

Histopathological study for the effect of vitamin C on the some mice tissues treated with nitrate

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SUMMARY

Thirty two mice were used to study the histopathological changes resulted from the effect of vitamin C on the some mice tissues treated with nitrate.

Mice were divided randomly in to four equal groups. The 1st group was given Vit C and potassium nitrate via water. The 2nd group was given potassium nitrate and the 3rd group received Vit C only, while the 4th group represented the control.

Two animals from each group were scarified weekly. The results showed similar pathological changes in the 1st and 2nd group especially in the stomach and intestine which gave severe changes that characterized by hyperplastic changes in addition for the hemorrhages and the infiltrations of inflammatory cells in all layers of both organs. Less alteration was seen in the liver and kidney. On the other hand, no pathological changes in the 3rd and 4th group were recorded.

دراسة نسيجية مرضيه لتاثير فيتامين C على بعض الاعضاء الداخليه للفئران المعاملة بالنترات

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الخلاصة

تم استخدام (32) فار لدراسة التغيرات النسيجية المرضيه الناتجة من تاثير فيتامين C على بعض انسجة الفئران المعاملة بالنترات. قسمت الفئران عشوائيا الى (4) مجاميع متساوية .

المجموعة الاولى اعطيت فيتامين C و نترات البوتاسيوم عن طريق الماء . و المجموعة الثانية اعطيت نترات البوتاسيوم , و المجموعة الثالثة اعطيت فيتامين C فقط . المجموعة الرابعة تمثل مجموعة السيطرة .

تم قتل حيوانين من كل مجموعة اسبوعيا. النتائج اظهرت تغيرات مرضية متشابهة في المجموعتين الاولى و الثانية وخاصة في المعدة والامعاء حيث كانت شديدة تميزت بفرط التنسج والنزف وارتشاح للخلايا الالتهابية في كل طبقات العضوين. كانت التغيرات اقل في الكبد و الكلية . من جانب اخر لم تسجل تغيرات مرضية في المجموعتين الثالثة و الرابعة.

Introduction

Nitrates contamination of water from fertilizers or from the breakdown of organic wastes is a common problem (1).local studies have shown that rivers of Iraq are polluted with different levels of nitrate (2). Nitrate may react in the stomach with nitro stable compounds (e.g. secondary and tertiary amines or amides in food) to form N- Nitroso compounds , most of which have been found to be carcinogenic in all animal species test , so that they are probably also carcinogenic to humans. It has been clearly established that the intake of certain dietary components presenting vegetables such as vitamin C decreased the risk of gastric cancer due to decrease in the formation of N-Nitroso compounds (3).

It appears that vitamin C fights off these pollutants by stimulating enzymes in the liver that detoxify the body. Another way in which Vit C protects us is by preventing the development of nitrosamines, the cancer –causing chemicals that stem from the nitrate contained in many foods (4).

This work aimed to study the effect of vitamin C on stomach, intestine, liver and kidney with nitrate treated mice.

Material and method

Thirty two mice were divided randomly in to four equal groups the 1st group was given vitamin C in concentration 200mg\liter freely via water and potassium nitrate in concentration 2gm\liter (4, 5). The 2nd group was given potassium nitrate in concentration 2gm\liter .the 3rd group was given vitamin C in concentration 200mg\liter ,the 4th group represented the control.

The experimental period was (4) weeks. Two animals were killed after each week of this period. Tissue specimens from stomach, intestine, liver, and kidney were fixed in 10% neutral buffered formalin and then tissue specimens were processed histopathology and paraffin blocks were cut at 4-6mm.thickness and tissue sections were stained with routine hariss haematoxylin and eosin stain (6).

Results

Macroscopic alteration:

There is alteration in the 1st and 2nd group characterized by appearances of congestion in the blood vessels on the surface of stomach, intestine and there is no obvious changes in the liver and kidney. No alteration in the 3rd and 4th group.

