

# HISTOPATHOLOGICAL EFFECTS OF HIGH NITRATE INGESTION ON LUNGS IN RABBIT

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## ABSTRACT

Nitrate when ingested are converted to nitrite in the digestive system and absorb in blood causing methemoglobinemia. 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, 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 anaesthetised & 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 and cyanosis appeared on nails, lips, tongue & sclera on 90th day (mainly in rabbits of group D & E). The respiration rate & heart rate were increased with loss of weight. The rabbits of experimental groups passes stool with mucous on 45th day and had diarrhea on 90th day. 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.** Nitrate, Nitrite, Methemoglobin, Cyanosis, Histopathology, Lungs

## INTRODUCTION:

The majority of Indian population is exposed to nitrate through ground water and dietary sources. Excessive nitrate concentration in drinking water is reported to have caused methaemoglobinemia in infants up to 6 month of age<sup>1-5</sup>. Maximum permissible limit for nitrate ion in drinking water have been set at 50mg/liter by WHO<sup>6</sup> and 45 mg/liter by Bureau of Indian standard (IS-10500)<sup>7</sup>. In several developing countries high nitrate concentration at times up to 500mg/liter is not uncommon.

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. Some studies suggested that reduction of nitrate to nitrite starts even in the oral

cavity<sup>8</sup>. The reviewed literature includes animal study reporting correlation among drinking water nitrate concentration, high methaemoglobinemia and pathological changes in bronchi and lung parenchyma<sup>9,10</sup>. The 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<sup>11</sup>. 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<sup>12</sup>.

**AIM OF STUDY:** As the above reported data, it was planned to study the toxicological effects of nitrate exposure in an appropriate animal study under laboratory conditions. Rabbits were chosen as the animal as their stomach pH is similar to that of infants (3.05.0).

## MATERIAL AND METHODS

The study was conducted on five groups of 2 rabbits

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each. The age of rabbits was three and half to four months and 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 NaNO<sub>3</sub>) and food 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 appearance of Cyanosis In various regions and changes in physical activity of the animal's .After 120 days the animals were anaesthetized with Pentothal sodium 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 .The results were noted.

**OBSERVATION AND DISCUSSION**

[A] GENERAL OBSERVATION- Cyanosis was appeared in rabbits of group C,D & E only. In group C, the cyanosis was seen in nails on 105th day. in group D ,the cyanosis was observed in nails & sclera on 90th day and on lips it appeared on 105th day .In group E, the cyanosis was observed in nails, sclera & lips on 90th day in both rabbits but in tongue & mucous membrane of oral cavity ,the cyanosis was observed in one rabbit only on 105th day.(Table-I)

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

Water Nitrate(mg/l)	Congestion	Inflammatory cells	Breakdown of Alveoli	Bronchiole
45	NIL	NIL	NORMAL	NORMAL
100	NIL	+	NORMAL	Reactive type of epithelial Hyperplasia
200	++	+	NORMAL	<b>A</b>
400	++	++	Acute inflammatory cells in alveolar space	<b>B</b>
500	+++	+++	Severe infiltration of lymphocytes with prominence of Type-2 pneumocytes.	<b>C</b>

