

Histopathological Changes in the Duodenum and Jejunum of Rabbits Exposed to High Nitrate Intake in Drinking Water

Manoj Kumar Sharma¹, Hemlata Sharma²

Abstract

Introduction: In India particularly in Rajasthan state, peoples drink water having high level of nitrates and concentration up to 500 mg of nitrate ion per liter is common. The ingested nitrate is converted to nitrite in the digestive system and absorb in blood causing methemoglobinemia. The peak of methemoglobin 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 duodenum and jejunum of rabbits of different groups. **Materials & Methods:** An experimental study was conducted in 10 rabbits according to guideline of ICMR, New Delhi between three and a half months to four months of age having weight ranging from 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 given water orally having 06 mg/liter. Groups B to E (experimental groups) were administered water orally having a concentration of 100 mg/Liter, 200 mg/Liter, 400 mg/Liter & 500 mg/Liter of nitrate respectively for 120 days. Then all rabbits were anaesthetized and sacrificed according to the guidelines of the ICMR and duodenum and jejunum were removed and processed for paraffin sections. Hemotoxyllin and eosin staining was done for microscopic observations. **Results:** The results showed mononuclear infiltration and ulceration of epithelium in mucosa of duodenum and jejunum which started in Group B. The histopathological changes appeared in sub-mucosa, muscularismucosa, muscularisexterna and serosa, started from Group C as exfoliation, crowding and stratification of epithelium. The changes were more pronounced in duodenum and jejunum of group D & E in the form of congestion of blood vessels in sub mucosa and mild infiltration of lymphocytes in muscularisexterna and abnormal villi.

Keywords: Duodenum; Jejunum; Small intestine; Nitrate; Nitrite; Histopathology; Rabbits.

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Introduction

The majority of population of India is exposed to nitrate through ground water and dietary sources.¹ Excessive nitrate concentration in drinking water is reported to have caused methemoglobinemia in infants up to 6 months of age.^{2,3} Maximum

permissible limit for nitrate ion in drinking water has been set at 50 mg/Liter by WHO and 45 mg/Liter by Bureau of Indian Standard (IS-10500).⁴⁻⁶

In few developing countries high nitrate concentration at times up to 500 mg/liter is not uncommon.⁷ In our body, the nitrates are reduced to nitrite and leads to methemoglobinemia 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 condition conducive but also to their reduction to nitrites.⁷ Few studies explained that reduction of nitrate to nitrite starts even in the oral cavity.^{8,9}

The present animal study on rabbits fed on nitrate rich water reports lymphocytic infiltration, atrophy of mucosa, interstitial round cell infiltration and fibrosis at certain areas.

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In some Indian villages, people have been consuming water containing high nitrate concentration at time up to 500 mg/liter is not uncommon. As the above reported data, it was planned to study the toxicological effects of nitrate exposure in an appropriate animal under laboratory conditions.

Aim of study

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

Materials and Methods

The study was conducted in the Anatomy department, S.M.S Medical College and attached group of hospitals, Jaipur, Rajasthan 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).^{9,10} The age of rabbits was three and a 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. The water containing 45, 100, 200, 400 and 500 mg/Liter nitrate (in the form of NaNO₃) were provided ad libitum and food soaked in the same water were also given to group A to E respectively. The group consuming 45 mg/Liter named as a control group. The observations were made during the experimental period of 120 days for the changes in physical activity of the animals on a predesigned pro forma after every 15 days. After 120 days, all animals were sacrificed according to the guidelines of ICMR and dissected. The duodenum and jejunum

were removed and biopsy was taken from the organ. These tissues were fixed in 10% formalin solution and stored for histopathological examination.

Results

Histopathological Changes in Duodenum: The mucosa, sub-mucosa, muscularisexterna and serosa was absolutely normal in Group A rabbits but changes starts in rabbits of Group B. There was ulceration of mucosa and moderate inflammation in duodenum. Some slides showed abnormal villi also. Sub serosa, muscularisexterna and serosa showed normal histology in Group C. In Group D, the duodenum showed moderate inflammatory inflammation and ulceration in mucosa and mild inflammatory inflammation in muscularis mucosa. The serosa was normal. In GI tract of Group E, the duodenum showed conversion of epithelium into low columnar cells. The mucosa and sub-mucosa showed dense collection of mononuclear cells. alceration of epithelium and abnormal villi were observed in mucosa and congested blood vessels was observed in serosa (Fig. 1, 3, 5, 7) (Table 1).

