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Research article

## TOXIC EFFECTS OF HIGH NITRATE INTAKE IN OESOPHAGUS AND STOMACH OF RABBITS

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### ABSTRACT

**Introduction:** 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. **Aim of study:** To find out the correlation between drinking water nitrate concentration and histopathological changes in esophagus and stomach of rabbits of different groups. **Material & Methods:** Therefore an experimental study was conducted in 10 rabbits according to guideline of ICMR, New Delhi between three and half month to four months 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 a concentration of 100mg/liter, 200mg/liter, 400mg/liter & 500mg/liter of nitrate respectively for 120 days. Then all rabbits were anaesthetized & sacrificed according to the guidelines of the ICMR and oesophagus and stomach were removed & processed for paraffin sections. Hemotoxyllin and eosin staining was done for microscopic observations. **Results:** The results showed mononuclear infiltration in the esophagus which started in group B and in stomach, histopathological changes appeared in sub-mucosa, muscularis mucosa, muscularis externa and seosa started from group c. The changes were more pronounced in stomach of group D & E in the form of congestion of blood vessels in sub mucosa and mild infiltration of lymphocytes in muscularis externa.

**Keywords:** Oesophagus, Stomach, Nitrate, Nitrite, Histopathology, Rabbits.

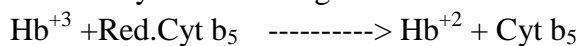
### INTRODUCTION

The majority of Indian population is exposed to nitrate through ground water and dietary sources<sup>1</sup>. Excessive nitrate concentration in drinking water is reported to have caused

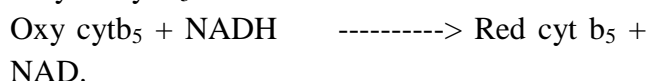
methaemoglobinemia in infants up to 6 months of age<sup>2, 3</sup>. The maximum permissible limit for nitrate ion in drinking water have been set at 50mg/liter by WHO and 45 mg/liter by Bureau of Indian standard (IS-10500)<sup>5,6,7</sup>. In several

developing countries with high nitrate concentration at times up to 500mg/liter is not uncommon<sup>8</sup>. In body, nitrate are reduced to nitrite & leads to methaemoglobinemia which occurs through microbial action either in the environment or in the body<sup>9</sup>. There are three stages of interaction between sodium nitrite and blood as :- an induction period , a reactionary period & a terminal period , often prolonged during which the product of the reaction, chiefly methemoglobin pass into hematin and other degradation products<sup>10</sup>. The health risk from exposure to nitrate is therefore related not only to their concentration in drinking water and food but also condition conducive to their reduction to nitrites<sup>11,12</sup>.

This action may be brought about in one of the following way<sup>13</sup>----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 the ferric state. Normally the methemoglobin is formed is reduced by the following reaction:



Reduced cytochrome b<sub>5</sub> is generated by the enzyme cyt.b<sub>5</sub> reductase:



Thus the enzyme cyt b<sub>5</sub> reductase plays a vital role in counteracting the effect of nitrate ingestion.

We choose the rabbits for the study because pH of their stomach is similar to human beings. so it is proposed to study the effect of nitrate toxicity in blood of rabbits .

## MATERIAL AND METHODS

The present research work was permitted by Departmental research committee and university of Rajasthan, Jaipur and principal & controller of SMS Medical College, Jaipur Rajasthan. 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)<sup>10</sup>. 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 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. After 120 days the animals were anaesthetized and sacrificed according to the guidelines of ICMR<sup>10,11</sup> and dissected. The Oesophagus and stomach were removed and biopsy was taken from the organ. These tissues were fixed in 10% formalin solution and subjected to histopathological examination.

## RESULTS

**Histopathological changes in oesophagus:** No changes were observed in oesophagus of rabbits subjected to water ingestion containing 45mg/liter (control group). The histopathological changes associated with high nitrate in drinking water indicated a change in mucosa which started at 200mg/L nitrate. The changes were more pronounced as the nitrate concentration increased, in the form of mononuclear infiltration. the changes in submucosa were observed in group E only. The changes were in the form of moderate infiltration.

