

Effects of Nitrate Toxicity on Vitamin A Concentration and the Thyroid Gland Status in Albino Rats

Atef Mohammed Khalil^{1*}, Seddik Hammad²

¹Department of Pathology and Clinical Pathology, Faculty of Veterinary Medicine, South Valley University, Qena, Egypt

²Departments of Forensic Medicine and Toxicology, Faculty of Veterinary Medicine, South Valley University, Qena, Egypt

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*Corresponding author: Atef Mohammed Khalil

Abstract

Background: Nitrates and nitrites are naturally found in fruits, vegetables and grains. In addition, they are used as food additives, preservatives and colour fixatives. Nitrite toxicity is induced by formation of methaemoglobin, and carcinogenic substances e.g. nitrozoamines. **Materials and methods:** In the current study we explored the impacts of chronic exposure of albino rats to potassium nitrate. Fourteen rats were randomly divided into 2 groups (n=7), control group and potassium nitrate treated group in dose of 20 mg/kg for 3 months. Subsequently, urine and blood samples were collected at 1, 2 and 3 months. At the end of the experiment (3 months), rats were sacrificed and thyroid glands were harvested. **Results:** Biochemical analysis exhibited significant decrease in the urinary iodine and blood-based thyroid hormones (T3 and T4), vitamin A compared with matched-time control group. However, calcium, nitric oxide and MDA showed significant increase in potassium nitrite treated rats. Marked thyroid injury was observed in the form of size and shape of the thyroid follicles, some follicles were enlarged and filled with colloid fluids, but other were completely empty upon sodium nitrite exposure. Moreover, in some cases hyperplasia with leukocytic infiltrations in the parafollicular cells replacing the atrophied follicles. **Conclusion:** Our results provide new evidences that toxic effect of potassium nitrite is possibly mediated by inhibition of iodine absorption, vitamin A level, thyroid destruction and depression of the antioxidant system.

Keywords: Biochemical analysis, Histopathological examination, Oxidative damages, Potassium nitrate, Thyroid gland, Urinary iodine.

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INTRODUCTION

Nitrates and nitrites are chemicals that can be found naturally in our environment. Nitrates are essential (needed) nutrients for plants to grow. Nitrates can be found in the air, soils, surface waters and ground water underground drinking water [1]. Organic and inorganic sources of nitrogen are transformed to nitrate by mineralization, hydrolysis and bacterial nitrification [2]. One of the most important sources of nitrate includes natural fertilizers, animal and human waste, nitrogen oxides from utilities and automobile, and leguminous crops that fix atmospheric nitrogen [3]. They are used as food additives, being added as preservatives and color fixatives to some processed foods (particularly cured meats, fish and cheese) [4]. Although the preservatives which are permitted in foods are considered to be without potential adverse effects there have been concerns about the safety of nitrites. Nitrite, in high concentrations, is undoubtedly toxic to humans. Acute effects have been observed from accidental ingestion, for example in contaminated

drinking water [5], sausages [6] and medicines [7]. Toxicological studies have documented that nitrite is toxic to animals [8]. The toxic effects of nitrites have been related to the formation of methaemoglobin, inducing hypertrophy of the adrenal zona glomerulosa in rats and the formation of cancerogenic nitrozoamines [8,9,4]. In man, previous studies have shown that a food iodine deficiency represents the principal factor of the development of the goitre [10, 11]. In animals, goitrogenic effects of iodine deficiency or nitrate ingestion were also observed. Indeed, for rats, a food iodine deficiency induced the goiter [12,13].

The consumption of nitrates induced histological modifications of the thyroid gland [14,15,16] as well as a decrease in the secretion of the thyroid hormones [17]. Also Nitrate toxicity causes many diseases of endocrine system which affects on one or all body systems [18]. Dietary nitrate accelerates the depletion of vitamin A through prevention its conversion from B- Carotene [19].

In this context, the major objective of the present study was to estimate the effect of increasing levels of the dietary intake of nitrites in laboratory rats on: (1) urinary iodine excretion, (2) changes in the morphology of thyroid follicles, and (3) thyroid gland hormonopoesis.

