ID: JCDR/2012/3068.3491:1844

Original Article

Experimental Research Section

Evaluation of the Haematological Responses to High Nitrate Exposure in Rabbits

MANOJ KUMAR SHARMA, HEMLATA SHARMA

ABSTRACT

Introduction: Nitrate is a common pollutant in the drinking water of Rajasthan as well as in the rest of India. The maximum permissible limit for the nitrate ion in drinking water has been set at 50 mg/l by the WHO and at 45 mg/ l by the Bureau of Indian Standards. In the body, nitrate is reduced to nitrite and the absorption of nitrite leads to methemoglobinaemia. Nitrate toxicity has been observed frequently in ruminants which has been fed on a variety of plant materials which had high nitrate content.

Aim: The present study examined a possible correlation between the nitrate concentration in drinking water and the blood profile changes in rabbits.

Materials and Methods: Rabbits were selected for the study because the pH of their stomachs was similar to that of human beings. We included 5 groups of rabbits, with 2 rabbits in each group. The groups were named A, B, C, D and E respectively. The nitrate concentrations in the drinking water were 45 mg/l, 100 mg/ l,200 mg / l,400 mg / l and 500 mg/ l in the groups A to E respectively. The group A was considered as the control group. After 120 days of the ingestion of sodium nitrate in drinking water, blood samples were collected from the rabbits of each group and were observed.

Results and Conclusion: The maximum haematological parameters such as WBC, lymphocytes, MCHC, RBC, Hb and basophills showed decreased values in the higher groups as compared to the values of the control group. The PCV and MCV showed increased values in the higher groups as compared to the values of the control group.

The present study indicated that the symptoms of nitrate toxicity were associated with the low oxygen carrying capacity of blood in the affected animals. Nitrate toxicity is a potential health hazard and the scarcity of data on the nitrate toxicity in blood created an interest in attempting the present study.

Key Words: Nitrate, Nitrite, Blood, Methemoglobin, Toxicity, Rabbits

INTRODUCTION

A major part of the Indian population is exposed to nitrate through dietary sources [1]. The maximum permissible limit for the nitrate ion in drinking water has been set at 50 mg/l by the WHO and at 45 mg/l by the Bureau of Indian Standards [2, 3, 4]. In several countries, especially in India, the consumption of water which contains high nitrate concentrations, sometimes as high as 500 mg/l, is common [4-6]. In the human body, nitrate is reduced to nitrites, which cause methemoglobinaemia [6]. When nitrate is ingested it is reduced to nitrites in the intestinal tract, although some studies suggested that this process may start in the oral cavity [6-7]. The nitrite ions are absorbed in to the blood stream where they convert haemoglobin to the methemoglobin [7]. The health risk from the exposure to nitrate is therefore related not only to its concentration in drinking water and food, but also to a condition which is conducive to its reduction to nitrites [8].

This action may be brought about in one of the following ways [9]: By the direct action of the oxidant or by the action of a hydrogen donor in the presence of oxygen, or by auto oxidation. In the presence of nitrites, the ferrous ion of haemoglobin gets directly oxidized to the ferric state. Normally, methemoglobin is formed by the following reaction:

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

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

$$Oxy\ cytb_5 + NADH \text{ ----------> } Red\ cyt\ b_5 + NAD.$$

Thus, the enzyme, cyt b_5 reductase plays a vital role in counter acting the effect of nitrate ingestion.

We chose rabbits for the study because the pH of their stomachs is similar to that of human beings [6]. So, it was proposed to study the effects of nitrate toxicity in the blood profile of rabbits .

MATERIALS AND METHODS

This study was conducted on five groups of rabbits, with 2 rabbits in each group. The rabbits were used for the study because the pH of their stomachs was similar to that of infants (pH= 3.0-5.0) [6]. The rabbits were three and a half to four months old and their weights varied from 1.310 kg to 1.720 kg. The 5 groups were identified as A, B, C, D and E. Ad libitum quantity of water which contained 45, 100, 200, 400 and 500 mg/liter nitrate (in form of NaNO3) and food which was soaked in the same water were given to the groups A, B, C, D and E respectively. The group which consumed 45mg/ liter of nitrate served as the control group. After the completion of the experimental period, the blood samples were collected from the rabbits of each group in heparinized vials according to the guidelines of ICMR and they were sent to the central lab of Swai Man Singh Hospital and Medical College, Jaipur for haematological estimation. The results of the blood estimation were recorded and shown by using an autoanalyzer [Tables/Fig-3, 4, 5, 6 and 7].