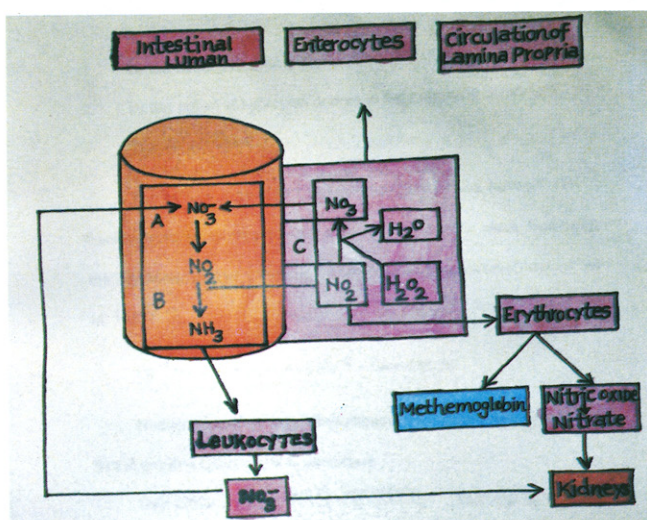
(+) = Mild, (++) = Moderate, (+++) = Severe  
 NIL = No clinical finding observed, NORMAL= Normal histology seen.  
 A = Mild Respiratory Epithelial Hyperplasia  
 B= Severe Respiratory Epithelial Hyperplasia and Shedding of Respiratory Epithelium  
 C= Severe Respiratory Epithelial Hyperplasia and Shedding of Respiratory Epithelium

Table-1: Changes in Lung parenchyma with different concentration of nitrate in Drinking water

FIVE GROUPS WITH ANIMAL NO.	LETHARGY										CYANOSIS										
	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	
Days of Observation (120 Days)	1 <sup>st</sup> Day	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	15 <sup>th</sup> Day	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	30 <sup>th</sup> Day	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	45 <sup>th</sup> Day	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	60 <sup>th</sup> Day	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	+	+
	75 <sup>th</sup> Day	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	+	+
	90 <sup>th</sup> Day	-	-	+	-	+	+	+	+	+	+	-	-	-	-	-	-	-	-	+	+
	105 <sup>th</sup> Day	-	-	+	-	+	+	+	+	+	+	-	-	-	-	-	+	+	-	+	+
	120 <sup>th</sup> Day	-	+	+	-	+	+	+	+	+	+	-	-	-	-	-	+	+	+	+	+

GP = Groups  
 + = Appearance of symptoms  
 - = No symptoms was observed

TABLE-II: Comparison in Physical activity of Rabbits in all groups during experimental period (120 Days).



A=Bacterial nitrate reductase  
 B=Bacterial nitrite reductase  
 C= Catalase

Figure 1: Conversion of ingested inorganic & organic nitrate to nitrite by microflora in the oral cavity and in the gastrointestinal tract by intestinal microflora, these activities result in increased oxidation of hemoglobin to methemoglobin and increased production of nitric oxide.

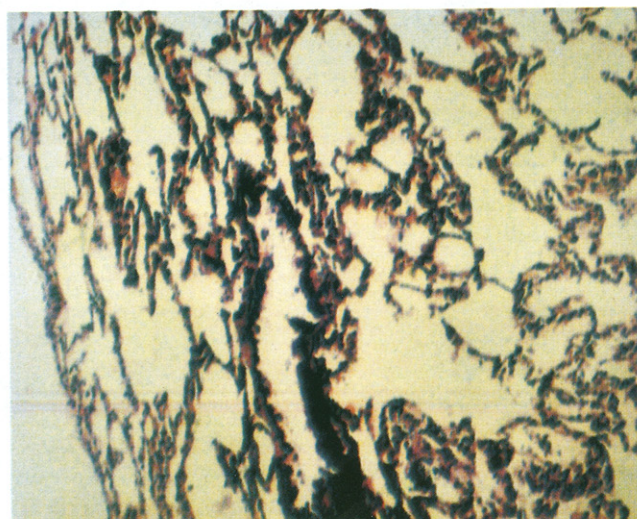


Figure 2: Microphotograph of Lung showing epithelial hyperplasia of Respiratory bronchiole.(10X)

Recurrent Diarrhea in children living in Ares with High levels of Nitrate in Drinking water. Archives of Environmental Health. August 2001;56(4):371.