MATERIALS AND METHODS

Experimental animals and housing

A total of fourteen (14) rats (average body weight =15-22 kg), at age 3- 6 weeks, purchased from the animal house, Cairo university. All animals were housed in clean cages and given standard diet and clean water ad libitum. Cages were placed in an air-conditioned room ($23 \pm 3^{\circ}\text{C}$) with 12:12 hour light: dark cycle. Animals were kept for two weeks before starting of the experiment for acclimatization, during which they subjected for clinical and laboratory examinations. All protocols were approved by the Institutional Review Board for Animal Experiments of South Valley University, Egypt.

Experimental design

The animals were allocated randomly into 2 groups (n=7), Group 1: served as control group the rats were received saline in by stomach tube. Group 2: received 20 mg/kg body weight of potassium nitrate (equivalent to 1/40 LD50) orally/day, dissolved in water and given for successive 3 months. The animals examined daily for any abnormality in their behavior throughout the experimental period. Blood was collected monthly from the jugular vein of all experimental groups for biochemical examinations. At the end of the experiment the rats were euthanized and dissected for collection of thyroid glands for histological examinations.

Urine and blood collection

Urine was collected quantitatively from each rat during the last 3 days (88–90 day) of the experiment. The pooled 3-day samples were stored at -20°C until analysis. At the end of the experiment (day 90), the rats were anaesthetized with thiopental. Blood was rapidly collected by cardiac puncture, transferred to centrifuge tubes with no anticoagulant, and serum was separated by low-speed centrifugation ($1500 \times g$, 15 min). The serum samples were stored at -20°C until analysis. After bleeding,

Iodine in urine

Iodine in urine was determined after digestion with chloric acid solution (potassium chlorate and perchloric acid, 70%) using the Sandell-Kolthoff spectrophotometric method [20].

Biochemical analyses

Iodine in urine was determined after digestion with chloric acid solution (potassium chlorate and perchloric acid, 70%) using the Sandell-Kolthoff spectrophotometric method [20]. Serum serum thyroid

hormones Triiodothyronin (T3) and thyroxin (T4) concentrations were measured using enzyme immune linked sorbent assay (ELISA) kit (Genway Biotech inc Co, America). Serum Vitamin A and calcium were analysed with standard kits (*Novus Biotech Brand, America* and *Biodiagnostic co, respectively*). Malondialdehyde (MDA) and nitric oxide (NO) were estimated colorimetrically by using a standard assay kit (Biovision Incorporated 155s. Milpatas Boulevard, Milpatas, USA and Biochain Institute, Inc, USA, respectively).

Histopathological examination

Pieces of thyroid gland were freshly prepared, fixed in 10% neutral buffered formalin at room temperature overnight and embedded in paraffin. Sections were cut and then stained with hematoxylin and eosin [21].

Statistical Analysis

The data were statistically analyzed by one-way (ANOVA) analysis of variance (pc-state computer program) and the least significant difference (L.S.D) was used to test the difference between treatments. Results were considered statistically significant when $P < (0.05)$.

RESULTS:

Urine iodine concentration

Administration of potassium nitrite for successive 3 months induced decrease in the concentration of iodine that excreted in urine which start from the first month and become highly significant in the second and third months when compared with control rats as shown in (Fig 1).

Thyroid hormones

(Fig. 2) reveals the effect of potassium nitrate toxicity on T3 and T4 hormones which characterized by significant decrease in the levels of both hormones was recorded in all inoculated animals in comparison to the healthy rats.

Correlation among urine iodine and thyroid hormones concentrations

The level of thyroid hormones (T3 and T4) correlate positively with the urine iodine concentration in the potassium nitrate inoculated animals. The correlation was positive in both hormones levels (T3 and T4), $R^2 = 0.9634$ and $R^2 = 0.9977$ respectively as shown in (Fig. 3).

Vitamin (A) and calcium levels

For vitamin (A) level the exposed group to potassium nitrate revealed significant decrease in the levels of the calcium and vitamin A in the serum starting from the first month and decrease continuously until the third month of administration as shown in (Fig. 4).