S. No	Blood Profile	Normal Range of Values	Group A (45mg/L)	Group B (100mg/L)	Group C (200mg/L)	Group D (400mg/L)	Group E (500mg/L)
1	WBC	6-12/cumm	5.40	9.97	4.28	4.96	5.16
2	N%	17-52	-	-	-	-	-
3	L%	42-80	35.2	-	44.9	45.0	20.0
4	M%	5-8	3.3	-	3.5	4.2	2.7
5	E%	0-3	-	-	-	-	-
6	B%	0-5	1.1	3.4	1.2	1.0	1.0
7	RBC	4-7/cumm	5.75	4.35	6.19	5.10	5.52
8	Hb	8-15 gm	12.8	11.7	12.9	12.6	12.3
9	PCV	30-50%	33.1	29.7	39.9	37.9	37.5
10	MCV	68µ3	63.3	68.3	64.5	74.3	67.9
11	MCH	24-7 µg	22.3	26.9	20.8	24.7	22.3
12	MCHC	35-0%	33.6	39.4	32.3	33.2	32.8

[Table/Fig-1]: Blood Values as observed in rabbits of all groups.
mg/l = Milligram per liter.

S. No	Blood Profile	Group A (45mg/L)	Group B (100mg/L)	Group C (200mg/L)	Group D (400mg/L)	Group E (500mg/L)
1	WBC	5.40/cumm	↑	↓	↓	↓
2	N%	-	-	-	-	-
3	L%	35.2	-	↑	↑	↓
4	M%	3.3	-	↑	↑	↓
5	E%	-	-	-	-	-
6	B%	1.1	↑	↑	↓	↓
7	RBC	5.75/cumm	↓	↑	↓	↓
8	Hb	12.8 gm	↓	↑	↓	↓
9	PCV	33.1%	↓	↑	↑	↑
10	MCV	66.3µ3	↑	↑	↑	↑
11	MCH	22-3 µg	↑	↓	↑	↔
12	MCHC	33.6%	↑	↓	↓	↓

[Table/Fig-2]: Comparison in blood values of rabbits of all groups with control group (Group A).
↑ = Increased value than control group; ↓ = Decreased value than control group; ↔ = same value as observed in control group;
- = No value was obtained; mg/l = Milligram per liter.

OBSERVATION AND RESULTS

The values of the blood for all the groups were obtained by using an autoanalyzer at the Swai Man Singh Hospital , Jaipur and they were compared with the values of the blood of group A (control group), separately one by one [Table/Fig-I and 2].

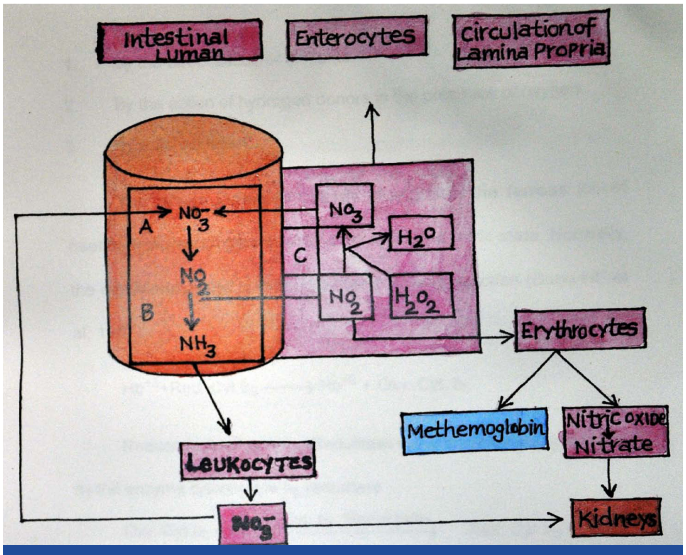
The WBC and MCHC showed increased values in the blood of group B only and continuous decreased values in that of group C, D and E respectively as compared to the values of the blood of group A.

The lymphocytes and monocytes showed increased values in the blood of groups C and D, but in group E, decreased values was observed as compared to the values in the blood of group A.

