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Effect of Potassium Nitrate (KNO3) and non-Nitrate Liver Meat Delicacy on Body Weight and Haematological Parameters of Rattus Norvegicus

Edoga C.O[1*], Njoku O.O[2] and Amadi E.N[3]

[1.] Department of Zoology and Environmental Biology, University of Nigeria, Nsukka, Enugu State NIGERIA.
[2.] Department of Applied Biology, Federal University of Technology, Owerri, Imo State NIGERIA.
[3.] Department of Zoology, Nnamdi Azikiwe University, Awka, Anambra State, NIGERIA.

*Corresponding Author: cyrilkachiedoga@gmail.com

Abstract: This research is aimed at assessing the effects of potassium nitrate on the body weights and the blood parameters of wistar rats. Thirty wistar rats of both sexes weighing between 142-147g, aged 3 months old were kept in Animal House of the Department of Zoology and Environmental Biology, University of Nigeria, Nsukka for 4 weeks for acclimatization before the commencement of the study. The study rats were divided into 5 groups (A to E) of six rats per group. Group A to D were treated with test substances while E which received no test substance served as control. They were fed orally with graded doses of potassium nitrate and liver meat (group A=250mg nitrate, B=250mg liver meat, C=125mg nitrate/ liver meat, D=125mg liver meat/kg/body weight respectively) once daily for 60 days in lower concentration compared to an oral LD50 of 500mg/kg/body weight was determined using Field and Wisconsin as modified by Shetty and Arika. At the end of the experiment (60 days), the rats were bled from the retro-bulbar plexus of medial centus and about 2.5ml of blood were collected from the five groups into K3-EDTA anticoagulant containers for hematological analyses. The analyses shows the significant decrease in the body weight of the rats treated with potassium nitrate when compared to other groups ($P<0.05$). The total white blood cell counts significantly decreased in potassium nitrate alone treated rats when compared with other groups ($P<0.05$). The platelet counts showed increase in potassium liver meat treated rats when compared with the other groups ($P<0.05$). The total blood films of the potassium nitrate treated rats showed stomatocytes, poikilocytes, crenated red cell, irregular contracted red cell and hypochromasia when compared with the control.
Keywords: Pottasium Nitrate, Rattus Norvrgicus, Leucocytes, Thrombocytes, Stomatocytes, Poikilocytes, Anaemia, Hypochromsia.

INTRODUCTION

The use of potassium nitrate in meat preservation is important to the meat industry; and nitrate is regarded as indispensable in most preservation chambers because of its ability to inhibit growth and toxin formation of Clostridium botulinum[1]. Nitrate is obtained both as a natural food component, an additive to fish; meat products and also to cheese milk in certain part of the world. The effect of nitrate depends on its reduction by microorganisms to nitrite, which acts as preservatives, a colour fixing agents, a flavoring substance. Nitrate occurs in the environment, in air, food (particularly in vegetables and fruits) and water. In nature, it exist either as ionic form or as conjugate of a compound[2]. Most of the ionic nitrate salts are soluble in water at standard temperature and pressure. Chemically, a nitrate is functional group with general chemical formula RONO2 where R stands for any organic residue. They are the esters of nitric acid and alcohols formed by the reactions of methanol and nitric acid[3], the nitrate of tartaric acid[4] and the appropriately named nitroglycerin. It has earlier been documented that nitrates are produced by natural biological and physical oxidations and therefore are ubiquitous in the environment[5]. Most nitrate compounds are strong oxidizing agents and some can react violently with oxidizable substances and may explode if exposed to heat or shock[6]. Organic molecules containing nitrates groups are manufactured primarily for explosives or for their pharmacological effect[7]. Ammonia from waste and septic tank can be oxidized to nitrate surface and ground water especially near areas of concentrated animal populations such as feedlots and diary barns[8]. The ground water in the agricultural southern eastern United states is not very vulnerable to NO3 contamination, whereas, it is a serious problem in the part of the mid- west[9]. In addition to drinking water, dietary source of nitrates include compounds used in meat curing processes and nitrates in vegetables. High concentration of nitrates in vegetables can reflect the over application of nitrate containing fertilizers. On-farm nitrogen tests improve fertilizer efficiency and protect underground water[10]. Inorganic nitrates can be reduced to nitrate (NO2) by the micro flora in saliva and the gastrointestinal tract. Nitrate is thought to be respposible for most of the toxic digestion[11]. Whether dietary exposure to nitrate metabolites is detrimental or beneficial to human health has long been a matter of controversy. In spite of no constant evidence, nitrate metabolites are associated with the formation of carcinogenic-nitrosamines and gastric cancer[12]. However, recent studies demonstrate that ingested nitrate plays a host defense against gastrointestinal pathogenic bacteria[13]. The justification of this present research is to assess the inherent damages of nitrate to human especially blood parameters since fairly used refrigerators or freezers which implored the application of nitrate to enhance preservation are commonly used in Africa and Nigeria in particular.