Serum oxidative stress markers (Nitric oxide and MDA)

Nitrate toxicity has great effect on the anti-oxidative system where nitrite administration for 3 months induced significant increase in the levels of oxidative stress markers (nitric oxide and lipid peroxidation) in all months of exposure as exhibited in (Fig 5)

Thyroid gland and parathyroid gland

Macroscopically, slight hypertrophy was detected in the thyroid gland in administered animals. Microscopically, the thyroid follicles appeared in different in size and shape, some of thyroid follicles were enlarged and filled with colloid fluids, but other were completely empty from colloid. Hyperplasia in the parafollicular cells were recorded which replaced the atrophied (necrosed) follicles. A large focal area of the gland showed collapsed follicles with presence of some leukocytes (Fig. 6).

Figure ligand

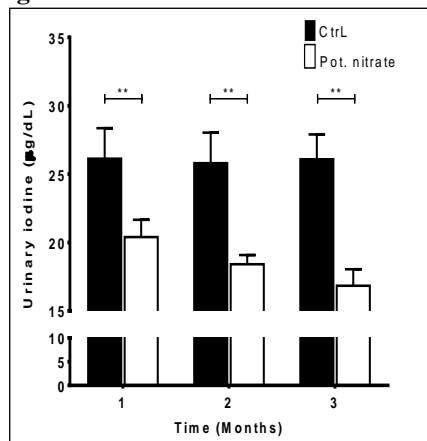


Fig-1: Effect of potassium nitrate administration for excessive three months on the urine iodine excretion . $P < 0.01$ compared with control values. Bars represent means \pm SEM (N=7).

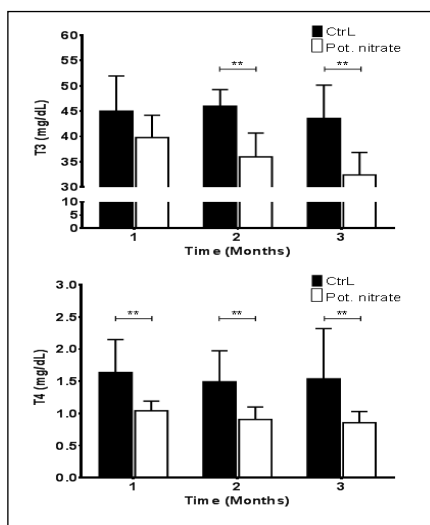


Fig-2: Effect of potassium nitrate administration for excessive three months on T3 and T4 hormones concentration. $P < 0.01$ compared with control values. Bars represent means \pm SEM (N=7).

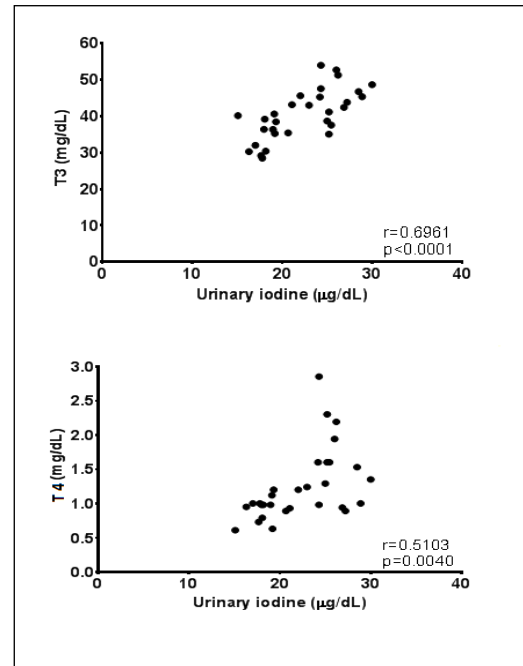


Fig-3: Correlation among level of thyroid hormones and urine iodine concentration in period of three months in potassium nitrate inoculated group. $P < 0.01$ compared with control values.