Microscopic alteration:

The 1st group (Vitamin C +Pot.nitrate) and second group (pot. nitrate) showed similar pathological alteration characterize by:

Stomach: - the pathological changes seen in the second weeks of the experiment .which characterize by hyperplasia and hypertrophy of the mucosa, in the 3rd and 4th weeks it become more hyper plastic, there is necrosis in the upper layer of mucosa with presence of necrotic cell in the lumen of stomach with infiltration of inflammatory cells "neutrophils" in the mucosa, sub mucosa and serosa. Also there is congestion of blood vessels and edema in the sub mucosa. In the last week there is hemorrhage in the sub mucosa (figure 1).

Intestine: the pathological alteration started in the 2nd week of the experiment, epithelial cells showed hypertrophy and hyperplasia with obvious aggregation of inflammatory cell in the sub mucosal area. In the last week there is congestion and hemorrhage in the sub mucosa, and wide degeneration and necrosis in the mucosal and sub mucosal layer. Figure (2, 3, and 4).

Liver: - pathological changes characterize by congestion and diltation of the central vein with infiltration by inflammatory cells "neutrophils" in and out the central vein, also there is degeneration and necrosis in some of hepatocytes and enlargement of the others (hyperatrophy) and in others there is proliferation. These alterations started in the 2nd week and continue to the end of the experiment (figure5).

Kidney: - the pathological changes seen in the 4th week, kidney parenchyma showed edema and some renal tubules showed complete degeneration of lining epithelium and more sever necrosis in few other tubules (figure 6).

3rd group (vitamin C)and4rd group (control):- no pathological changes.

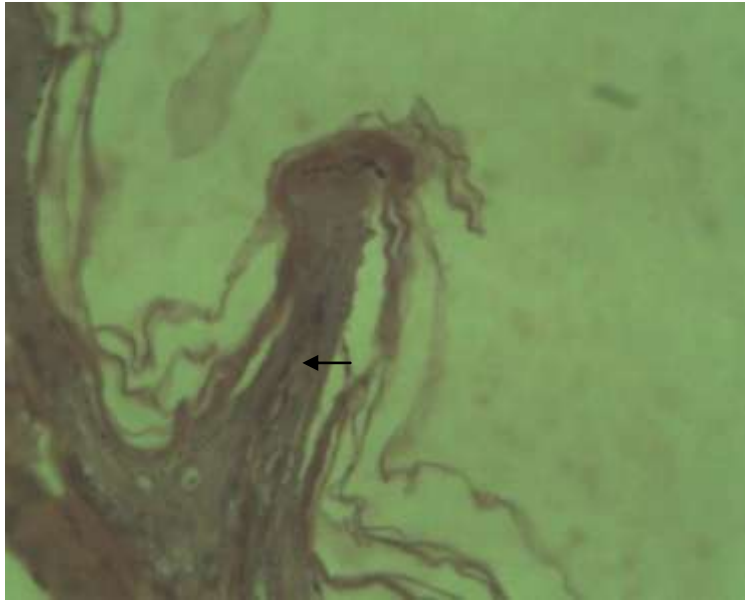


Figure (1): Stomach hyperplasia of epithelia lining no glandular stomach (H and E X400)