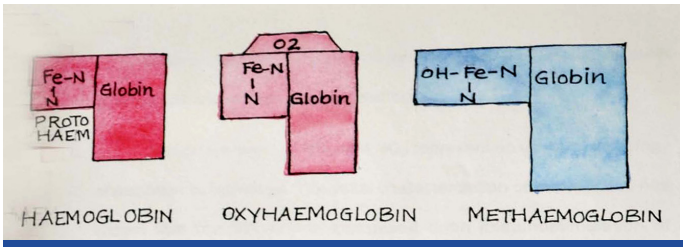
The basophils showed increased values in the blood of groups B and C and decreased values in the blood of groups D and E as compared to the values of the blood of group A respectively.

RBC and Hb showed decreased values in the blood of groups B, D and E as compared to the values of the control group, but PCV showed decreased values in blood of group B only as compared to the values of the blood of group A.

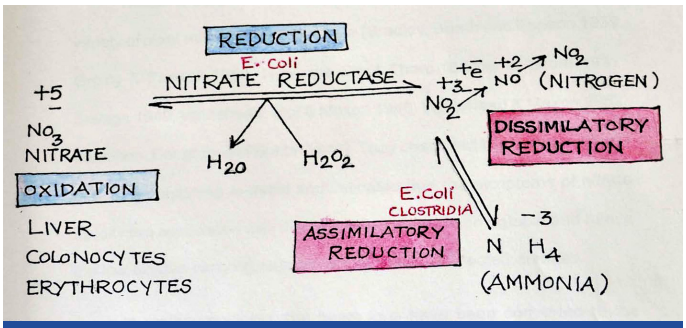
MCV showed increased values in the blood of the rabbits of all the groups.



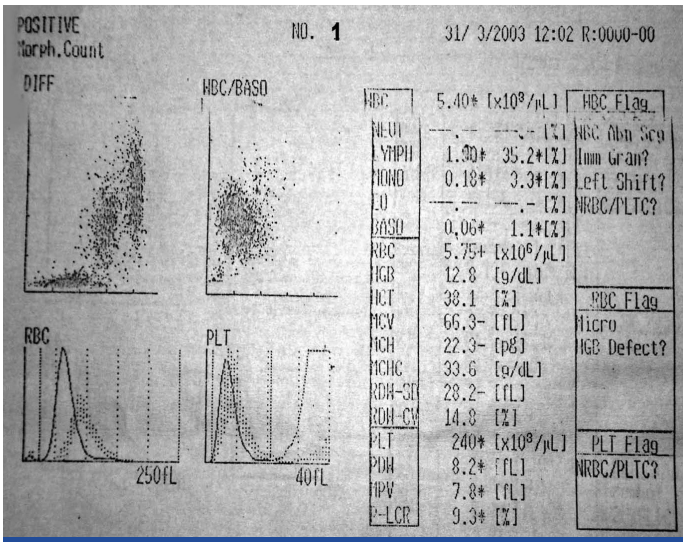
[Table/Fig-3]: Conversion of ingested inorganic & organic nitrate to nitrite by microflora in the oral cavity and in the gastro-intestinal tract by intestinal microflora, these activities result in increased oxidation of hemoglobin to methemoglobin and increased production of nitric oxide.
A=Bacterial nitrate reductase; B=Bacterial nitrite reductase; C= Catalase
(Recurrent Diarrhea in children living in Ares with High levels of Nitrate in Drinking water. *Archives of Environmental Health* 56(4):371. August 2001)



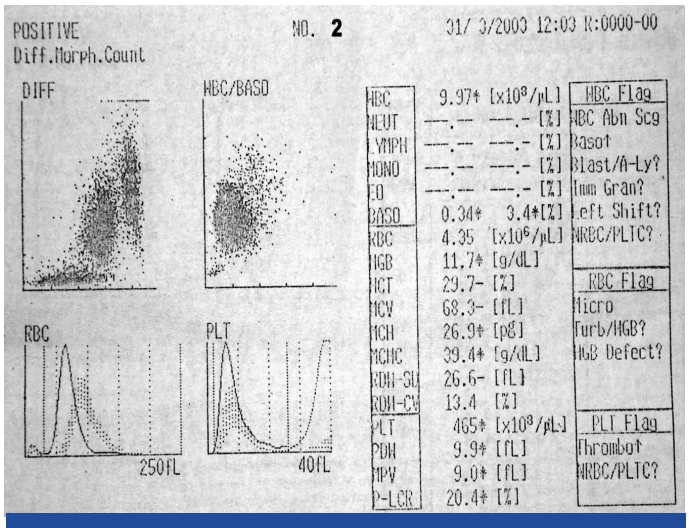
[Table/Fig-4]: Structure of Haemoglobin, Oxyhaemoglobin and Methaemoglobin (FARRANT-M, 1945)



