in osmotic fragility of rats exposed to 30 mg bromate group, and those treated with 500 mg nutmeg, 300 mg vitamin E and the combination in week 1, 2 and 3.

There was significant difference in osmotic fragility of rats exposed to 50 mg bromate group, and those treated with 500 mg nutmeg, 100 mg vitamin E and the combination in week 1, 2 and 3.

Table 9: Effects of *Nutmeg* extract and Vitamin E on osmotic fragility scores of potassium bromate induced toxicity

		0.8		treatment			weeks	
Treatment	**Week 1**	**Week 2**	**Week 3**	**p-value**	**p-value**	**p-value**	**p-value**	**f-value**
10mg Bromate	11.15±0.03 ^{e}	10.16±0.33 ^{d}	11.10±0.03 ^{e}	.000	.000	.000	.000	83.562
10mg Bromate+ 500mg Nutmeg	13.23±0.02 ^{g}	12.23±0.22 ^{f}	13.30±0.02 ^{g}	.000	.000	.000	.000	60.043
10mg Bromate +100mg Vit E	10.05±0.02 ^{d}	11.05±0.12 ^{e}	10.07±0.02 ^{d}	.000	.000	.000	.000	92.562
10mg Bromate+ 500mg Nutmeg+100mg Vit E	10.22±0.04 ^{d}	09.22±0.14 ^{c}	10.33±0.04 ^{d}	.000	.000	.000	.000	102.074
30mg Bromate	11.15±0.02 ^{e}	12.15±0.32 ^{f}	11.19±0.02 ^{d}	.000	.000	.000	.000	75.706
30mg Bromate+ 500mg Nutmeg	15.21±0.03 ^{h}	15.11±0.13 ^{h}	15.20±0.03 ^{h}	.000	.000	.000	.667	.434
30mg Bromate +300mg Vit E	10.16±0.03 ^{d}	10.17±0.23 ^{d}	10.19±0.03 ^{a}	.000	.000	.000	.990	.010
30mg Bromate+ 500mg Nutmeg+300mg Vit E	9.12±0.01 ^{c}	8.62±0.08 ^{b}	8.82±0.01 ^{c}	.000	.000	.000	.001	31.165
50mg Bromate	8.21±0.03 ^{b}	8.31±0.08 ^{b}	8.31±0.03 ^{a}	.000	.000	.000	.302	1.472
50mg Bromate+ 500mg Nutmeg	17.16±0.03 ^{i}	18.09±0.05 ^{i}	17.16±0.03 ^{i}	.000	.000	.000	.001	28.321
50mg Bromate +500mg Vit E	12.16±0.02 ^{f}	13.15±0.07 ^{g}	13.16±0.02 ^{f}	.000	.000	.000	.000	62.739
50mg Bromate+ 500mg Nutmeg+500mg Vit E	10.09±0.02 ^{d}	11.09±0.12 ^{e}	11.05±0.02 ^{d}	.000	.000	.000	.000	91.854
Control	7.21±0.01 ^{a}	7.21±0.01 ^{a}	7.21±0.01 ^{a}	.000	.000	.000	1.000	0.000

In week one, two and three, there was significant increase in osmotic fragility in all the treatment groups

compared with control. Osmotic fragility however did not vary significantly from week one to three in all the treatment groups except group 6, 7, and 9.

There was significant difference in osmotic fragility of rats exposed to 50 mg bromate group, and those treated with 500 mg nutmeg, 500 mg vitamin E and the combination in week 1, 2 and 3.

There was significant difference in osmotic fragility of rats exposed to 30 mg bromate group, and those treated with 500 mg nutmeg, 300 mg vitamin E and the combination in week 1, 2 and 3.

There was significant difference in osmotic fragility of rats exposed to 50 mg bromate group, and those treated with 500 mg nutmeg, 100 mg vitamin E and the combination in week 1, 2 and 3 except.

