HYPOTHYROIDISM IN MALE RATS OF DIFFERENT AGES EXPOSED TO NITRATE POLLUTED DRINKING WATER

BY

Azza M. El-Wakf; Hanaa A. Hassan; Fahmy G. El-said; Abeer E. El-Said
Department of Zoology, Faculty of Science, Mansoura University.

ABSTRACT

The effect of nitrate pollution on the thyroid hormonal levels, body weight and other health indices was studied in male rats of different ages. The tested rats were divided into young (3 weeks old) and adult (12 weeks old) groups exposed to pollution via sodium nitrate (NaNO3) intake in drinking water in concentrations 100, 250, 550 mg/L daily for 4 months. The study revealed a dose dependent decrease in serum levels of thyroid hormones thyroxine (T4) and triiodothyronine (T3) accompanied by increased levels of thyroid stimulating hormone (TSH) in all nitrate exposed rats, indicating development of hypothyroidism. A significant reduction of the body weight gain was also recorded in the young animals at different nitrate doses, but only in the adults with the high dose. Meanwhile, a significantly elevated nitric oxide (NO) level in the serum and urine were noticed that confined to the young animals with medium (250 mg/L) and high (550 mg/L) nitrate doses and the high dose adult animals. In addition, nitrate exposure resulted in a decreased serum concentrations of total proteins and protein fractions (albumin and globulin), accompanied by significant increases in the urea and creatinine levels in both serum and urine of the tested rats. This together with the above detected changes was found to exceed as the nitrate concentration increased and to be more pronounced in the young rats. Therefore, it can conclude that prolonged exposure to nitrate polluted drinking water is toxic to the thyroid hormonal activity and the other measured indices in both young and adult rats, but the young animals comprised the most age group susceptible to nitrate toxicity.

Key Words: Nitrate pollution - Thyroid hormonal levels - Nitric oxide.

INTRODUCTION

High consumption of nitrate polluted drinking water causes various health problems in human (Zaki et al., 2004). The most well known are methemoglobenemia (Gupta et al., 2000; Schnobrich et al., 2007), growth retardation (Mukhopadhyay et al., 2005) and gastric cancer (Vermeer et al., 1998).

Other target for nitrate exposure, such as thyroid gland has been recently researched; however results obtained in this respect were contradictory. Some workers showed an increase in thyroid volume
with signs of thyroid disorders when drinking water nitrate level is below (Zaki et al., 2004) or above (Van Maanen et al., 1994) the WHO standard of 50 mg/L (Avery, 1999), while others failed to show such effect (Knobeloch and Proctor, 2001). This contradiction could be related to different factors including level of nitrate exposure as well as age of exposure (Ogur et al., 2000, Zaki et al., 2004).

Therefore the present study was carried out on male rats of two ages; young (3 weeks-old) and adult (12 weeks-old) ingested sodium nitrate in drinking water at different doses (100, 250 and 550 mg/L) for continuous four months with the aim of evaluating nitrate effects on the thyroid hormonal status. Other health indices including body weight, total protein, urea and creatinine as well as nitric oxide (as a marker of nitrate accumulation in the body) were also investigated.

MATERIAL AND METHODS

1- Experimental animals:
Healthy male Wistar albino rats of two ages; young of 3-weeks old, weighing 35±5 g and adult of 12-weeks old, weighing 175±5 g were used in this experiment. All rats received standard diet and drinking water and lithium during the entire experimental period.

2- Experimental design:
Rats of each age were divided into four groups (each of 8 rats). The first one was the control group received tap water containing 4.9±2 mg NO3/L, and the other three groups received respectively drinking water containing sodium nitrate at doses (100 mg/L≤ 8.7 mg/kg body weight (bw), 250 mg/L≤ 21.7 mg/kg bw and 550 mg/L≤ 47.7 mg/kg bw) of sodium nitrate (National toxicology program 2001) daily for four months. These doses were chosen based on the previous experimental model for evaluating nitrate toxicity (Strateva et al; 1986, Ogur et al., 2000) as well as the recent ecological study carried by El-Wakf et al. (2008).