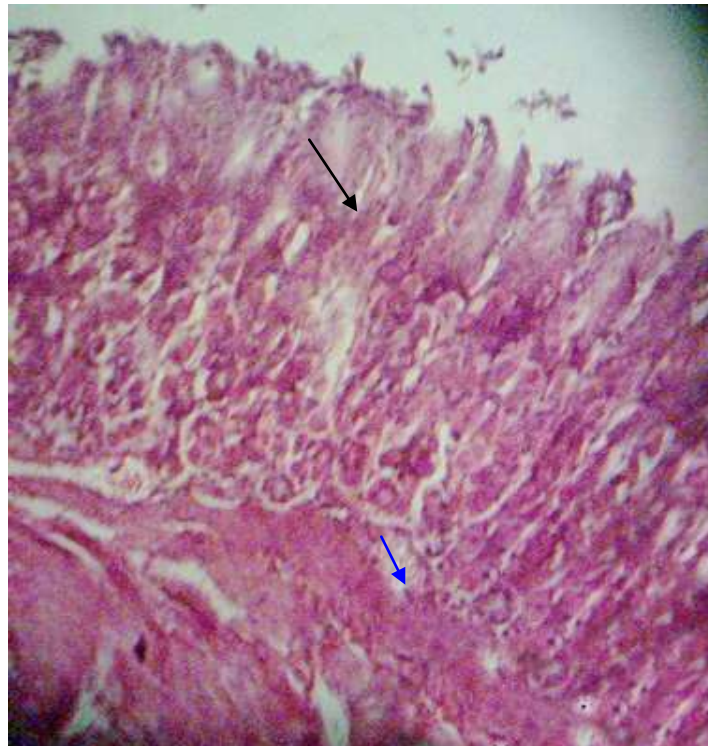


Figure (2): section of the intestine, showed hyperplasia, hypertrophy of the mucosa (↓) with sloughing of the epithelial cells, with infiltration of the inflammatory cells (↓).(H and E X 200)

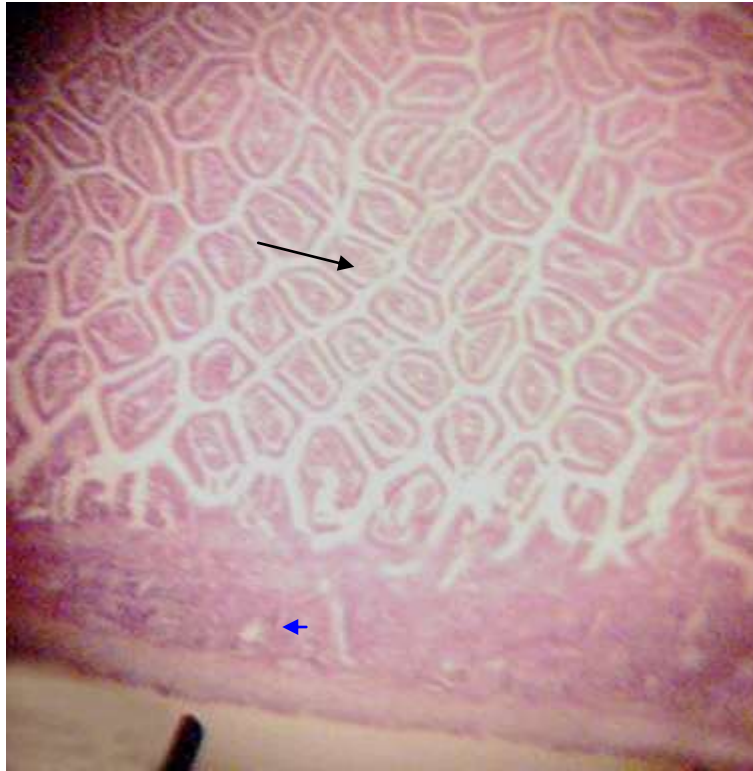


Figure (3) histological section of the intestine notices the hyperplasia, hypertrophy of the mucosa () with edema in the sub mucosa (). (H and E X100)