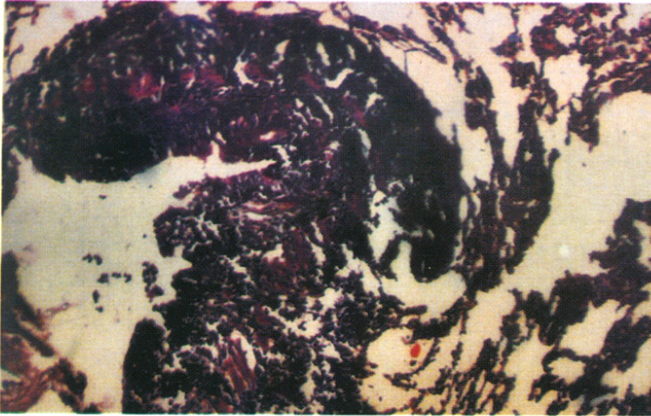


Figure 3: Microphotograph of Lung showing marked mononuclear infiltration around bronchiole and in the lung parenchyma. (10X)

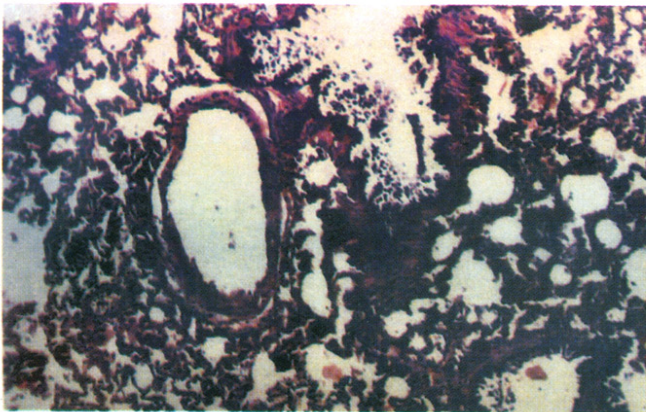


Figure 4: Microphotograph of Lung showing bronchiolar epithelial hyperplasia, desquamation and mild mononuclear infiltration.(10X)

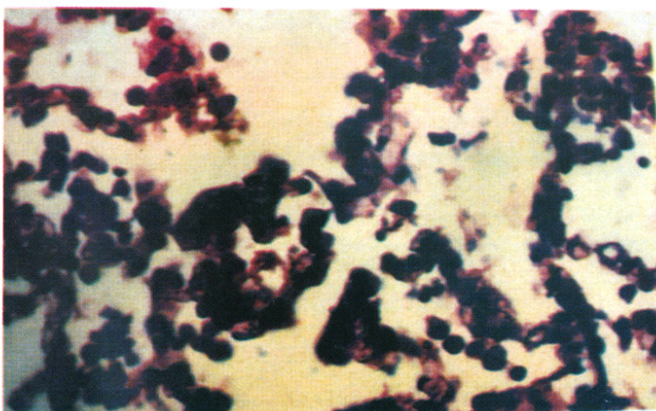


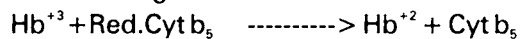
Figure 5: Microphotograph of Lung showing type-II pneumocytes hyperplasia in alveoli.(40X)

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-I)

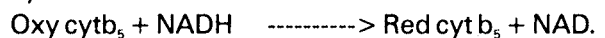
[B] HISTOPATHOLOGICAL CHANGES- No changes was observed in lung parenchyma of rabbits subjected to water ingestion containing 45mg/liter (control group).For other rabbits, the changes have been depicted in table-II.

The results revealed that the degree of damage in the tissue was progressing as the nitrate content of the ingested water increased<sup>13,14</sup>. It has been observed that ARI contributes to about 20% of mortality in children less than 5 year of age<sup>15</sup>.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<sup>16-18</sup>.

**PATHOPHYSIOLOGY OF NITRATE TOXITY:** The essential action in the formation of methemoglobin is an oxidation of the ferrous to ferric ion. This action may be brought about in one of the following way--- 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:



Reduced cytochrome  $b_5$  is generated by the enzyme cyt. $b_5$  reductase:



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.

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