3- Animals investigation:
a - Body weight gain:
In order to follow the effect of nitrate on the body weight gain of rats, the body weight (g) was recorded at the start and end of the experiment for each tested group.

After four months of nitrate exposure, rats were kept individually in metabolic cages for 24 hours. Urine was frequently collected, centrifuged and stored at -20°C until analysis.

At the end of the experimental periods, the animals were then sacrificed and the blood samples were collected, centrifuged at 800 Xg for 15 minutes and non hemo-
lysed sera were separated for biochemical and hormonal analysis.

**b- Biochemical and hormonal analysis:**

Total protein and albumin concentrations were estimated using Diamond Diagnostic Kit as described by Henry (1964) and Doumans et al. (1971), respectively. Globulin concentration was calculated by subtracting albumin concentration from the total protein value. Urea and creatinine levels were measured using Diamond diagnostic Kits based on the methods of Patton and Crouch, 1977 and Henry (1974), respectively. Nitric oxide level was measured colorimetrically in both serum and urine using Diagnostic kit from ABC according to Fox et al. (1981). In addition, serum thyroid hormones [triiodothyronin (T3), thyroxine (T4) and thyroid stimulating hormone (TSH)] levels were measured by in vitro diagnostic radioimmunoassay with the immunoHite 2000 analyzer for the quantitative measurement as described by Ahmed et al. (1974), Wenzel (1981) and Babson (1991), respectively.

4- Statistical analysis:

All data are represented as means ± SE. One way analysis of variance (One-way ANOVA) followed by Least Significant Difference (LSD) test were used to determine differences among means of investigated groups. The level of statistical significant was set at P < 0.05 (Snedecor and Cochran, 1989).

**RESULTS**

1- Body weight gain:

As shown in table 1, the present data recorded a significant and dose-dependent decrease in the body weight gain of the young animals with different nitrate doses, besides the high dose adult animals if compared to their respective control groups.

2- Serum analysis:

As shown in table 2, the chronic four months treatment by different nitrate doses induced significant and dose dependent reduction in serum T3 and T4 levels of all young and adult animal groups except for the low dose adult animals which showed non significant decrease compared to their respective control groups. In contrast, TSH showed increased serum levels that were significant with medium and high dose groups of both investigated ages. Meanwhile, by comparing the two studied age groups, all adult animals exhibited significant decreases in serum T3 and T4 levels compared to their respective young groups, while no significant differences were detected with the measured TSH level.

Moreover, the present biochemical analysis showed dose dependent decreased
serum levels of total protein, albumin and globulin accompanied with corresponding increases in serum urea and creatinine concentrations, which in all tended to be significant in the younger animals with medium and high nitrate doses and the adults with high dose only. It was also showed that all adult groups had significantly increased total protein, albumin, globulin, urea and creatinine values if compared to their respective young groups (Table 2).

Additionally, all nitrate exposed groups of both ages exhibited a dose-dependent increase in serum nitric oxide levels which was significant in the young rats with medium and high nitrate doses, as well as the high dose adult animals. Also, nitric oxide levels in all adult groups showed lower but non-significant values comparing to their respective young groups.

3- Urine analysis:
As shown in table 3 obtained data indicated significant and dose dependent increase in urine total protein concentration of all nitrate exposed groups. Similarly, a dose dependent elevation in the urine levels of both urea and creatinine levels were observed, but this was only significant with both medium and high dose young groups, as well as high dose adult group. Moreover, all adult groups exhibited significant elevation in the urine total protein, urea and creatinine levels compared to their respective control groups.

With regard to nitric oxide obtained data illustrated increased urine nitric oxide concentrations of all investigated groups, which were significantly observed with both medium and high dose young groups, as well as the high dose adult group. By comparing the two studied ages, urine nitric oxide markedly decreased in all adult animals compared to their respective young groups, but this decrease was non-significant, except for the high dose group.