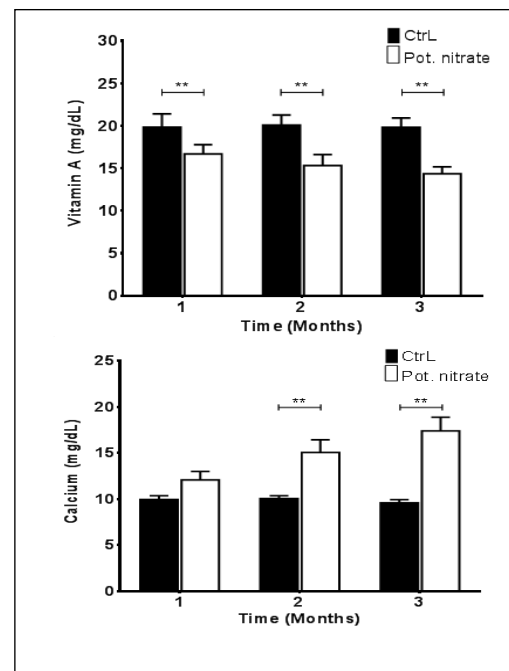


Fig-4: Effect of potassium nitrate administration for excessive three months on serum Vitamin A and serum calcium levels. $P < 0.01$ compared with control values. Bars represent means \pm SEM (N=7).

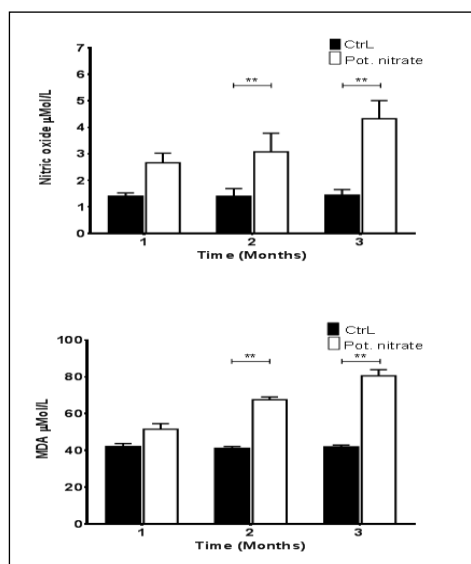


Fig-5: Effect of the potassium nitrate administration for excessive three months on the oxidative stress markers (NO and MDA). $P < 0.01$ compared with control values. Bars represent means \pm SEM (N=7).

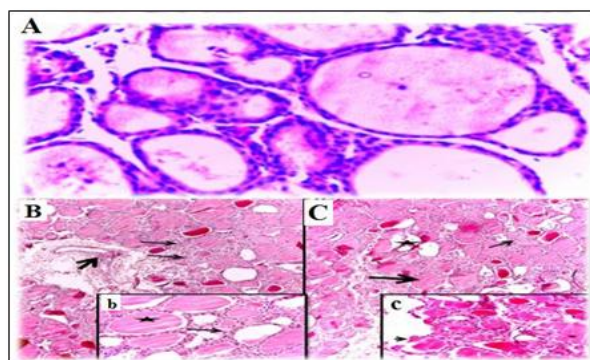


Fig-6: Histological changes (B and C) in hematoxyline and eosin stained section in thyroid gland following potassium nitrate oral administration for excessive three months compared with normal histological structure of thyroid gland (A).

In picture (B) thyrotoxicity was marked by necrosis and atrophy in some thyroid follicles (thin arrow) associated with vascular destruction (Thick arrow). In picture (b) a high power of picture (B) showing hyperplasia in the Parafollicular epithelium (thin arrow) with presence of some follicles fully distended with colloid fluids (Star). In picture (C) some follicles appeared atrophied (thin arrow) and some others appear fully distended (thick arrow) while some other follicles completely empty (Stars). In the high power of picture C (c) there are some empty follicles and ruptured follicular epithelium. (H&E, X.400)

DISCUSSION

Nitrate is the most important known environmental pollutants. It pollutes food, water (especially underground water), plants and soil [22]. There is a high risk for animal and human to be exposed for nitrate toxicity [23].