MATERIAL AND METHODS

Thirty 90-day old wistar rats (Rattus norvegicus) weighing approximately 146g were used for this experiment. The rats were marked for identification and held in stainless wire-rats-cages in clean experimental animal house of the Department of Zoology and Environmental Biology, University of Nigeria, Nsukka where the physical changes of the study rats were observed and the body weight recorded. The cages were labeled A to E corresponding to the five groups and each group has six rats. Diet 1 was given to six rats in cage A which contained complete pelleted standard Top feed and equally treated with 250mg KNO3. Diet 2 was given to six rats in cage B which contained complete pelleted standard Top feed and also fed with 250mg of liver meat powder. Diet 3 was used to feed six rats in cage C which contained pelleted standard Top feed and 125mg KNO3.125mg liver meat powder. Diet 4 was used to feed six rats in cage D which contained complete pelleted standard Top feed and 125mg of liver meat powder and diet 5 was used to feed rats in cage E which contained Top feed without any treatment and/or supplement, served as control. Potassium nitrate (KNO3) used for this study was procured from chemical/reagent store of Ariken's scientific co. E16 Head Bridge Market, Onitsha, Nigeria. The KNO3 was weighed at the Biochemistry Department, Nnamdi Azikiwe University, Awka, with electronic balance (Mettler®). The liver meat was bought from Ogige Market Nsukka, Enugu State NIGERIA. It was dried in hot air oven at 160o for 10 minutes thoroughly and grinded with grinding machine. The liver meat was turned into powered form. Then the required quantity measured with electronic balance ready for the experiment. At the end of 60 days experiment, the rats were bled from the retrobulbar plexus of medial cantus and about 2.5 ml of blood sample were collected from the five groups into K3- EDTA anticoagulant containers for haematological analyses.

Statistical Analysis: The changes in mean weight and blood parameters of the study rats were determined using 2-way ANOVA and data were reported as mean value.

RESULTS

Table 1. Body Weight (g) of the experimental Rats

Groups	Group A (Infected with250 mg of KNO_3)	Group B (Infected with 250mg of liver meat)	Group C (125 mg nitrate nad liver meat)	Group D (125mg of liver meat)	Group E (Control)
	130.5	150.0	150.0	150.5	155.5
	130.0	165.0	160.0	160.0	154.5
	129..5	150.5	165.0	170.5	155.5
	-	170.5	155.0	165.5	152.5
	-	150.5	-	162.0	155.5
	-	-	-	-	160.5
Total	390.0	787.0	630.0	808.5	934.0
Mean	130.0g	157.5g	157.5g	161.7g	155.6g

Table 2. White blood cell count ($X10^9/l$) of the experimental Rats

Groups	Group A	Group B	Group C)	Group D	Group E (Control)
	4.30	5.00	3.50	5.80	5.80
	3.70	4.20	5.10	3.60	5.60
	3.9	14.50	5.30	8.60	5.50
	-	6.00	7.8	5.10	5.80

	-	6.2	-	4.9	5.50
	-	-	-	-	5.2
Total	11.90	35.90	21.70	28.00	33.40
Mean	3.96	7.18	5.43	5.60	5.56

Table 3. Platelet Count ($X10^9/L$) of the experimental Rats

Groups	Group A	Group B	Group C	Group D	Group E (Control)
	136.0	162.0	177.0	156.0	139.0
	128.0	152.0	160.0	175.0	125.0
	140.0	140.0	189.0	179.0	135.0
	-	147.0	172.0	125.0	140.0
	-	168.0	-	122.0	142.0
	-	-	-	-	135.0
Total	404.0	769.0	698.0	757.0	816.0
Mean	134.5	153.8	174.5	151.4	136.0

Table 4. Packed cell volume count (%) of the experimental Rats

Groups	Group A	Group B	Group C	Group D	Group E (Control)
	35.1	45.2	46.2	46.2	48.0
	37.2	48.2	49.2	49.3	50.2
	36.5	49.3	50.3	47.6	52.4
	-	50.3	50.3	49.1	49.1
	-	50.5	-	45.0	50.2
	-	-	-	-	50.3
Total	108.8	243.5	196.0	237.2	300.2
Mean	36.26%	48.70%	49.00%	47.44%	50.03%

Table5. Haemoglobin (g/dl) of the experimental Rats

Groups	Group A	Group B	Group C	Group D	Group E (Control)
	8.9	11.5	11.8	11.8	12.3
	9.4	12.3	13.3	12.5	12.8
	9.2	12.5	13.3	12.1	13.3
	-	12.8	12.8	12.5	12.5
	-	12.8	-	11.2	12.8
	-	-	-	-	12.8
Total	27.5	61.9	51.2	60.1	76.5
Mean	9.16	12.38	12.80	12.02	12.75