**DISCUSSION**

Body weight is one of the basic indices for assessment of the health status of an organism (Gatseva et al., 1999; Zaki et al., 2004). In the present study, long term exposure to drinking water with different nitrate concentrations (100, 250, 550 mg/L) showed decreased body weight gain in the tested rats of different ages, with the most pronounced decrease being demonstrated among the younger animals, particularly with the high nitrate concentration. Similarly, other investigations indicated that nitrate exposure causes body weight reduction in a dose-dependent manner (Zaki et al., 2004) that was greater in the young growing animals (Ogur et al., 2000). Primary, this reduced body weight can be explained by an increased protein
catabolism revealed by the low serum total protein and protein fractions (albumin and globulin) along with high levels of urea and creatinine in both serum and urine of nitrate exposed rats, however an increased urinary protein loss was also found. This protein loss probably resulting from nitrate-induced kidney dysfunction (Al-Ayed, 2000) seemed to be a second factor affecting the body weight in the present study. Additionally, nitrate could induce body weight loss by the way of inhibiting food intake through its influence on the nervous regulation of feeding behaviour (Jahreis et al., 1991) that was found to cause a decrease of growth hormone receptors within the liver, thus causing lack of plasma somatomedins (Jahreis et al., 1987) in turn affect body growth.

Beside this, a number of investigations have focused on the influence of nitrate on the thyroid status. Some workers showed thyroid hypertrophy with decreased thyroid hormones in people using drinking water with nitrate below (Eskiocak et al., 2005) concentrations or above (Tajtakova et al., 2006) the WHO nitrate standard of 50 mg/L (Gateva et al., 1998 and Tajtakova et al., 2006). Similarly, the animal studies by Jahreis et al. (1991) and Eskiocak et al. (2005) showed decreased serum levels of T₄ and T₃ with increased secretion of more TSH from pituitary to compensate for thyroid dysfunction in nitrate consumed rats. Such an effect is in keeping with the present study showing decreased serum levels of both T₄ and T₃ along with increased TSH in all tested rats, with the greatest effect being detected among the younger animals exposed to the high nitrate concentration.

Regarding the mechanism by which nitrate causes thyroid hormones deficiency, several studies indicated that nitrate seems to exert its effect in the organism after their gastric reduction into nitrite, then nitric oxide known to alter the thyroid gland (Zaki et al., 2004) as evidenced by: first the use of an inhibitor of NO synthesis reduces the vascular expansion observed in the human thyroid gland during goiter formation induced by a low iodine diet (Coline et al., 1995), as NO is a biogenic messenger that converts guanosin triphosphate (GTP) to cyclic guanosine monophosphate (cGMP) that induces vasodilatation (Zaki et al., 2004). Second, the long-term exposure to NO donors (nitroprosside and S-nitroglutathione) significantly inhibits iodine transport and organification in cultured bovine thyroid cells and reduces the differentiation of these cells (Costamagna et al., 1998). This possible inhibition of iodine transport by NO would be exerted on Na+/I- symporter (NIS), an intrinsic membrane protein that mediates the active transport of iodide into the thyroid cells (De La Vieja et al., 2000). Additionally, it was explained that decreased accumulation of iodide in
thyroid gland by nitrates might be responsible for the decreased activity of thyroid peroxidase, the enzyme that catalyzes the biosynthesis of thyroid hormones at different levels, including organization of iodide, iodination of tyrosine residues in thyroglobulin and coupling reactions for synthesis of T₄ and T₃ (Mukhopadhyay et al., 2005). These data thus indicated that increased NO production by nitrate exposure probably inhibits iodide transport into thyroid gland that consequently causes thyroid hormones deficiency. The present study confirmed this as the nitrate induced decrease of thyroid hormones (T₃ and T₄) goes in parallel with the increased NO levels in both serum and urine of all tested rats of both ages.

In this concern, several researchers indicated that thyroid hormones are produced as thyroglobulin and stored in the thyroid follicles for months. The thyroglobulin store of thyroid gland is sufficient to meet the needs of the body for 1-3 months (Guiles, 2000). For this reason, even if synthesis of thyroid hormones stops completely, a decrease in thyroid hormones levels in circulation and thus development of clinical insufficiency takes a long time (Guiles, 2000). This may be the cause for the lack of nitrate effect in a number of studies with short term nitrate exposure (Katti and Sathyanesan, 1987; Boas et al., 2006) in contrast to the long-term influence of nitrate, where the reserve iodide presumably became exhausted and thus resulted in a decreased serum levels of thyroid hormones, similarly as described in the present study and other earlier investigations (Zaki et al., 2004 and Eskicioglu et al., 2005).