This study was designated to investigate the biochemical and histopathological effects of nitrite toxicity in rats. Data in our study revealed that exposure to potassium nitrite for three months induced decrease in the urine iodine excretion, same result was obtained by other study [24], which described the decreases in the urine iodine excretion could be explained by decreased iodine absorption in the digestive tract. This negative effect of nitrite could be either indirect *i.e.* by inhibiting Na^+/K^+ ATP-ase complex and energy generation for iodine transmembrane transport [25] or direct *i.e.* by inhibiting sodium-iodide symporter Na^+/I^- [26, 27, 28], both involved in iodine trapping in gastric mucosa. For the thyroid hormones metabolism nitrite toxicity induced significant decrease in the circulating thyroid hormones (T3 and T4). Decrease the ratio of the thyroid hormones was due to the fact that, in the synthesis of thyroxine iodine must first be absorbed by the gland as iodide latter converted into an organic compound by combination with tyrosine. The nitrate inhibits these processes via uptake of the iodide, and prevents the conversion into an organic compound and so prevents the formation of thyroxine. The low level of thyroid hormones stimulates the production of thyrotrophic hormone which cause hypertrophy and hyperplasia of the follicular epithelium as sign of toxic thyroiditis [29, 30].

The correlation among urine iodine excretion and thyroid hormones was strongly positive which means the decrease of iodine absorption leads to decrease the thyroid hormones synthesis. In accordance nitrite toxicity induced significant decrease in the calcium level. The changes in the function of the parathyroid gland may be induced by wide varieties of chemical substances that either elevate or lower the blood calcium concentration [29]. Hypertrophy and hyperplasia reported in our cases was a feature of the decrease of calcium concentration in the blood [18, 31].

For better understanding the correlation between nitrite toxicity and thyroid gland disturbances, vitamin A was analyzed. Exposure to nitrite induced significant decrease in Vitamin A level. Vitamin A Deficiency (VAD) occurred as nitrate is thought to interfere with the conversion of plant carotene to vitamin A [2]. VAD decreases thyroidal iodine uptake, impairs thyroglobulin (Tg) synthesis, and increases thyroid size [32, 33], which confirm our theory of urine iodine excretion. Furtherly, nitrite toxicity induced significant increase in lipid peroxidation (MDA) and nitric oxide level this comes in agreement with the study done by Hanaa *et al.*, 2009 [34]. Nitrite toxicity increase the oxidative stress in the tissue this could be attributed to that nitrite can also be an important source of NO, particularly under acidic conditions [35]. In turn, the produced nitric oxide (free radical) plays an important role in the pathogenesis of cellular injury [36] that increases the MDA production. In our study administration of potassium nitrite induced significant

increase in the nitric oxide level. In this work, the histopathological changes in the endocrine gland were: the thyroid gland revealed hyperplasia in the follicular and parafollicular cells. Some follicles were hypertrophied and contain large colloid volume. Vacuolation is prominent in the cytoplasm of the hypertrophied follicles. Flattening appeared in the epithelium of some follicles with pressure atrophy in the surrounding follicles. In the long term treatment, the same lesions appeared with necrosis and degeneration in the collapsed follicles. Extensive desquamation of the follicular cells, proliferation in the interstitial connective tissues that replaced the atrophied tissues thickening and hyalinization in the arterial wall of the thyroid gland was observed. Similar lesions were reported by [30, 37] who showed follicular hyperplasia, vacuolization, congestion and increase amount of colloid in some follicles (toxic thyroiditis). Other studies revealed that toxic thyroiditis manifested morphologically by hypertrophy and increase in size of the thyroid gland [15, 30, 38]. Hypertrophy and hyperplasia of the thyroid follicles was to compensate the deficiency of the thyroid enzymes in the blood (feedback mechanism) as the level of T3 and T4 was found to be significantly decreased in the blood in comparison to control animals.

CONCLUSION

In conclusion, these results provide new evidences that toxic effect of potassium is possibly mediated through depression the antioxidant system and subsequent cellular damage.