**Histopathological changes in stomach:** No changes were observed in stomach of rabbits subjected to water ingestion containing 45mg/liter (control group) & 100mg/litre (group B). The changes appeared in submucosa, muscularis externa and serosa of rabbits in group C in the form of mild mononuclear infiltration. The changes were more pronounced in group D & group E in thr form of congestion of blood vessels in sub mucosa and mild infiltration of lymphocytes in msclaris externa.

**Table.1: Comparative histopathological changes in Oesophagus of Rabbits in all groups.**

OESOPHAGUS		MUCOSA	SUB-MUCOSA	MUSCULAR IS EXTERNA	ADVENTITIA
5 Groups of	GROUP A	NORMAL	NORMAL	NORMAL	NORMAL
	GROUP B	NORMAL	NORMAL	NORMAL	NORMAL
	GROUP C	+	NORMAL	NORMAL	NORMAL
	GROUP D	++	NORMAL	NORMAL	NORMAL
	GROUP E	++++	++++	NORMAL	NORMAL

(+) = Mild inflammation, (++) = Moderate inflammation, (+++) = Severe inflammation

**Table-2: Comparative histopathological changes in stomach of Rabbits in all groups.**

STOMACH		MUCOSA	SUB-MUCOSA	MUSCULARI S EXTERNA	SEROSA
5 Groups of Rabbits	GROUP A	NORMAL	NORMAL	NORMAL	NORMAL
	GROUP B	NORMAL	NORMAL	NORMAL	NORMAL
	GROUP C	++	+	+	+
	GROUP D	++	++	+++	NORMAL
	GROUP E	++++	++++	\$\$\$	NORMAL

(+) = Mild inflammation, (++) = Moderate inflammation, (+++) = Severe inflammation

(\$\$) = Congestion of blood vessels, NORMAL= Normal histology.

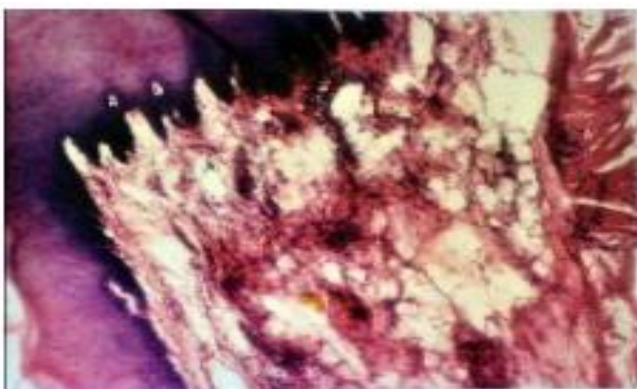


Fig. 1: Oesophagus showing mononuclear infiltration in the sub-epithelial region. (10 x ) group- c



Fig.2: Oesophagus showing severe mononuclear infiltration in the mucosal region.(10 x) Group D

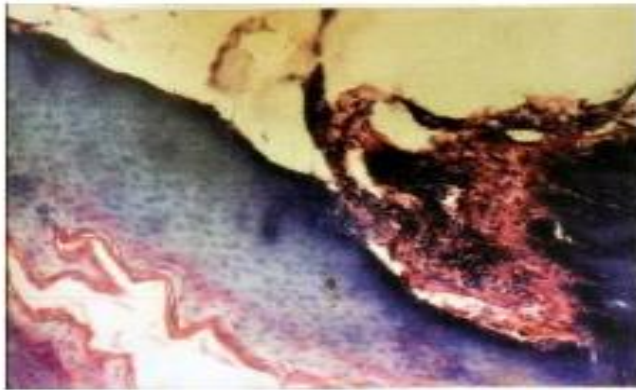


Fig.3: Oesophagus showing mononuclear infiltration in the sub-epithelial region and sub mucosal region (10 x)

