



## NITRATE INDUCED TOXICITY ON SOME HAEMATOLOGICAL PARAMETERS OF CHARLES FOSTER RATS

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### ABSTRACT

Nitrate can cause multiple physiological effects if allowed to build up to high concentrations in animals. This work is concerned with the study of short term (14 days oral intoxication) nitrate exposure to male and female Charles foster rats that were divided into 4 groups. I<sup>st</sup> group was allowed free access to distilled water whereas II<sup>nd</sup>, III<sup>rd</sup> and IV<sup>th</sup> groups were provided with water possessing nitrate concentration 45 mgL<sup>-1</sup>, 90 mgL<sup>-1</sup> and 135 mgL<sup>-1</sup> NO<sub>3</sub><sup>-</sup> respectively. Under these conditions the haematological parameters such as T-RBC count, Hct and Hb showed increasing trend while TLC and blood platelets showed decrease in response to the stress imposed. Certain variations were observed in differential leucocytes count. Nitrate contaminated water seems to have disturbed the physiology and metabolism of experimental mammals (rats).

**KEY WORDS** Charles foster rats, Haematological parameters, Nitrate contaminated water, Nitrate toxicity

### INTRODUCTION

Excessive use of nitrogenous fertilizers subsequently leads to ecosystem pollution by the accumulation of nitrates in vegetables and fodder as well as the contamination of surface and ground water [1]. Especially, nitrates and nitrites are of great importance and concern to man and animals because they possess mutagenic, carcinogenic, teratogenic and embryotoxic activities [2]. Nitrates/nitrites are likely to exert harmful effects on the respiratory function as a consequence of acute intoxication while being responsible for methaemoglobinemia [3,4]. The major metabolites of nitrates are nitric oxide and nitrosamine. The latter one is highly carcinogenic and associated with a high risk of stomach, liver and esophagus carcinomas [5]. Several clinical studies documented hepatorenal and/or hepatotoxicity in humans were associated with excess nitrate intake [6]. Nitrates, in case of their oral absorption, are reabsorbed rather quickly in intestines and over 80% are released in a mass in the urine [7] and the high rate of absorption is may be due to the shortness of the nitrate ion radius. Nitrites are formed in the buccal cavity by bacterial reduction of about 20% of the nitrates ingested which escaped from elimination, circulating between the buccal cavity and the digestive system [8]. It has been shown that nitrite treatments in intact RBC causes a noticeable oxidation of oxyhaemoglobin to methaemoglobin by radical generation along with a decrease in glutathione level in the intracellular

medium associated with membrane lipid peroxidation [9].

Nitrate transformation into *N*-nitroso compounds in the stomach in the presence of secondary amines makes them still more dangerous [10,11]. Although secondary amines represent the essential constituents of human food, risk of cancer can only be reduced if the nitrate or even nitrite levels in food remains as low as possible [12]. [13] have stated the importance of haematological parameters because of their relationship with respiration (RBC, Hct, and Hb levels) and defense mechanism (WBC level).

The aim of the study was to investigate the effect of nitrate on some haematological parameters of Charles foster rats at different oral doses i.e. 45, 90 and 135 mgL<sup>-1</sup> NO<sub>3</sub><sup>-</sup> dissolved in water.

### MATERIAL AND METHODS

The animal clearance was obtained from Institutional Animal Ethics Committee (IAEC) of Central Drug Research Institute (CDRI), Lucknow.

A total of 32 rats (16 males and 16 females), weighing 200-250 g each, were divided into 4 groups. Each group contained 4 male and 4 female rats, kept in different cages. The II, III and IV group of rats were provided with water possessing 45 mgL<sup>-1</sup>, 90 mgL<sup>-1</sup> and 135 mgL<sup>-1</sup> NO<sub>3</sub><sup>-</sup> concentration respectively. The I group (control group), was

provided with distilled water during entire duration. Different nitrate concentrations were maintained by employing requisite amount of Potassium nitrate salt to water.