REFERENCES

- Gehle, K. (2009). Agency for Toxic Substances and Disease Registry. Nitrate/nitrite toxicity. Case studies in environmental medicine (CSEM).
- Allison, D. (1998). Nitrate poisoning of livestock, Cooperative Extension Service. Colleague of Agriculture and Home Economics. Guide. B-807, 112-117.
- Fields, S. (2004). Global nitrogen: cycling out of control.
- WHO.(1995). Evaluation of Certain food additives and contaminants. Forty-fourth report of the Joint FAO/WHO Expert Committee on Food Additives. Geneva, Technical Report Series 859, 29–35.
- Bradberry, S.M., Gassard, B., & Vale, J.A.(1994). Methemoglobinemia caused by accidental contamination of drinking water with sodium nitrite J. Toxicol. Clin. Toxicol, 32 (2), 173-178.
- Kennedy, N., Smith, C. P., & McWhinney, P. (1997). Faulty sausage production causing methaemoglobinaemia. *Archives of disease in childhood*, 76(4), 367-368..
- Ellis, M., Hiss, Y., & Shenkman, L. (1992). Fatal methemoglobinemia caused by inadvertent contamination of a laxative solution with sodium nitrite. *Israel journal of medical sciences*, 28(5), 289-291.
- Jensen, F. B. (1995). Uptake and effects of nitrite and nitrate in animals. *Nitrogen metabolism and excretion*, 289-303.
- McKnight, G. M., Duncan, C. W., Leifert, C., & Golden, M. H. (1999). Dietary nitrate in man: friend or foe?. *British Journal of Nutrition*, 81(5), 349-358.
- Aquaron, R., Nguessi, P., Ben Eno, L., & Rivière, R. (1985). Le goitre endémique au Cameroun (Provinces de l'Est et du Nord Ouest). *Rev. Franc. Endocrinol*, 26, 537-545.
- Arnese, A., Colapietro, M., De Riu, S., Prospero, E., Saggiocco, G., Ullucci, R., & Grasso, G. M. (1992). Study of an endemic goiter area in northern Campania. *Journal of endocrinological investigation*, 15(2), 103-108.
- Stübner, D., Gärtner, R., Greil, W., Gropper, K., Brabant, G., Permanetter, W., ... & Pickardt, C. R. (1987). Hypertrophy and hyperplasia during goitre growth and involution in rats separate bioeffects of TSH and iodine. *European Journal of Endocrinology*, 116(4), 537-548.
- Mooij, P., De Wit, H. J., Bloot, A. M., Wilders-Truschnig, M. M., & Drexhage, H. A. (1993). Iodine deficiency induces thyroid autoimmune reactivity in Wistar rats. *Endocrinology*, 133(3), 1197-1204.
- Höring, H., Ellinger, C., Nagel, M., Paldy, A., & Desi, I. (1986). The action of phenylmercuriacetate and nitrate in combined application in rats: the thyroid gland, liver enzymes and morphologic findings in the brain and kidney. *Die Nahrung*, 30(7), 713-721.
- Gatseva, P., Marinova, S., & Vürtigova, L. (1992). Changes in the thyroid and other internal organs of experimental animals exposed to a drinking regimen with a high nitrate content. *Problemi na khigienata*, 17, 43-47.
- Gatseva, P., Lazarova, A., Maximova, S., & Pavlova, K. (1996). Experimental data on the effect of nitrates entering the organism with the drinking water. *FOLIA MEDICA-PLOVDIV AND SOFIA*, 38, 75-83.
- Jahreis, G., Hesse, V., Rohde, W., Prange, H., & Zwacka, G. (1991). Nitrate-induced hypothyroidism is associated with a reduced concentration of growth hormone-releasing factor in hypothalamic tissue of rats. *Experimental and Clinical Endocrinology & Diabetes*, 97(01), 109-112.
- Klassen, D.C. (2001). Casarett and Doull's, Toxicology. The basic science of poisons. 6th ed. McGraw-Hill medical publition. New York and Toronto. 20;415-428.
- Hatfield, E.E., Smith, G.S., Neumann, A., L., Forbes, R. M., & Garrigus, U.S. (1961). Interactions of nitrite, alpha-tocopherol, and tapazole upon the vitamin A nutrition of lambs fed "high nitrate" silage. *J. Animal Sci.* 20:676.

