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A STUDY OF TOXICOLOGICAL EFFECTS OF HIGH NITRATE INGESTION IN RABBITS

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Abstract:

In India, especially in Rajasthan people drink water containing high level of nitrates and concentration up to 500 mg of nitrate ion per liter is not unusual. The ingested nitrate is converted to nitrite in the digestive system and absorb in blood causing methemoglobinemia. Methaemoglobin is not restricted to infants alone but it is prevalent in higher age groups also. The peak of methaemoglobin is observed at 45-95 mg/liter of nitrate concentration of water. Some recent studies have indicated the prevalence of a high percentage (40 – 82 %) of cases of acute respiratory tract infection with history of recurrence in children drinking high nitrate in water than that reported for areas of low nitrate concentration. Therefore an experimental study was conducted in 10 rabbits between three and half month to four month of age having weight ranging 1.310 kg to 10720 kg. Five groups A, B, C, and D & E were formed with two rabbits in each group. The control group A was administered water orally having 06 mg/liter .Group B to E (experimental groups) were administered water orally having concentration of 100mg/liter,200mg/liter,400mg/liter & 500mg/liter of nitrate respectively for 120 days. During experimental period the difference in general behavior of rabbits were noted. After that rabbits were anaesthetized & sacrificed according to guidelines of ICMR and lungs were removed & processed for paraffin sections, hemotoxyllin and eosin staining was done for microscopic observations. During experimental period, the animals were lethargy on 75th day .The respiration rate & heart rate were increased with loss of weight. The microscopic study revealed epithelial hyperplasia and lymphoid aggregation in the respiratory parenchyma. In higher group the lungs were infiltrated with inflammatory cells in inter alveolar space with abundance of Type -2 pneumocytes in the alveolar epithelium.

Key Words. : Lungs, Methaemoglobin, Nitrate, Nitrite, rabbits, histopathology.

Introduction:

The majority of Indian population is exposed to nitrate through ground water and dietary sources[1]. Excessive nitrate concentration in drinking water is reported to have caused methaemoglobinemia in infants up to 6 month of age[2, 3]. Maximum permissible limit for nitrate ion in drinking water have been set at 50mg/liter by WHO and 45 mg/liter by Bureau of Indian standard (IS-10500)[5,6,7].In several developing countries high nitrate concentration at times up to 500mg/liter is not uncommon[8].In body, nitrate are reduced to nitrite & leads to methaemoglobinemia which occurs through microbial action either in environment or in the body so, the health risk from exposure to nitrate are therefore related not only to their concentration in drinking water and food but also condition conducive to their reduction to nitrites[9]. Some studies suggested that reduction of nitrate to nitrite starts even in the oral cavity[9]. The reviewed literature includes animal study reporting correlation among drinking water nitrate concentration, high methaemoglobinemia and pathological changes in bronchi and lung parenchyma[10,11]. A animal study was performed on rats fed on nitrate rich water & reported frequent dilatation of bronchi with lymphocytic infiltration ,atrophy of mucosa and muscles and intestinal round cell infiltration at certain areas[12]. The present animal study on rabbits fed on nitrate rich water reports frequent dilatation of bronchi with lymphocytic infiltration, atrophy of mucosa, interstitial round cell infiltration and fibrosis at certain areas. Epidemiological study on nitrate toxicity indicated an association of increased asthmatic attacks and high air borne nitrate concentration[13]. In several Indian villages, people have been consuming water containing high nitrate concentration at time up to 500 mg/liter & indicated a high percentage (40 -82 %) of cases of acute respiratory tract infection with history of recurrence in children[14].

As the above reported data, it was planned to study the toxicological effects of nitrate exposure in an appropriate animal under laboratory conditions.

Material and Methods:

The study was conducted in Department of Anatomy at SMS Hospital and Medical College, Jaipur (INDIA) on five groups of 2 rabbits each. The rabbits were used for the study because their stomach pH is similar to infant (pH= 3.0-5.0) [10]. The age of rabbits were three and half to four months & weight varied from 1.310 kg to 1.720 kg. These groups were identified as A,B,C,D & E. Ad libitum quantity of water containing 45,100,200,400 and 500 mg/liter nitrate (in form of NaNO3) and food