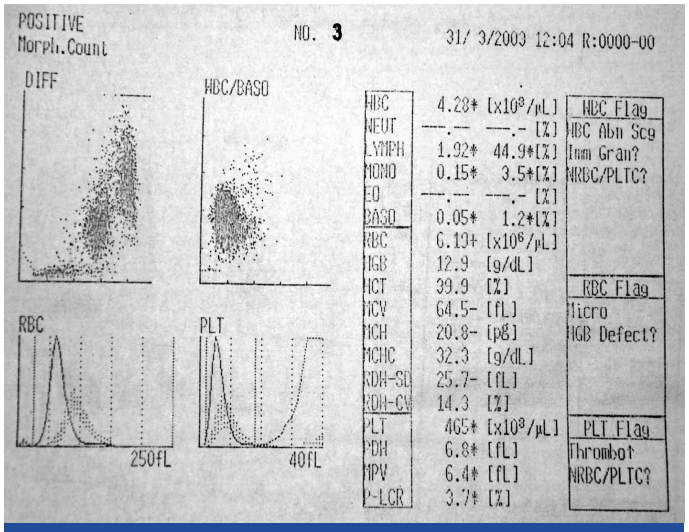
[Table/Fig-5]: Aerobic and anaerobic bacteria, body tissues and the oxidation – reduction state of nitrogen. Role of Nitrite and Nitrate as a redox couple in the rat colon. *Gastroenterology*, 916; Vol. 94(4), 1988.



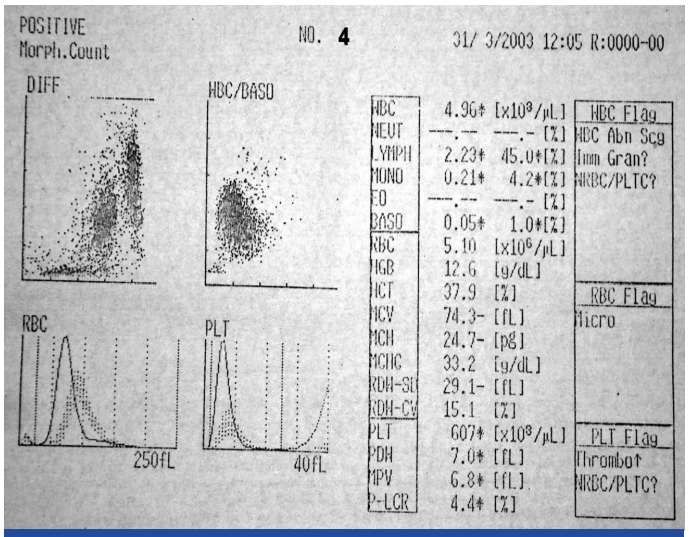
[Table/Fig-6]: Laboratory examination of blood in Rabbits of Group - A (45 mg/l)



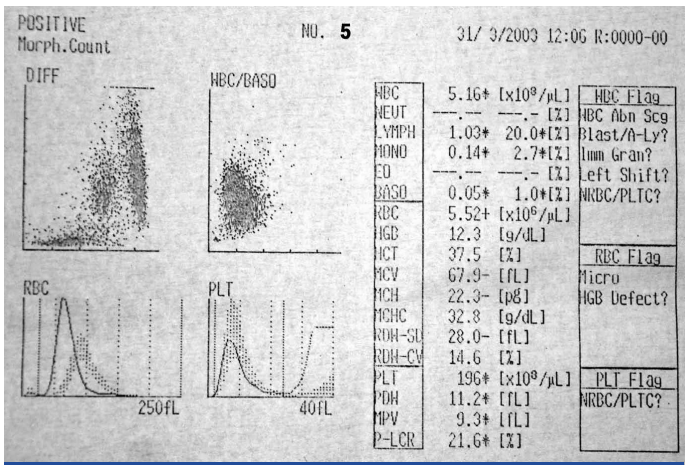
[Table/Fig-7]: Laboratory examination of blood in Rabbits of Group- B (100 mg/l)



[Table/Fig-8]: Laboratory examination of blood in Rabbits of Group - C (200 mg/l)



[Table/Fig-9]: Laboratory examination of blood in Rabbits of Group - D (400 mg/l)



[Table/Fig-10]: Laboratory examination of blood in Rabbits of Group - E (500 mg/l)

MCH showed decreased values only in the blood of group C and increased values in the blood of groups B and D respectively, but group E showed the same value as was observed in the blood of group A.