Table 6. Red Blood Cell Count ($X10^{12}$) of the experimental Rats

Groups	Group A	Group B	Group C	Group D	Group E (Control)
	3.37	4.55	4.82	4.70	5.17
	3.90	5.57	5.62	4.76	4.19
	3.23	4.67	4.90	5.67	5.90
	-	4.19	5.12	6.87	5.80
	-	5.15	-	4.01	5.72
	-	-	-	-	5.12
Total	10.50	24.28	20.46	26.01	31.90
Mean	3.50	4.86	5.12	5.20	5.32

DISCUSSION

The result obtained showed that there was progressive decrease in body weight gain in treatment groups, but was statistically significant (P<0.05) only in group A 130.00+ 1.6g/kg when compared with other groups body weight, see table 1. The group of rats fed with potassium nitrate alone progressively became severely lean, anaemic and began to die by the third week and only three rats used finally. The decrease body weight gain in the nitrate-treated group is in agreement with the earlier result[14]. Table-2 shows that there was a decrease in the total white blood cell count in the nitrate alone treated rats when compared with the nitrate-treated/liver meat and control group. The mean total white cell counts were 3.96X109/l for group B, 7.18X9/l for group C, 5.60X109/l for group D and 5.56X109/l for group E which served as control. The above feature could be as a result of the interference in the conjugation of iron necessary for haemoglobin formation. Table 3,4,5, and 6 showed that there were progressive decrease in the platelet count value, packed cell volume, haemoglobin and red blood cell count in the treatment groups, but were statistically significant (P<0.005) only in group A when compared with other groups platelet count, packed cell volume, haemoglobin and red blood cell count. This report is consistent with the experiment conducted with Long-Evans rats. Animals that were receiving nitrate had lower body and liver weights, decreased vitamins E levels in serum and higher red blood cells reduced glutathione levels while the lungs of all animal exhibited severe lesions[15].

From the research, we thus conclude that the indiscriminate and uncontrollable use of nitrate as a preservative on consumables portends a serious health damages on human blood parameters and some visceral organs.

REFERENCES

1. FAO/WHO. (1996). Evaluation of certain food additives. Twentieth report of the joint ,FAO/WHO. Expert committee on food additives. FAO food and nutrition Series no, 11996WHO tech. rep, Ser, 599.
2. Marschner, H. (1999). Mineral nutrition of higher plants. Academic Press London, 889.
3. Black, A.P., Babers, F.H. (1989). Methyl nitrate (http:www.org/orgsyn/Prep. content.asp? Org. synth coll, 12: 412.
4. Snyder, H.R., Hendricks, R.G, Brooks, L.A. (1992). Imidazole, Org. Synth: College, 3: 471
5. Ridder, W.E. & Oehme, F.W. (1994). Nitrates as an environmental animal and human, hazard. Clin.Toxical .7(2): 145-159.
6. Sax, N.I. & R.J. Lewis. (1989). Dangerous properties of Industrial Materials. 7th Ed, Van Nostrands, Reinbold, New York. 2:2494.

7. Stokinger, H.E. (1982). Aliphatic nitro compounds, nitrates, nitrites. In: Patty's,industrial Hygiene and Toxicology.2A Eds. G Clayton and F.E.Clayton, John Wiley & Sons. New York.2: 4169-4201.
8. Bouchard, D.C., Williams, M.K. & Surampalli, R.Y. (1992). Nitrate concentration of ground water sources and potential health effects. Am. Water Works Assoc .J. 84(9):85-90.
9. Spalding, R.F. & Exner, M.E. (1993). Occurrence of nitrate in ground water-a review .J. Environ Qual. 22: 392-402.
10. Hartz, T.K., Smith, R.F., Schulback, K.F. & Lestrange, M. (1994). On-farmnitrogen tests improve fertilizer efficiency, protect groundwater. California Agriculture, 48 (4): 29-32.
11. Johnson, C.J. & Kross, N.C. (1990). Continuing importance of nitrate contamination of groundwater and wells in rural areas. Am.J.Ind.Med, 18 (4): 449-456.
12. Hartman, P.E. (1983). Review: Putative mutagens and carcinogens in foods, Nitrate/nitrite ingestion and gastric cancer mortality. Environ. Mutagen 5: 111-1211.
13. Isabel, C., Luisa, O. & Ana V. (2005). The need for reference materials when monitoring nitrate intake. Analytical and Bioanalytical Chemistry. 378(5).
14. Grant, D. & Nutler, W.H. (2005). Chronic toxicity of sodium nitrate in the male F 344 rat. Food Chem. Toxic. 27:565-571.
15. Chow, C.K., Chen, C.J. & Gairola, C. (2008). Effects of nitrate and nitrite in drinking water on rats. Toxicol. Letters, 6: 199-206.