Table 1: Microscopic changes in Lung with different concentration of nitrate

Water Nitrate(mg/l)	Congestion	Inflammatory cells	Breakdown of Alveoli	Bronchiole
45	NA	NA	NORMAL	NORMAL
100	NA	+	NORMAL	Reactive type of epithelial Hyperplasia
200	++	+	NORMAL	X
400	++	++	Acute inflammatory cells in alveolar space	Y
500	+++	+++	Severe infiltration of lymphocytes and prominence of Type-2 pneumocytes.	Z

X = Mild Respiratory Epithelial Hyperplasia; Y= Severe Respiratory Epithelial Hyperplasia and Shedding of Respiratory Epithelium; Z= Severe Respiratory Epithelial Hyperplasia with Shedding of Respiratory Epithelium; (+) = Mild, (++) = Moderate, (+++) = Severe; NA = No clinical finding observed, NORMAL= Normal histology seen.

soaked in the same water were given to group A to E respectively. The group consuming 45mg/liter served as a control group. Observations were made during the experimental period of 120 days for the changes in physical activity of the animals on a predesigned Performa after every 15 days. After 120 days the animals were sacrificed according to the guidelines of ICMR[15] and dissected. The lungs were removed and biopsy was taken from the organ. These tissues were fixed in 10% formalin solution and subjected to histopathological examination.

Results and Discussion:

[A] General Observation

The rabbits of group A did not show lethargy throughout the experimental period. In group B,C & D lethargy appeared on 90th day. The rabbits of group E become lethargic on 60th day. Rabbits of all groups i.e. A to E showed continuous increase in heart rate and respiration rate. The diarrhea was observed in both the rabbits of group D on 120th day and 90th day in rabbits of group E respectively (Table-2)

These findings are in accordance with Gupta SK et al[9] (1999) and Gupta SK et al [10] (2001). The findings indicate that high nitrate creates problem with oxygen carrying capacity of blood. These findings are in accordance with the results of Gupta SK et al[9] (1999),Gupta SK et al[10] (2001), Comly et al[16] (1945), Ferrant et al[17] (1945) and Greenberg et al[18] (1945). Increase in heart rate and respiration rate was proportional to the nitrate concentration in drinking water. These findings are similar as those of Kielbase et al[19] (2000) .

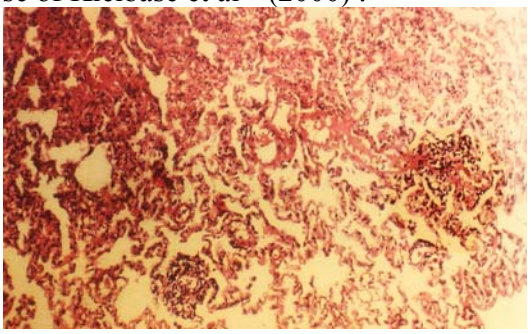

Figure 1: Microphotograph of Lung showing marked mononuclear infiltration in the lung parenchyma. (10X), Group III

[B] Histopathology - No changes was observed in lung parenchyma of rabbits subjected to water ingestion containing 45mg/liter (control group).The changes were more conspicuous as nitrate concentration increased. Lymphoid aggregation in parenchyma and respiratory epithelial hyperplasia was seen with shedding of respiratory epithelium at 400 mg/ liter nitrate

concentration .The densely arranged focal collection of lymphocytes and acute inflammatory cells in alveolar space was seen in group D and E only (Table- 1).

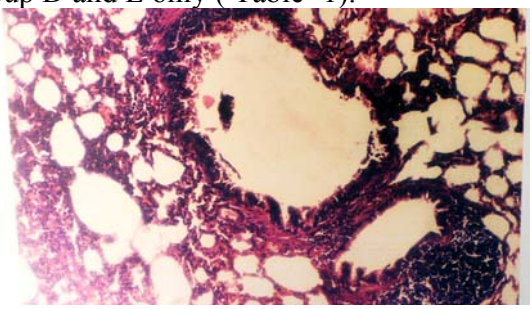

Figure 2: Microphotograph of Lung showing bronchiolar epithelial hyperplasia, desquamation and severe lymphocytic infiltration.(10X), Group IV

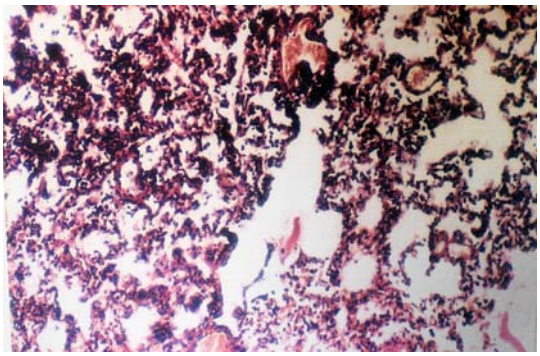

Figure 3: Microphotograph of Lung showing severe lymphocytic infiltration and congestion of alveolar space. (10X), Group V

These findings are in accordance with Gupta SK et al[10] (2000) and Greenberg et al[18] (1945). The results revealed that the degree of damage in the tissue was progressing as the nitrate content of the ingested water increased[20]. It has been observed that ARI contributes to about 20% of mortality in children less than 5 year of age[10].