DISCUSSION

The presence of high nitrate concentrations in drinking water causes severe methemoglobinaemia and clinical cyanosis in infants

and adults. The present study indicates that the excess intake of nitrate adversely affects the blood profile in rabbits.

Marshall W et al [9] observed the action of nitrites on blood. The interaction of sodium nitrite and blood takes place in three stages:

(a) An induction period
(b) A reactionary period
(c) A terminal period which is often prolonged, during which the products of the reaction, chiefly methemoglobin, pass into haematin and other degradation products.

Darling and Roughton [10] described that the action of nitrite on haemoglobin was extremely complicated. It varies with the molecular ratio of nitrite to haemoglobin, pH, the presence or absence of O2 and reducing agents and possibly with other factors.

In the present study, Hb showed increased values in the blood of group C as compared to the values of the control group. This finding was consistent with the findings of Ogur R et al [11] .

The WBC showed decreased values in groups C, D and E (in the higher concentration group) as compared to the values of the controls. This finding was consistent with the findings of Tepperman J et al [12].

RBCs showed decreased values in the higher groups as in the groups D and E. These findings were in accordance with the results of Bodannsky O et al [13] who also reported that the repeated administration of methemoglobin producing agents like nitrate, acetanilide ,acetophanetidine, etc. may cause haemolytic anaemia but that it may be dose dependant.

MCV showed continuous increased values in the higher groups as in the groups A, B, C, D and E in the present study, which showed similarity with the findings of Barry N. Madison et al [14] who studied the haematological responses of acute nitrite exposure in fishes. They observed that nitrite became more lethal in the presence of high bacterial activity due to the waste build up in the body.

Van Loon et al [15] found that the chronic administration of acetanilide (up to 36 mg/kg) caused little or no haematological changes in dogs, but that larger doses produced haemolytic anaemia.

Nitrates are potential health hazards and they have been known to cause methemoglobinaemia and other changes in the blood profile [16]. The scarcity of data on the nitrate toxicity in blood created an interest in attempting the present study, but a more elaborative study with larger number of samples is required to establish a positive correlation .

ABBREVIATIONS

1. Cyt b_5	Cytochrome b_5
2. Hb^{+2}	Ferrous state of hemoglobin
3. Hb^{+3}	Ferric state of hemoglobin
4. ICMR	Indian Council of Medical Research
5. Kg	Kilogram
6. mg	Milligram
7. NAD	Nicotinamide adenine dinucleotide
8. NADH	Nicotinamide adenine dinucleotide hydrogenase
9. pH	Negative logarithm of hydrogen ion
10. Red cyt b_5	Rduced cytochrome b_5
11. Oxy $cytb_5$	Oxidized cytochrome b_5
12. WHO	World Health Organization
13. WBC	White Blood Cells
14. RBC	Red Blood Cells
15. Hb	Hemoglobin
16. PCV	Packed Cell Volume
17. MCV	Mean Cell Volume
18. MCH	Mean Cell Hemoglobin
19. MCHC	Mean Cell Hemoglobin Concentration.
20. N%	Neutrophills
21. L%	Lymphocytes
22. M%	Monocytes
23. B%	Basophills

ACKNOWLEDGEMENT

I am extremely thankful to my esteemed teacher ,distinguished guide and true mentor Dr. (Mrs.) Neelam Bapna, Ex professor and Head, Department of Anatomy, Swai Man Singh Medical College, Jaipur, India for the precise guidance, valuable suggestions and constant encouragement without which it would not have been possible for me to start and finish this research work.

I am also thankful to Dr. Suresh Joshi, Ex Deputy Director, University Grant Commission Academic staff college and Associate Professor, Department of Zoology, University of Rajasthan, Jaipur, India whose initiative gave me a sense of professional duty and pride.

I am equally thankful to Dr. Dhindsa , Superintendent Chemist, Public Health and Engineering Department for his cooperation in the estimation of nitrate in drinking water and other chemical evaluations during my research work.

Mrs. Hemlata sharma, Lecturer, Department of Anatomy, Jhalawar Medical College, Jhalawar, India was helpful in preparation of this manuscript and helped me in quite a lot with the leg work.