Histopathological Changes in Jejunum: The mucosa, sub-mucosa, muscularisexterna and serosa was absolutely normal in Group A rabbits. The changes starts in rabbits of Group B in form of ulceration of epithelium and mild lymphocytic infiltration and plasma cells infiltration in mucosa. In GI tract of Group C, the jejunum showed ulceration, exfoliation, crowding and stratification of epithelium. There was moderate infiltration of lymphocytes, plasma cells, eosinophils and

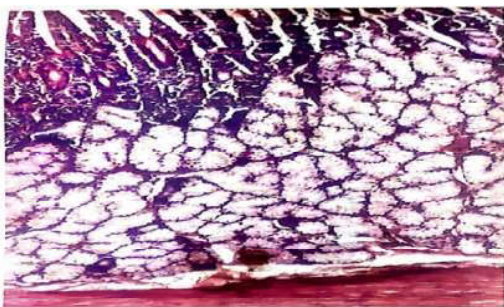


Fig. 1: Microphotograph of duodenum showing mild lymphocytic infiltration in lamina propria. (10X), Group B



Fig. 2: Microphotograph of jejunum showing epithelial shedding. (10X), Group B

neutrophils in mucosa. The submucosa, muscularis mucosa and serosa were observed normal. The Group D rabbits showed ulceration of epithelium and moderately inflammatory inflammation in

mucosa and severe infiltration of lymphocytes and plasma cells in submucosa. The congestion of blood vessels in mucosa and serosa were observed. The muscularisexterna was normal. The maximum

pathological changes were observed in Group E, in which jejunum showed marked inflammatory inflammation in mucosa and submucosa. There was marked ulceration of epithelium in mucosa.

The severe congestion of blood vessels in mucosa and serosa were observed. The muscularisexterna did not showed any significant change (Figs. 2, 4, 6, 8, 9) (Table 2).

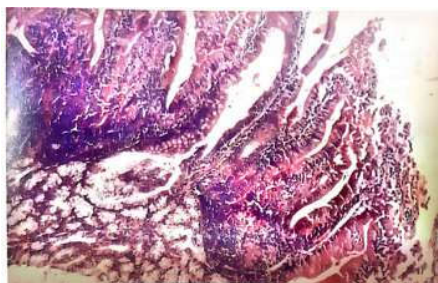


Fig. 3: Microphotograph of duodenum showing marked epithelial ulceration and moderate mononuclear infiltration in lamina propria. (10X), Group C

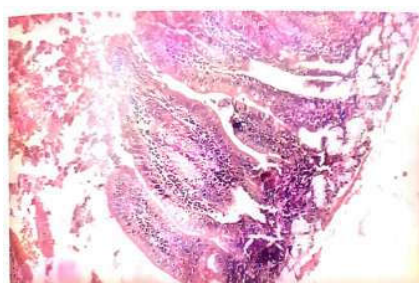


Fig. 4: Microphotograph of jejunum showing marked epithelial ulceration and moderate mononuclear infiltration in lamina propria. (10X), Group C



Fig. 5: Microphotograph of duodenum showing lymphoid aggregation in lamina propria. (10X), Group D

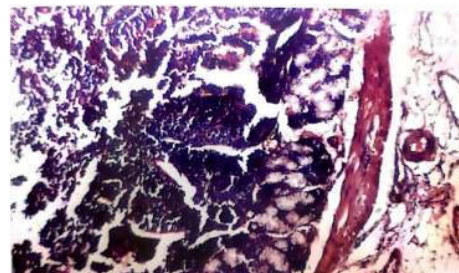


Fig. 6: Microphotograph of jejunum showing marked ulceration of epithelium and mononuclear infiltration in lamina propria. (10X), Group D



Fig. 7: Microphotograph of duodenum showing lymphoid aggregation in sub mucosa and shortening of villi. (10X), Group E



Fig. 8: Microphotograph of jejunum showing epithelial ulceration and dilated congested blood vessels in the lamina propria. (10X), Group E

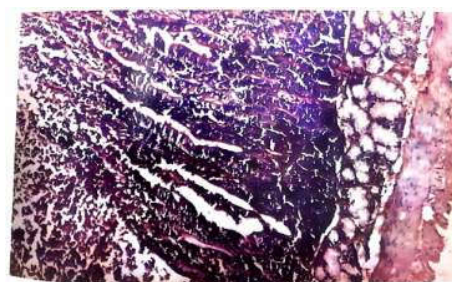


Fig. 9: Microphotograph of jejunum showing marked epithelial ulceration. (10X), Group E