The essential action in the formation of methemoglobin is an oxidation of the ferrous to ferric ion[21]. This action may be brought about in one of the following way[10,22.23] ----By direct action of the oxidant **or** by the action of hydrogen donor in the presence of oxygen **or** by auto oxidation. In the presence of nitrites, the ferrous ion of hemoglobin gets directly oxidized to ferric state. Normally the methemoglobin is formed is reduced by the following reaction:

Hb^{+3} +Red.Cyt b_5 ----------> Hb^{+2} + Cyt b_5

Reduced cytohrome b_5 is generated by the enzyme cyt.b5 reductase:

Oxy cytb_5 + NADH ----------> Red cyt b_5 + NAD.

Thus the enzyme cyt b_5 reductase plays a vital role in counteracting the effect of nitrate ingestion. Bacteria causing non specific diarrhea are generally considered responsible for conversion of nitrate to nitrite. Lower stomach pH of adult acts as an inhibitor of these bacteria. However they can multiply in relatively high pH of the stomach.

Conclusion:

The results of present study proved strong interdependence between high nitrate concentration and histopathological changes of lungs in rabbits. The degree of damage was more pronounced as nitrate concentration increased in drinking water. A possible cause could be the reverse of cyt.b5 reductase activity and its adaptation with increasing water nitrate concentration to compensate methaemoglobinemia. While an isolated study can be not extrapolated to humans it highlights the need for conducting further studies in population consuming nitrate rich water.

These findings are of interest since humans even under normal circumstances can consumes such levels of nitrate and may be a possible cause of recurrent acute respiratory infection, which is a common disease in our country[22,23].

The nitrate problem has not been taken up seriously in our country. It is expected that the finding of this study will draw attention of decision maker to take note of this serious problem and take adequate step to ensure that safe drinking water is available to public.

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TABLE-2

Comparison in Physical activity of Rabbits in all groups during experimental period of 120 Days

FIVE GROUPS WITH ANIMAL NO.		HEART RATE (135/MIN)										RESPIRATION RATE (55/MIN.)										LETHARGY									
		GPA		GPB		GPC		GPD		GPE		GPA		GPB		GPC		GPD		GPE		GPA		GPB		GPC		GPD		GPE	
		1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
Days of observation (120 Days)	1st Day	140	144	140	142	142	140	142	140	142	140	56	58	56	58	56	56	56	58	56	56	-	-	-	-	-	-	-	-	-	-
	15th Day	□	-	-	□	-	□	□	~	-	-	□	-	~	-	~	□	~	-	□	-	-	-	-	-	-	-	-	-	-	-
	30 th Day	□	□	□	□	□	□	□	□	□	~	□	-	□	-	□	□	□	□	□	-	-	-	-	-	-	-	-	-	-	-	-
	45th Day	□	□	□	□	□	□	□	□	□	□	□	-	□	-	□	□	□	-	□	□	-	-	-	-	-	-	-	-	-	-
	60th Day	□	□	□	□	□	□	□	□	□	□	□	-	□	-	□	□	□	□	□	□	-	-	-	-	-	-	-	-	+	+
	75th Day	□	□	□	□	□	□	□	□	□	□	□	-	□	-	□	□	□	□	□	□	-	-	-	-	-	-	-	-	+	+
	90th Day	□	□	□	□	□	□	□	□	□	□	□	-	□	-	□	□	□	□	□	□	-	-	+	-	-	+	+	+	+	+
	105th Day	□	□	□	□	□	□	□	□	□	□	□		□	-	□	□	□	□	□	□	-	-	+	-	-	+	-	-	-	-
	120th Day	158	158	152	158	166	168	192	198	216	218	60	60	58	60	64	60	64	64	72	84	-	+	+	-	+	+	+	+	+	+

GP = Groups; + Appearance of symptoms; - No symptoms was observed; □ Increase in Parameter; □ Decrease in Parameter; □ change was observed