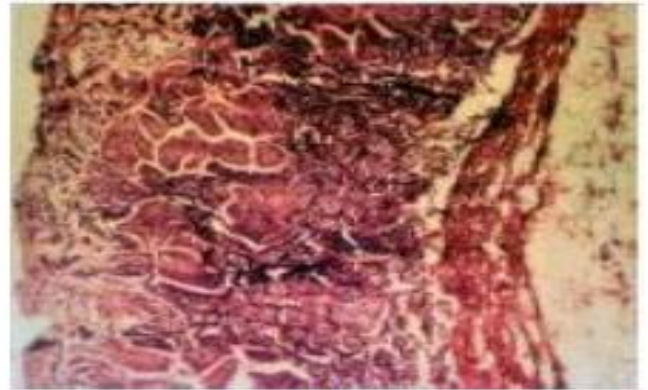


Fig.4: Stomach showing mild mononuclear infiltration in the lamina propria . (10 x ) Group B

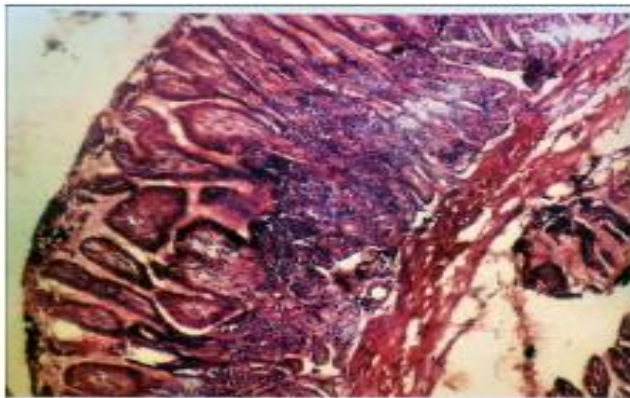


Fig.5: Stomach showing moderate mononuclear infiltration in the lamina propria. (10X) Group C



Fig.6: Stomach showing severe mononuclear infiltration Group D

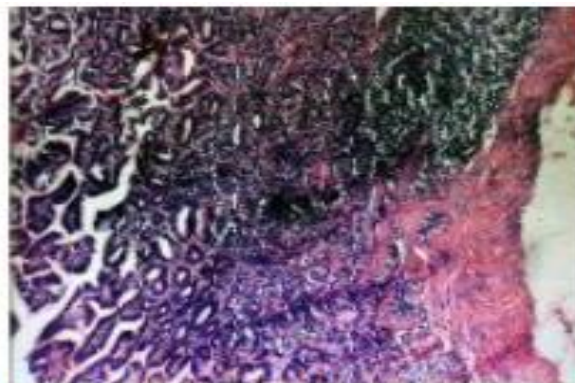


Fig.7: Stomach showing marked lymphocytic infiltration in the sub-mucosa and muscular layer.(10 X )Group E

## DISCUSSION

**OESOPHAGUS-** No references regarding study of histopathological changes in oesophagus associated with ingestion of high nitrate

**STOMACH-** The findings of present research work are consistent with Xu G et al<sup>14</sup> (1992) who analyzed 178 samples of drinking water for nitrate and nitrite. The results suggested that nitrate in drinking water probably plays an important role in gastric mucosal lesions and even carcinogens.

concentration in the drinking water could be found inspite of our best efforts made to search the literature.

No more study has performed regarding histopathological analysis of stomach when high nitrate ingested in drinking water till date

## CONCLUSION

Nitrate are reduced to nitrite by micro flora in the oral cavity & increased consumption of nitrite

leads to : increased production of nitrates, excess nitric oxide generation which has vasodilator effects , enhanced absorption of sodium from intestinal lumen , and increased production of oxygen which will react with other cell constituents possibly causing irreversible damage

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