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Original Article

HISTOPATHOLOGICAL CHANGES IN KIDNEYS OF RABBIT EXPOSED TO HIGH NITRATE IN DRINKING WATER

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ABSTRACT

Background: 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. The peak of methaemoglobin is observed at 45-95 mg/liter of nitrate concentration in water..

Aims and Objects: To find out the correlation between drinking water nitrate concentration and histopathological changes in kidneys of rabbits of different groups.

Materials and Methods: 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 and E were formed having 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. Then all rabbits were anaesthetized & sacrificed according to guidelines of ICMR and kidneys were removed & processed for paraffin sections. Hemotoxyllin and eosin staining was done for microscopic observations.

Results: During experiment, animals were lethargic on 75th day .Intake in quantity of food and water was not altered in the rabbits. Rabbits of all group i.e. A to E showed continuous increase in heart rate (up to 218/minute in group E) and respiration rate (up to 84/minute in group E) respectively. The microscopic study of kidney showed focal collection of lymphocytes in interstitial tissue, particularly in pericapsular area of kidney which appeared in rabbits of group B.

The changes were more pronounced in group C &D in the form of focal collection of plasma cells. The hyaline and cellular casts were observed in proximal & distal tubules which started appearing in animals of group C. The congestion of blood vessels and damaged tubular epithelium were observed in tubules of group E rabbits.

Discussion and Conclusion: The Results occurred possibly due to cytochrome b_5 reductase activity and its adaptation with increasing water nitrate concentration to compensate methaemoglobinemia.

KEYWORDS: Kidney, Nitrate, rabbits, histopathology, Methemoglobin, Cyanosis.

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BACKGROUND

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 [1,2]. 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 [3,4,5]. The peak of methaemoglobin is observed at 45-95 mg/liter of nitrate concentration of water. Maximum permissible limit for nitrate ion

in drinking water have been set at 50mg/liter by WHO [6] and 45 mg/liter by Bureau of Indian standard (IS-10500)[7]. In several developing countries high nitrate concentration at times up to 500mg/liter is not uncommon [6,7,8].

Rabbits were chosen because their stomach pH is similar to that of infants (3.0—5.0)[9]. Oxygen is essential for formation of methemoglobin by nitrite. Nitrate are reduced to nitrite by micro flora in the oral cavity & increased consumption of nitrite leads to increased production of nitrates with 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 [10].

There are three stages of interaction between sodium nitrite and blood as: an induction period, a reactionary period & a terminal period, chiefly methemoglobin pass into hematin and other degradation products[10].

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[11].

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

MATERIALS AND METHODS

The study was conducted in the department of Anatomy, 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 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 the form of NaNO3) 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 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 kidneys were removed and biopsy was taken from the organ. These tissues were fixed in 10% formalin solution and subjected to histopathological examination.

OBSERVATIONS AND RESULTS

A. GENERAL OBSERVATION

In rabbits of all groups, the heart rate were 140/minute to 144/minute in the starting of the experimental period which increased up to 218/minute in rabbits of group E after completion of 120 days period (Table-I). The respiration rate was noted 56/minute to 60/minute in all rabbits on first day of experimental period which increased up to 84/minute in rabbits of group E on 120 the day of the experiment (Table-I).

It was observed that tachycardia and tachypnoea precedes the cyanosis even in groups where lethargy and cyanosis were not present.

Table 1: 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.)									
		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)	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, ↑ = Increase in Parameter, ↓ = Decrease in Parameter

Increase in heart rate and respiration rate was proportional to the nitrate concentration in the drinking water. These findings are similar as those of Kielbase et al [12] (2000) and Walton et al (1951) [13]. Kielbase et al (2000)[12] observed that isobutyl nitrate caused rapid dose dependent and parallel reduction in systolic and diastolic pressure with increase heart rate in experimental animal.

Walton et al (1951)[13] observed that in infants, the high nitrate concentration in drinking water produce an increase in respiratory rate.

B. HISTOPATHOLOGICAL CHANGES

No changes was observed in kidneys of rabbits subjected to water ingestion containing 45mg/ liter (control group). The changes in the interstitium occurred at 100 mg/L nitrate concentration administered to rabbits in group B (FIGURE -1). The changes were more marked in the form of mono nuclear infiltration of cells in intestitium in group C (FIGURE -2). The changes in tubules appeared in group D at 400 mg/l nitrate concentration in the form of hyaline and cellular casts and damaged tubular epithelium (FIGURE -3). The congestion of blood vessels was observed in group E only (FIGURE -4).