Table 10:

	0.85			treatment			weeks	
Treatment	**Week 1**	**Week 2**	**Week 3**	**p-value**	**p-value**	**p-value**	**p-value**	**f-value**
10mg Bromate	8.26±0.10 [c]	9.26±0.10 [f]	8.66±0.10 [f]	.000	.000	.000	.000	99.576
10mg Bromate+ 500mg Nutmeg	8.23±0.02 [c]	8.20±0.02 [e]	8.33±0.02[e]	.000	.000	.000	.211	2.040
10mg Bromate +100mg Vit E	7.10±0.03 [b]	7.60±0.03 [d]	7.10±0.03 [c]	.000	.000	.000	.000	47.295
10mg Bromate+ 500mg Nutmeg+100mg Vit E	7.21±0.03 [b]	7.30±0.03 [c]	7.1±0.03 [c]	.000	.000	.000	.040	5.800
30mg Bromate	6.19±0.09 [a]	6.20±0.19 [a]	6.29±0.09 [b]	.000	.000	.000	.177	2.347
30mg Bromate+ 500mg Nutmeg	11.20±0.02 [e]	11.25±0.12 [h]	11.20±0.82 [i]	.000	.000	.000	.825	.199
30mg Bromate +300mg Vit E	8.16±0.02 [c]	8.20±0.09 [e]	8.06±0.02 [d]	.000	.000	.000	.176	2.354
30mg Bromate+ 500mg Nutmeg+300mg Vit E	7.14±0.02 [b]	7.54±0.07 [d]	7.04±0.02 [c]	.000	.000	.000	.000	40.027
50mg Bromate	8.21±0.01 [c]	8.31±0.41 [e]	8.41±0.01 [a]	.000	.000	.000	.068	4.344
50mg Bromate+ 500mg Nutmeg	13.12±0.03 [f]	14.18±0.63 [i]	13.12±0.13 [j]	.000	.000	.000	.000	61.811
50mg Bromate +500mg Vit E	9.07±0.04 [d]	9.99±0.14 [g]	9.07±0.14 [g]	.000	.000	.000	.000	96.062
50mg Bromate+ 500mg Nutmeg+500mg Vit E	9.15±0.08 [d]	9.05±0.48 [f]	9.75±0.28 [h]	.000	.000	.000	.000	49.485
Control	6.22±0.03 [a]	6.82±0.33 [b]	5.23±0.13 [a]	.000	.000	.000	.000	515.514

In week one, two and three, there was significant increase in osmotic fragility in all the treatment groups compared with control. Osmotic fragility however did not vary significantly from week one to three in all the treatment groups except group 2, 5, 6, 7, and 9.

There was significant difference in osmotic fragility of rats exposed to 50 mg bromate group, and those treated with 500 mg nutmeg, 500 mg vitamin E and the combination in week 1, 2 and 3.

There was significant difference in osmotic fragility of rats exposed to 30 mg bromate group, and those treated with 500 mg nutmeg, 300 mg vitamin E and the combination in week 1, 2 and 3.

There was significant difference in osmotic fragility of rats exposed to 10 mg bromate group, and those treated with 100 mg vitamin E alone and in combination with 500 mg nutmeg in week 1, 2 and 3.

DISCUSSION

Oxidative agents are established causes of haemolysis (Akomas et al., 2015; Devasagayam *et al*., 2004; Abel – Aziz *et al*., 2014). This oxidative effect of potassium bromate may be the reason for the increased osmotic fragility in the groups administered the agent. Co-treatment with nutmeg and vitamin E may have increased the integrity of the RBC cell membranes and reduce haemolysis. Vitamin E plays an important role in stabilizing the structure of membranes by forming complexes with destabilising molecules so as to prevent disturbance of the amphipathic balance within the structure (Wang, 1999). Adenkola and Oluremi (2014) indicated that normal function of the erythrocytes depends highly on their membrane stability and ability to resist lysis. Reactive oxygen species can attack erythrocyte membrane and reduce its ability to resist lysis (Devasagayam *et al*., 2004). Thus the higher membrane stability observed in rats treated with nutmeg may be attributed to the antioxidant potentials of the nutmeg extract. Antioxidants have greatly been implicated in the prevention of cellular damage and generally consolidate the integrity of erythrocyte membrane by reducing their oxidative damage due the impact of free radicals (Adenkola and Oluremi 2014). Thus, the extract may have inhibited osmotic fragility effects of potassium bromate via this mechanism. Also.

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 moreprotection against these toxicity. The protective effects of both nutmeg and vitamin E is linked with their anti-oxidant properties and as such can provide protection against toxicants whose mode of action involves generation of reactive oxygen species and oxidative stress.

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