Equally important was the friendly guidance and cooperation of Dr. Vijay Shekhawat, Animal Surgeon and Mr. Manohar singh, Animal House attendant, Swai Man Singh Medical College, Jaipur, India for their help in providing a healthy environment for the rabbits during my experiments.

The whole research work was funded by myself only.

REFERENCES

[1] 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.
[2] World Health Organization. Nitrates, Nitrites and N-Nitroso compounds. *Environmental Health criteria*,Geneva. 1977;5:77.
[3] World Health Organization. Guidelines for drinking water quality, Volume 1.Geneva. 1993; 52-53.
[4] Bureau of Indian Standards. *Drinking water specification IS 10500, 1991*, New Delhi, India.1995;3.
[5] Ferrant M, Black PM, Jolesz F, Kikinis R, Macq B. et al. Methemoglobinemia. Two cases in new born infants which were caused by the presence of nitrates in well water. *The journal of pediatrics*.1945: 585-91.
[6] Gupta SK, Gupta RC, Gupta AB, Seth AK, Bassin JK, Gupta A. Recurrent acute respiratory tract infection in areas with high nitrate concentration in drinking water. *Environmental Health Perspectives*. 2001; 108(4):363-66.
[7] Gupta SK, Gupta RC, Gupta AB, Seth AK, Bassin JK, Gupta A. Epidemiological evaluation of recurrent stomatitis, nitrates in drinking water and the cytochrome b_5 reductase activity. *The American Journal of Gastroenterology*. 1999; 94(7):1808-12.
[8] Gupta SK, Gupta RC, Gupta AB, Seth AK, Bassin JK, Gupta A. Adaptation of the cytochrome b5 reductase activity and methemoglobinemia in areas with high nitrate concentration in drinking water. *Bulletin of The World Health Organization* 1999;77 (9) : 749- 753.
[9] Marshall CR, Marshall W. The action of nitrates on blood. Journal of Biological Chemistry. 1945; 158:187-208.

[10] Darling RC, Roughton FJW. The effect of methemoglobin on the equilibrium between oxygen and haemoglobin. *Am. J. Physiol* 1948; 137:56-6.
[11] Ogur R, Korkmaz A, Hasde M. Effect of high nitrate intake in rats. *Journal of Basic & Clinical Physiology and Pharmacology* 2000;11(1): 47-56.
[12] Tapperman J,Marquardt R, Reifenstin GH. Methemoglobinemic cyanosis –Report of an epidemic which was caused due to the corning extract which was substituted for maple syrup. *JAMA* 1951; 146 (10): 983-85.
[13] Bodansky O. Methemoglobinemia and methemoglobin producing compounds. *Pharmacol. Rev*. 1951; 3:144-78.
[14] Madison BN, Wang YS. Haematological responses of acute nitrite exposure in various fishes. *Aquatic Toxicity*. 2006;79 (1): 16-23.
[15] Van L, Clark EJ , Byron B, Blair D. Haematologic action of acetanalid and acetophenetidine in dogs. *Journal of Lab and Clinical Medicine* 1944;89 : 948-56.
[16] Shuval HI, Gruener N. Epidemiological and toxicological aspects of nitrates and nitrites in the environment. *Am J Public Health*. 1972; 62:1045-52.

AUTHOR(S):
1. Dr. Manoj Kumar Sharma
2. Mrs. Hemlata Sharma

PARTICULARS OF CONTRIBUTORS:
1. Assistant Professor, Anatomy
   Jhalawar Medical College,
   Jhalawar - 326001 (Rajasthan), India.
2. Lecturer, Anatomy
   Jhalawar Medical College,
   Jhalawar - 326001 (Rajasthan), India.

NAME, ADDRESS, TELEPHONE, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Manoj Kumar Sharma
III/5, Staff quarters, Medical college campus,
Jhalawar Medical college, Jhalawar - 326001 (Rajasthan), India.
E-mail: drmanojsharma2002@yahoo.co.in
Phone: 07432-233412; Mob. 09667334455; 09929391333

FINANCIAL OR OTHER COMPETING INTERESTS:
None.

Date of Submission: **Aug 28, 2011**
Date of Peer Review: **Oct 21, 2011**
Date of Acceptance: **Dec 20, 2011**
Date of Publishing: **Feb 15, 2012**