Fig. 1: Microphotograph of Kidney showing moderate interstitial mono-nuclear infiltration. **(10X), GROUP- B.**

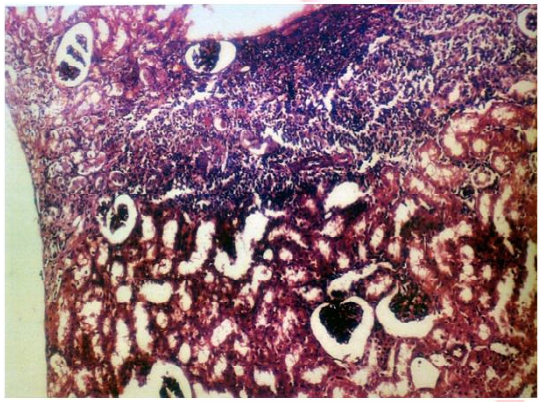

Fig. 2: Microphotograph of Kidney showing marked lymphocytic infiltration in interstitium. **(10X), GROUP-C**

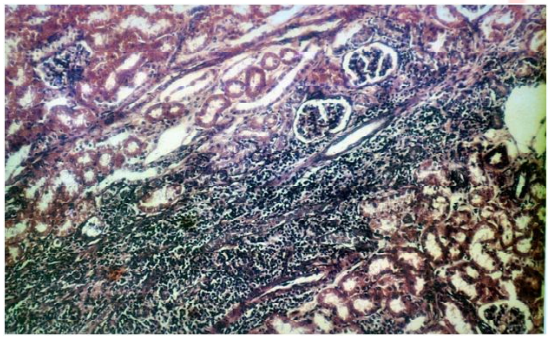

Fig. 3: Microphotograph of Kidney showing marked Hyaline and Cellular casts in renal tubules.
(10X), GROUP-D

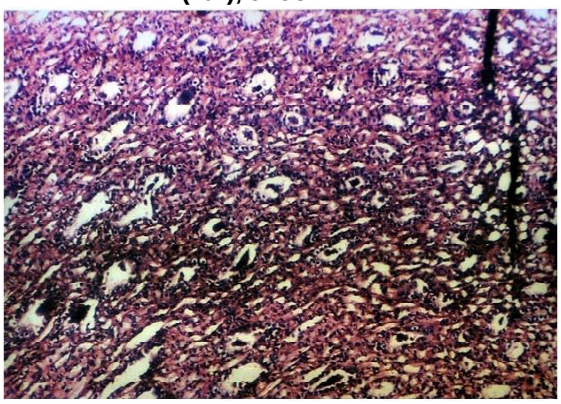

Fig. 4: Microphotograph Of Kidney Showing Hyaline And Cellular Casts In Renal Tubules And Marked Shedding Of Tubular Epithelium. **(40X), GROUP-E.**

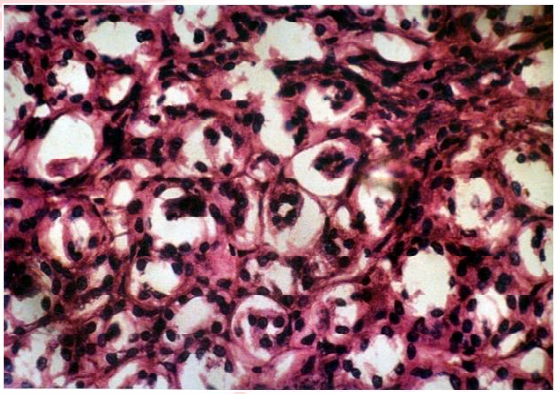

Table 2: Comparative histopathological changes in Kidneys of Rabbits in all groups.

KIDNEYS		GLOMERULUS	INTERSTITIUM	TUBULES	BLOOD VESSELS
5 Groups of Rabbits	GROUP A	NORMAL	NORMAL	NORMAL	NORMAL
	GROUP B	NORMAL	+	NORMAL	NORMAL
	GROUP C	NORMAL	++	NORMAL	NORMAL
	GROUP D	NORMAL	+++	&& ##	NORMAL
	GROUP E	NORMAL	++++	&&& ###	$$

NORMAL= Normal histology
(+) - Mild inflammation
(++) - Moderate inflammation
(+++) - Severe inflammation
& - Damaged tubular epithelium.
# - Presence of cellular and hyaline cast in proximal and distal tubules.
$ - Congestion of blood vessels.

The Histopathological changes have been depicted in table-2.

These findings are in accordance with Gataseva P at al (1996) [14] and Haley et al (1982) [15]. Gataseva P at al (1996) did experiment on 48 white female rats. The microscopic data showed parenchymatous granular dystrophy.

Haley et al (1982) [15] studied the effect of uranyl nitrate ingestion in rats and showed diffuse brush border loss in cortical proximal tubules. The proximal tubules were completely necrotic. The distal nephron segments exhibited considerable cellular swelling and vacuolization.

The results revealed that the degree of damage in the tissue was progressing as the nitrate content of the ingested water increased.

PATHOPHYSIOLOGY OF NITRATE TOXITY [16,17]:

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:

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.

DISCUSSION AND CONCLUSION

The results of present study proved strong interdependence between high nitrate concentration and changes in physical activities with histopathological changes of kidneys 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 methaemo globinemia. While an isolated study can be not extrapolated to humans it highlights the need for conducting further studies in population consuming nitrate rich water.

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.

ABBREVIATIONS

Cyt b_5 - Cytochrome b_5
Hb^{+2} - Ferrous state of hemoglobin
Hb^{+3} - Ferric state of hemoglobin
ICMR - Indian Council of Medical Research
Kg - Kilogram
mg - Milligram
NAD - Nicotinamide adenine dinucleotide
NADH -Nicotinamide adenine dinucleotide hydrogenase
pH - Negative logarithm of hydrogen ion
Red cyt b_5 - Reduced cytochrome b_5
Oxy $cytb_5$ - Oxidized cytochrome b_5
WHO - World Health Organization

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