In conclusion, the present study demonstrated that prolonged ingestion of nitrate in drinking water represents a risk factor for the development of hypothyroidism in all tested rats that seems to exceed with increasing nitrate concentration and to be more obvious in the younger rats. So, sanitary activities should be applied for decreasing nitrate concentrations in the drinking water in the agricultural areas with intensive farming, with the aim of preventing incidence of hypothyroidism among population living in these areas, particularly the younger ones.
Table (1): Body weight gain (g) of young and adult male rats after 4 months exposure to different nitrate doses.

<table>
<thead>
<tr>
<th>Animal Groups</th>
<th>Young Rats</th>
<th>Adult Rats</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Nitrate Exposed Groups</td>
<td>Control</td>
</tr>
<tr>
<td></td>
<td>Low dose</td>
<td>Medium dose</td>
<td>High dose</td>
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<tr>
<td>Initial body weight</td>
<td>Mean ± SE (N=8)</td>
<td>39.63 ± 1.17</td>
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<td>Final body weight</td>
<td>Mean ± SE (N=8)</td>
<td>315.13 ± 8.66</td>
<td>279.88 ± 5.11 (a)</td>
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<tr>
<td>Body weight gain</td>
<td>Mean ± SE (N=8)</td>
<td>275.5 ± 7.50</td>
<td>241.25 ± 4.34 (a)</td>
</tr>
</tbody>
</table>

Data are means ± SE, N= number of animals
\(a\) = significant difference between young nitrate exposed groups and their respective control.
\(b\) = significant difference between adult nitrate exposed groups and their respective control.
Table (2): Serum biochemical parameters level of young and adult male rats after four months exposure to different nitrate doses.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Animal Groups</th>
<th>ANOVA</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Young Rats</td>
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<tr>
<td></td>
<td>Control Group</td>
<td></td>
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<tr>
<td></td>
<td>Nitrate Exposed Groups</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Low Dose</td>
<td>Medium Dose</td>
</tr>
<tr>
<td>T₃ (ng/dl)</td>
<td>Mean ± SE (N=5)</td>
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<tr>
<td></td>
<td>142.90 ± 6.08</td>
<td>118.60 ± 7.76</td>
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<tr>
<td>T₄ (ng/dl)</td>
<td>Mean ± SE (N=5)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>12.07 ± 0.679</td>
<td>10.06 ± 0.334</td>
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<tr>
<td>TSH (mU/ml)</td>
<td>Mean ± SE (N=5)</td>
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</tr>
<tr>
<td></td>
<td>0.050 ± 0.005</td>
<td>0.058 ± 0.003</td>
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<tr>
<td>Total protein (g/dl)</td>
<td>Mean ± SE (N=8)</td>
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</tr>
<tr>
<td></td>
<td>8.06 ± 1.012</td>
<td>7.69 ± 0.115</td>
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<tr>
<td>Albumin (g/dl)</td>
<td>Mean ± SE (N=8)</td>
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</tr>
<tr>
<td></td>
<td>5.12 ± 0.247</td>
<td>4.45 ± 0.141</td>
</tr>
<tr>
<td>Globulin (g/dl)</td>
<td>Mean ± SE (N=8)</td>
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<tr>
<td></td>
<td>2.94 ± 0.206</td>
<td>3.24 ± 0.059</td>
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<tr>
<td>Urea (mg/dl)</td>
<td>Mean ± SE (N=8)</td>
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<tr>
<td></td>
<td>48.70 ± 3.034</td>
<td>58.64 ± 5.00</td>
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<tr>
<td>Creatinine (mg/dl)</td>
<td>Mean ± SE (N=8)</td>
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<tr>
<td></td>
<td>0.73 ± 0.024</td>
<td>0.81 ± 0.028</td>
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<tr>
<td>NO (μM/L)</td>
<td>Mean ± SE (N=5)</td>
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</tr>
<tr>
<td></td>
<td>27.05 ± 2.57</td>
<td>29.60 ± 2.00</td>
</tr>
</tbody>
</table>