**Table. 1.** Nitrate toxicity on some haematological parameter of Charles foster rats intoxicated orally for 14 days.

Parameters	<u>Groups</u>							
	I (control)		II (45 mgL <sup>-1</sup> )		III (90 mgL <sup>-1</sup> )		IV (135 mgL <sup>-1</sup> )	
	M	F	M	F	M	F	M	F
<b>Hb (g%)</b>	13.58±1.20	13.67±1.40	14.43±0.41	13.99±0.87	15.95±0.85	14.20±0.22	16.63±0.49	14.87±1.87
<b>T-RBC</b> (×10 <sup>6</sup> /mm <sup>3</sup> )	7.16±0.35	6.62±0.89	7.60±0.58	6.87±0.76	7.97±0.37	6.99±0.41	8.12±0.33	7.31±0.50
<b>Hct (%)</b>	40.63±1.68	37.40±3.06	42.50±1.83	37.92±3.84	42.48±1.90	38.28±2.45	44.10±1.20	38.90±4.28
<b>MCV</b> (micron <sup>3</sup> )	56.75±2.27	57.55±3.26	54.08±2.08	57.63±3.59	53.90±1.59	56.68±1.29	53.60±3.29	55.60±1.22
<b>MCHC</b> (g%)	33.43±2.79	35.08±1.44	31.68±1.20	33.10±3.20*	31.10±1.09	33.15±27.88	30.95±1.65	27.87±3.65*
<b>TLC</b> (×10 <sup>3</sup> /mm <sup>3</sup> )	14.40±1.36	11.28±1.60	14.19±1.65	10.43±1.14	11.91±0.23*	9.21±0.97	11.22±1.45	5.63±0.78*
<b>DLC</b>								
<b>Poly. (%)</b>	11.50±1.94	12.00±1.68	13.00±1.45	12.47±1.31	15.25±1.91	13.00±0.04	16.75±1.72	13.67±1.96
<b>Lympho.</b> (%)	81.50±7.25	82.25±6.85	83.01±5.21	82.75±6.05	83.56±4.52	83.75±5.63	85.25±5.97*	83.67±5.58
<b>Mono. (%)</b>	1.00±0.06	1.50±0.08	2.25±0.02*	1.50±0.08	2.25±0.05	1.75±0.06	2.25±0.06	2.00±0.00
<b>Eosino.</b> (%)	2.50±0.03	1.75±0.06	4.25±0.29*	4.25±0.20	4.75±0.30	3.00±0.31	4.75±0.37	3.33±0.41
<b>Baso. (%)</b>	0.50±0.02	0.50±0.05	0.45±0.00	0.48±0.08	0.45±0.00	0.44±0.04	0.43±0.00	0.40±0.03
<b>Platelets</b> (×10 <sup>3</sup> /mm <sup>3</sup> )	383.50±30.47	430.75±31.21	381.75±24.43	422.75±22.84	369.75±21.9*	378.00±31.9*	354.00±34.7*	355.33±36.15*

Values shown are mean of 4 replicates ± S.D., significance at p ≤ 0.05. \* = significant values

After 14 days rats were decapitated and blood samples were collected for determination of some haematological parameters such as Haemoglobin (Hb) content as described by Standard Sahli's method [14], Total RBC count using improved Standard Neubauer Haemocytometer [15] and Haematocrit (Hct) or Packed Cell Volume (PCV) by Wintrobe method [16]. Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin Concentration (MCHC), Total Leucocytes Count (TLC), Platelets and Differential Leucocytes Count

(DLC) were analyzed with the help of MS-9 Autohaemato-Analyzer.

The recorded data was subjected to one way ANOVA. Difference between individual means was tested using Least Significance Difference (LSD) tests at 0.05 significance level.

#### RESULTS AND DISCUSSION

In response to 45 mgL<sup>-1</sup>, 90 mgL<sup>-1</sup> and 135 mgL<sup>-1</sup> NO<sub>3</sub><sup>-</sup> concentration Charles foster rats showed increase in the Hb, T-RBC count and Hct value after 14 days (Table 1). This may be due to the lowering of

arterial O<sub>2</sub> saturation of blood which acts indirectly as stimulus for marrow erythrocyte production [17]. Thus, resulting for the development of hypoxic condition (lack of O<sub>2</sub>) due to the conversion of nitrate circulating between the buccal cavity and the digestive system into nitrite by bacterial reduction [4,8]. Nitrite when present at high concentration in blood, reacts with iron (II) of the Hb, forming methaemoglobin [NO<sub>2</sub><sup>-</sup> + oxyHb (Fe<sub>2</sub><sup>+</sup>) → methHb (Fe<sub>3</sub><sup>+</sup>) + NO<sub>3</sub><sup>-</sup>], which has no oxygen-carrying ability [18].

The decrease in the erythrocyte indices such as MCV and MCHC was noted. This decrease is correlated with increase in T-RBC count accompanied by Hb concentration and Hct value causing hypoxic condition in Charles foster rats. A similar result on these parameters has been reported in African catfish and rats by [13].

For all 4 groups of rats, reduction in the TLC was observed with increasing nitrate concentration in water. However, maximum TLC (14.40×10<sup>3</sup>/mm<sup>3</sup>) was recorded in males of control group, whereas least (5.63×10<sup>3</sup>/mm<sup>3</sup>) was found in the IV group of female rats (Table 1). This decrease in the TLC was due to the possible reason of their getting used up while encountering a variety of inflammation, injury and subsequent infections resulting due to the nitrate contaminated water [19].

In DLC, the polymorphs, lymphocytes, monocytes and eosinophils have resulted to their increased production in the experimental animals due to the allergic reactions occurring in the body, may be caused by the pollutant to which they were exposed. [20] observed the increased lymphocytes in the rats, under the impact of toxic nature of pollutant gases. Decrease in the basophiles might have resulted due to their involvement in neutralizing the toxic effects [13]. The platelets count of rats treated with nitrate contaminated water, significantly lowered than that of control. Minimum platelets count (354×10<sup>3</sup>/mm<sup>3</sup>) was noted in the males of IV group, on the other hand, highest (430.75×10<sup>3</sup>/mm<sup>3</sup>) was recorded in the females of control group. It is clearly indicated that nitrate polluted water have deleterious effect on blood platelets production.