20. Dunn, J. T., Crutchfield, H. E., Gutekunst, R., Dunn, A. D., Bourdoux, P., Gaitan, E., ... & Suwanik, R. (1993). Methods for measuring iodine in urine.
21. Bancroft, J. D., & Gamble, M. (Eds.). (2008). *Theory and practice of histological techniques*. Elsevier health sciences.
22. Spalding, R. F., & Exner, M. E. (1993). Occurrence of nitrate in groundwater—a review. *Journal of environmental quality*, 22(3), 392-402.
23. [23] WHO (2006). World Health Organization, Nitrate and potential endogenous formation of N-nitroso compounds Food Additives Series: no 50 Who. Geneva, P1-9
24. Kostogrys, R. B., Pisulewski, P. M., & Pecio, A. (2006). Nitrates affect thyroid status and serum triacylglycerols in Wistar rats. *Polish journal of food and nutrition sciences*, 15(1), 71-76.
25. Grudziński, I. P. (1998). Effect of nitrates and nitrites on small intestine. *Roczniki Panstwowego Zakladu Higieny*, 49(3), 321-330.
26. Kotani, T., Ogata, Y., Yamamoto, I., Aratake, Y., Kawano, J. I., Suganuma, T., & Ohtaki, S. (1998). Characterization of gastric Na⁺/I⁻ symporter of the rat. *Clinical immunology and immunopathology*, 89(3), 271-278.
27. Chung, J. K. (2002). Sodium iodide symporter: its role in nuclear medicine. *Journal of Nuclear Medicine*, 43(9), 1188-1200.
28. Szökeová, E., Tajtakova, M., Mirossay, L., Mojzis, J., Langer, P., Marcinova, E., ... & Bodnar, J. (2001). Effect of nitrates on active transport of iodine. *Vnitřní lékařství*, 47(11), 768-771.
29. Hayes, A. W. (2007). *Principles and methods of toxicology*. Crc Press.
30. Zaki, A., Chaoui, A. A., Talibi, A., Derouiche, A. F., Aboussaouira, T., Zarrouck, K., ... & Himmi, T. (2004). Impact of nitrate intake in drinking water on the thyroid gland activity in male rat. *Toxicology letters*, 147(1), 27-33.
31. Thrall, M. A., Weiser, G., Allison, R., & Campbell, T. (Eds.). (2012). *Veterinary hematology and clinical chemistry*. John Wiley & Sons.
32. OBA, K., & KIMURA, S. (1980). Effects of vitamin A deficiency on thyroid function and serum thyroxine levels in the rat. *Journal of nutritional science and vitaminology*, 26(4), 327-334.
33. Ingenbleek, Y. (1983). Vitamin A-deficiency impairs the normal mannosylation, conformation and iodination of thyroglobulin: a new etiological approach to endemic goitre. In *Nutritional Adequacy, Nutrient Availability and Needs* (pp. 264-297). Birkhäuser, Basel.
34. Hassan, H. A., El-Agmy, S. M., Gaur, R. L., Fernando, A., Raj, M. H., & Ouhtit, A. (2009). In vivo evidence of hepato-and reno-protective effect of garlic oil against sodium nitrite-induced oxidative stress. *International journal of biological sciences*, 5(3), 249.
35. Zweier, J. L., Wang, P., Samouilov, A., & Kuppusamy, P. (1995). Enzyme-independent formation of nitric oxide in biological tissues. *Nature medicine*, 1(8), 804.
36. Marletta, M. A., Yoon, P. S., Iyengar, R., Leaf, C. D., & Wishnok, J. S. (1988). Macrophage oxidation of L-arginine to nitrite and nitrate: nitric oxide is an intermediate. *Biochemistry*, 27(24), 8706-8711.
37. Gupta, S. K., Gupta, R. C., Chhabra, S. K., Eskiocak, S., Gupta, A. B., & Gupta, R. (2008). Health issues related to N pollution in water and air. *Current Science*, 1469-1477.
38. Sell, J. L., & Roberts, W. K. (1963). Effects of dietary nitrite on the chick: growth, liver vitamin A stores and thyroid weight. *The Journal of nutrition*, 79(2), 171-178.