Data are means ± SE, N= number of animals

a = significant difference between young nitrate exposed groups and their respective control.
b = significant difference between adult nitrate exposed groups and their respective control
c = significant difference between different adult groups and their respective young groups
Table (3): Urine biochemical parameters of young and adult male rats after four months exposure to different nitrate doses.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Animal Groups</th>
<th>ANOVA</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Young Rats</td>
<td>Adult Rats</td>
</tr>
<tr>
<td></td>
<td>Control Group</td>
<td>Nitrates Exposed Groups</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Low dose</td>
</tr>
<tr>
<td>Total protein (ng/dl)</td>
<td>Mean ± SE</td>
<td>N=8</td>
</tr>
<tr>
<td></td>
<td>0.0013 ± 0.0002</td>
<td>0.240 ± 0.025</td>
</tr>
<tr>
<td>Urea (mg/dl)</td>
<td>Mean ± SE</td>
<td>N=8</td>
</tr>
<tr>
<td></td>
<td>60.02 ± 1.69</td>
<td>75.92 ± 3.08</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>Mean ± SE</td>
<td>N=8</td>
</tr>
<tr>
<td></td>
<td>3.57 ± 0.274</td>
<td>3.92 ± 0.142</td>
</tr>
<tr>
<td>NO (µM/L)</td>
<td>Mean ± SE</td>
<td>N=5</td>
</tr>
<tr>
<td></td>
<td>111.27 ± 6.8</td>
<td>158.28 ± 11.32</td>
</tr>
</tbody>
</table>

Data are means ± SE, N= number of animals
a = significant difference between young nitrate exposed groups and their respective control.
b = significant difference between adult nitrate exposed groups and their respective control.
c = significant difference between different adult groups and their respective young groups.
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إنخفاض نشاط الدققيبة في ذكور جرذان مختلفة الأعمار تعرضت لمياه الشرب الملوثة بالنترات

المشتركون في البحث

د. عزه محمد الوقق
د. فهمي جاد السعيد

قسم علم الحيوان - كلية العلوم - جامعة المنصورة - مصر

تم دراسة تأثير التلوث بالمياPASSWORD_REDACTION به نسب عدد من مستويات هرمونات الدرقية ووزن الجسم وكذلك بعض المعايير الأخرى في ذكور جرذان مختلفة الأعمار. وقد قسمت الحيوانات لجامعة صغيرة الأعمار وأخرى كبيرة تعرضت للتناثر من طريق تناول مياه شرب تحتوي نترات الصوديوم بتركيزات TSH 0.100, 0.250, 0.500 ملليجرام / لتر لمرة أربعة أشهر. وأظهرت الدراسة أن نسبة مستوي كل من T4 و T3 مصحوبة بزيادة في مستوى TSH في أمثال جميع الحيوانات المعرضة للترات، مبيناً حدوث انخفاض في نشاط الدققيبة. وسجلت الدراسة أيضاً تناقصاً في وزن الجسم في الحيوانات الصغرى المعرضة لتركيزات النترات المختلفة والحيوانات الكبيرة المعرضة للتركيز المرتفع فقط. وفي نفس الوقت ارتفاعًا في مستوى T4 المستويات في الحيوانات الصغرى المعرضة للجرعات المرتفعة (0.250 ملليجرام / لتر) والحيوانات الكبيرة المعرضة للجرعة عالية، بالإضافة إلى ذلك ارتفاع التوتر للترات نقصاً في مستوى البروتينات الكلية والألبومين والجليبيدين في الفصل مصحوبًا بزيادة في مستوى البروتينات الكبيرة في كل من مصل وبروتياويات النترات. وقد كتبت هذه التغيرات بالإضافة إلى ملاحظتهم من تغييرات أخرى تزيد مع زيادة تركيز الترات وكان ذلك واضحاً في الحيوانات الصغرى.

ويكذلك يمكن استنتاج أن التعرض لبيئة شرب عالية التراث بالمياها تعتبر شديدة السمية لنشاط الهرموني للدقيقة وكذلك باقي المعايير الأخرى في كل من الجزء الصغير والكبيرة، ولكن كانت الحيوانات الصغرى أكثر إستجابة لتأثير الترات السام.