## CONCLUSION

Looking the findings reported in this paper, it appears that nitrate affected all the haematological parameters studied, which correspond to the increasing content of nitrate in water. Toxicity of nitrate increases as a function of it's level in water and the duration of exposure.

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## REFERENCES

1. Testud, F. (2005). Inorganic fertilizers. EMC-Toxicol. Pathol., 1: 21-28.
2. Krishnamoorthy, P., Sangeetha, M. (2008). Hepatoprotective effect of vitamin C on sodium nitrite-induced lipid peroxidation in albino rats. Indian Jr. of Biochem and Biophys., Vol. 45: 206-208.
3. Jensen, (2005). Nitrite transport into pig erythrocytes and its potential biological role. Acta Physiol. Scand., 184: 243-251.
4. Azeez, O.H., Mahmood, M.B. and Hassan, J.S. (2011). Effect of nitrate poisoning on some biochemical parameters in rats. Iraqi Jr. of Veterinary Sciences, 25(2): 47-50.
5. Kim, H.J., Chang, W.K., Kim, M.K., Lee, S.S., Choi, B.Y. (2002). Dietary factors and gastric cancer in Korea: Case-control study. Int. Jr. of Cancer, 97: 531-535.
6. Parada, B., Alves, R., Piloto, N., Sereno, J., Figueiredo, A., Pinto, R., Carvalho, L., Rocha- Pereira, P., Belo, L., Santos-Silva, A. and Teixeira, F. (2009). Characterization of a rat model of moderate chronic renal failure focus on haematological, biochemical, and cardio-renal profiles. Ren. Fail, 31: 833-42.
7. Hill, M. (1991). Nitrates and nitrites in food and water. Ellis Horwood, New York, pp. 131-132.
8. L'Hirondel, J.L. (1998). L'innocuité des nitrates alimentaires. Med Sci. 14: 636-639.
9. Gladwin, M.T., Raat, N.J.H., Shiva, S., Dezfulian, C., Hogg, N., Kim-Shapiro, D.B. and Patel, R.P. (2006). Nitrite as a vascular endocrine nitric oxide reservoir that contributes to hypoxic signalling, cytoprotection, and vasodilation. Am. Jr. Physiol. 291: H2026-H2035.
10. Yarube, I.U. Halim, M.A., Osaka, M.E., Ayo, J.O., Olorunshola, K.V. (2009). Antioxidant Vitamins C and E Alleviate the Toxicity Induced by Chronic Sodium Nitrate Administration on Sperm Count and Serum Testosterone Level in Wistar Rats. European Jr. of Scientific Res., 25(1): 35-41.
11. [Buehler, P.W.](#), [Butt, O.I.](#), [D'Agnillo, F.](#) (2011). Sodium nitrite induces acute central nervous system toxicity in guinea pigs exposed to systemic cell-free haemoglobin. [Biochem. Biophys. Res. Commun.](#), 409(3): 412-17.
12. Apfelbaum, M. (2001). Nitrates: une norme au pied d'argile. La recherche, 339: 31-34.
13. Taofik, O., Sunmonu, Oyelola, B., Oloyede (2008). Haematological response of African catfish (*Clarias gariepinus*) and rat to crude

- oil exposure. *The Internet Jr. of Haematol.*, 4(1).
14. Srinivasan, N.M., Kasturba, M.P. (2010). Intra-operative point of care haemoglobin estimation: A comparison of three methods. *Sri Lankan Jr. of Anaesthesiol.*, 18(1): 15-19.
  15. Dacie, T.V. and Lewis, S.M. (1968). *Practical Haematology*, 4<sup>th</sup> edition. J and A Churchill Ltd. London, pp. 568.
  16. Wintrobe, M.M., Lee, G.R., Boggs, D.R., Bithell, T.C., Forerster, J., Athens, J.W., Lukens, J. N. (1981). *Clinical Haematology* 8<sup>th</sup> edition. K.M. Varchere Company, Bombay.
  17. Zabulyte, D., Uleckiene, S., Kalibatas, J., Paltanaviciene, A., Jascaniniene, N., Stosik, M. (2007). Experimental studies on effect of Sodium fluoride and nitrate on biochemical parameters in rats. *Bull. Vet. Inst. Pulawy*, 51: 79-82.
  18. Dejam, A., Hunter, C.J., Pelletier, M.M., Hsu, L.L., Machado, R. F., Shiva, S., Power, G.G., Kelm, M., Gladwin, M.T. and Schechter, A.N. (2005). Erythrocytes are the major intravascular storage sites of nitrite in human blood. *Blood*, 106: 734-739.
  19. Alonso, A. and Camargo, J.A. (2009). Effects of pulse duration and post exposure period on the nitrite toxicity to a freshwater amphipod. [Ecotoxicol. and Environ. Safety](#), 72(7): 2005-2008.
  20. Kaur, S., Pande, R.K. (2006). Studies on haematological parameters of female rats under the stress of exhaust pipe emission from petrol and diesel vehicles in summer season. *Int. Jr. Poll. Res.*, 25(1): 105-108.