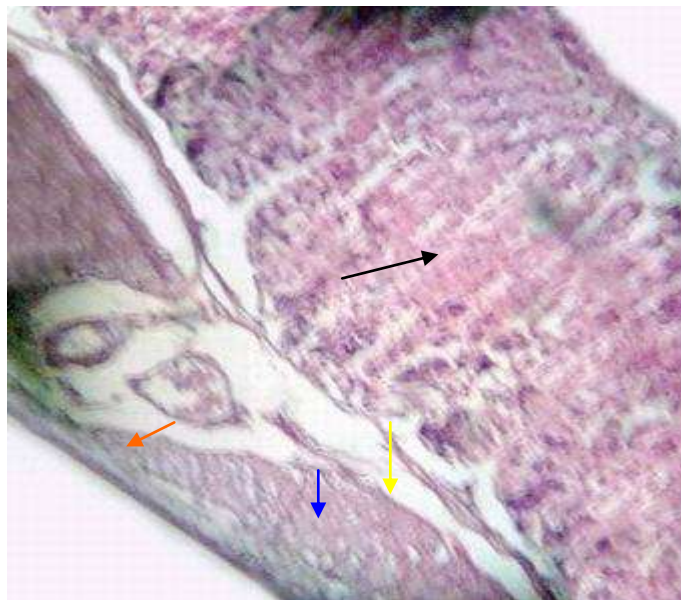


Figure (4): histological section of the intestine notice the hyperplasia and hypertrophy of the mucosa with infiltration of inflammatory cells in the sub mucosa also there is edema and congestion of the blood vessels. (H and E X200)

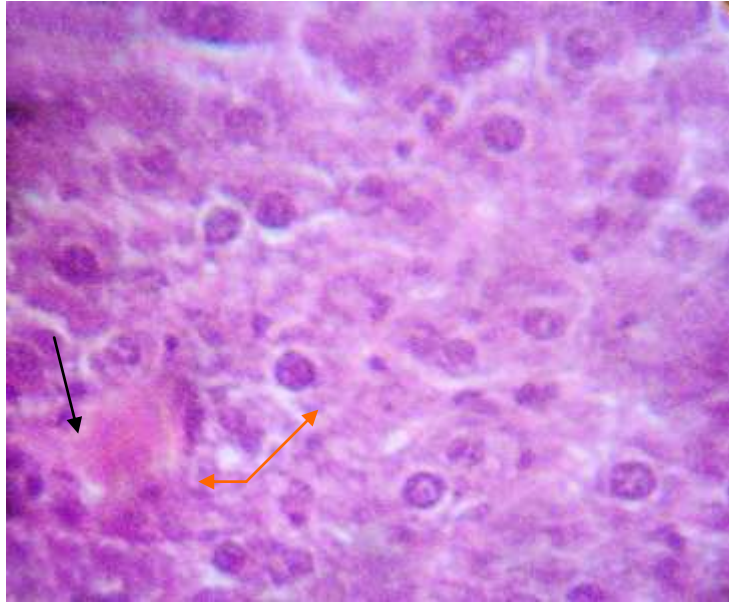


Figure (5): histological section of liver from the 1st group showing congested central vein surrounding by hepatocyte which appeared enlarged, degenerated and some are necrotized. (H&E: X200)

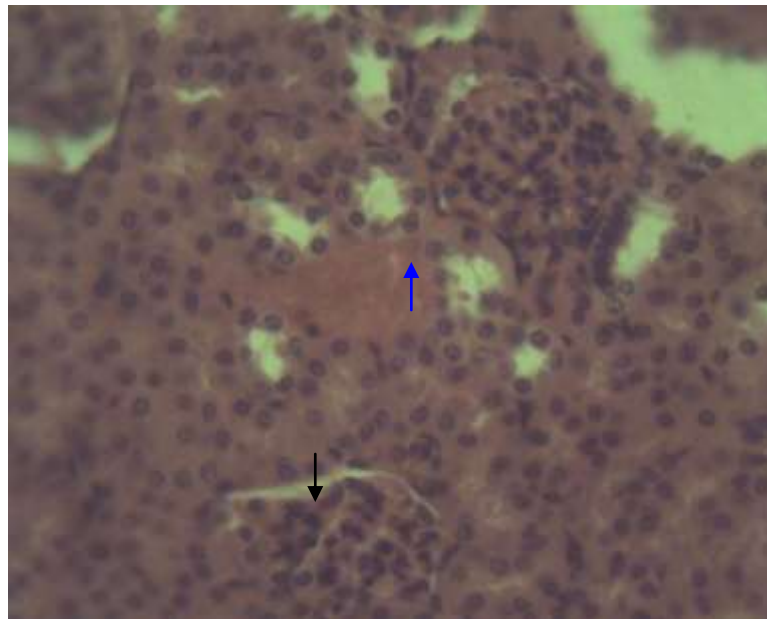


Figure (6): Kidney showing enlargement of glomeruli and the enlargement of the cells lining urinary tubules (H and E X200).