Table 1: Microscopic Changes in Duodenum with Different Concentration of Nitrate

	Group A	Group B	Group C	Group D	Group E
Mucosa	-	0+	0+	0++×	0+++×
Submucosa	-	-	-	+++	+++
Muscularis Externa	-	-	-	-	-
Serosa	-	-	-	×	×
	- Normal histology	+ Mild inflammation		++ Moderate inflammation	
	+++ Severe inflammation	0 Ulceration of epithelium		× Congestion of blood vessel	

Table 2: Microscopic Changes in Jejunum with Different Concentration of Nitrate

	Group A	Group B	Group C	Group D	Group E
Mucosa	-	0+	0++*	0++	0+++*
Submucosa	-	-	-	++	+++
Muscularis externa	-	-	-	+	-
Serosa	-	-	-	-	×
	- Normal histology	+ Mild inflammation		++ Moderate inflammation	
	+++ Severe inflammation	0 Ulceration of epithelium		* abnormal villi	

Discussion and Conclusion

The duodenum of rabbits in Group A did not any pathological changes. The histopathological changes were observed in mucosa of Group B in the form of ulceration of epithelium and mild infiltration of lymphocytes with plasma cells. The changes were more pronounced as the nitrate concentration increased in drinking water. In Group E, the mucosa showed ulceration of epithelium, abnormal villi, severe infiltration of lymphocytes and plasma cells in submucosa.

The histopathological changes started in Jejunum in Group B in form of ulceration of mucosa and mild inflammation. The increased nitrate concentration causes severe changes consisting of congestion of blood vessels, severe inflammation and ulceration of epithelium as observed in Group E. severe inflammation of sub mucosa was seen in Group D & E only which also showed involvement of the serosa in the form of congestion of blood vessels.

Farrant M *et al.* (1945) studied the two cases of methaemoglobin in newborn caused by nitrates in well water. The microscopic data of intestinal wall showed confluent ulceration with marked thickening.¹⁰

No more references regarding the study of histopathological changes in duodenum and jejunum associated with ingestion of high nitrate concentration in the drinking water could be found inspite of best efforts made to search the literature.

The results of present study proved strong interdependence between high nitrate concentration and histopathological changes in duodenum

and jejunum. The damage in tissue was more pronounced as nitrate concentration increased.^{11,12} The nitrate problem is not taken up seriously in our country till date. It is expected that the findings will draw attention about the seriousness of problem and people will get safe water for drinking.

References

1. Austin JH and Drabkin DL. The reaction of hemoglobin with nitrite. *Journal Bio. Chem.* 1936;112:67.
2. Bodansky O. Methemoglobin and Methemoglobin producing compounds. *Pharmacol. Rev.* 1951;3:144-95.
3. Comblath M and Hartmann AF. Methemoglobinemia in young infants. *J Pediat.* 1948;33: 421-25.
4. World Health Organization. Guidelines for research on acute respiratory infection. Memorandum from a WHO Meeting Bulletin of the World Health Organization. 1982;60:521-33.
5. World Health Organization. A programme for controlling acute respiratory infection in children. Memorandum from a WHO Meeting Bulletin of the World Health Organization. 1984;62:47-58.
6. World Health Organization. Respiratory infection in children. Management in small hospitals. Manual for Doctors. 1998. WHO; Geneva.
7. WHO. Guidelines for drinking water quality. 1993;1:52-53.
8. American Academy of Pediatrics. Transient organic aciduria and methaemoglobinemia

- with acute gastroenteritis. *Pediatrics*. April 1990;85(4):589-91.
9. Barton GMG. A fatal case of sodium nitrite poisoning. *The Lancet*. 1954 Jan 23;263(6804):190-91.
 10. Farrant M. Methemoglobinemia: two cases in newborn infants caused by nitrates in well water. *The Journal of Pediatrics*. 1945. pp.585-91.
 11. Gatseva P, Lazariva A *et al*. Experimental data on the effect of nitrates entering the organism with the drinking water. *FolicaMedica*. 1996; 38(1):75-83.
 12. Richmilewitz D, Stamler JS *et al*. Peroxynitrite induced Rat colitis— a new model of colonic inflammation. *Gastroenterology*. 1993;105: 1681-88.
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