Discussion

Vitamin C can reduce the development of nitrosamines from nitrates, chemicals that are commonly used in processed foods. Once formed, nitrosamine can become a carcinogen. But several human studies, in which the subjects consumed a nitrosamine precursor, the urinary levels of nitrosamines were significantly reduced by vitamin C. Three animal studies also support the preventive effect of ascorbic acid on nitrate induced cancer. In all three cases, the formation of tumors was inhibited, suppressed or reduced in frequency in the animals treated with vitamin C (4).

But recent investigations have demonstrated that nitrate causes severe pathological changes in the stomach and intestine due to its direct caustic action on alimentary mucosa and the hyperplastic change in stomach can be considered as a precancer change in mice (7,8).

A nitrate is the most irritating diuretic that is responsible for the damage of renal tubules. While the main function of the liver in the biotransformation of toxicants explains the pathological alteration in the liver (2, 3, 5, 7, 9 and 10), it appears that vitamin C does not work as a preventive effect on nitrate in the 1st group (vitamin C + pot. nitrate) due to the irritating action of nitrate on the mice tissues.

Animal studies reveal that ascorbic acid is not toxic after a single or repeated administration of relatively large doses, also histological examination of various organs showed no definite changes in mice, guinea-pig, rat tissues (11, 4). These studies supported our results that addition of vitamin C only (3rd group) makes no difference in the pathological alteration.

References

1. Dalefield, R.R., and Oehme, F.W. (1997). Stability of water nitrate levels during prolonged boiling. *Vet. Human, Toxicol*, 39(5): 97.
2. Abdul-razzaq, H.A. (1992). The effect of nitrate in drinking water on the liver function in laying hens. *Iraq J. Vet. Med.* 16:30-40.
3. World Health Organization. (1996). Guide lines for drinking –water quality. Second Edition, Health Criteria and other supporting information, Geneva: 269.
4. Nxtfrontier.Com Pte Ltd. (2005). Vitamin C:39.
5. Al-saadi, R.N. and Al-sultan, I.I. (2004). Experimental pathological study of nitrate toxicity in broilers. *Iraq J. Vet. Med.* (28) 1:22-31.
6. Luna, H.T. and Lee, G. (1968). Manual histological staining method of the armed forces. Institute of pathology. 3rd .ed. The blakiston division McGraw-Hill Co. New York. Toronto, London, Sydney:20-131.

7. Jubb,K.V.F.;Kennedy,P.C.and Palmer,N.(1985).Pathology of domestic animals . 3rd Edition, Academic press Inc.,Orland,London,Sydney,pp.37.
8. Radostits, O.M.; Blood, D.C.; Gay, C.C. (1997).Veterinary Medicine. A text book.Stomach,Neoplasm.8ed.WBSaunders company Ltd. London , Philadelphia , Toronto , Sydney, Tokyo. (2):pp198-231.
- 9.Amstutz,H.E.;Anderson,D.P.;Armour,S.J.;Jeffcott,L.B.,loew,F.M.; Wolf ,A. M. (1998).The Merck veterinary manual. Eighth Edition, Merck &Co. Inc.White house station, N.J., U.S.A., pp.2091-2094.
10. Al-aboudi, A.R. (1999).Pathology Study of experimental nitrate toxicosis in chickens. Iraq J.Vet.Med.Sci.2 (12):341-345.
11. World Health Organization. (1974).Ascorbic acid and its potassium and sodium salts. WHO food additives series No.5.Geneva.