Evidence for Androgenic Activity of Tumeric and Curcumin in Male Rats Exposed to Water Nitrate Pollution

Azza M. El-Wakf*; EL-Said M. Elhabiby; Waffa M. El-kholy and Eman Abd El-Ghany

Zoology Department, Faculty of Science, Mansoura University, Mansoura, Egypt
*dr_azzaelwakf@yahoo.com

Abstract: Nitrate pollution was recognized to inhibit gonadal androgen production that causes reproductive disorders in many vertebrates. The present study was undertaken to evaluate androgenic activity of tumeric and curcumin in male rats exposed to nitrate pollution. Nitrate pollution was achieved via NaNO3 intake in drinking water at dose 550 mg/L for four months. Tumeric was given as 1% (w/w) in diet, while curcumin was administered orally at dose of 20 mg/kg b.w. The study revealed a significant decrease in serum levels of androgens, testosterone (T) and dehydroepiandrosterone (DHEA), as well as in the activity of testicular steroidogenic enzyme, 3β-hydroxysteroid dehydrogenase (3β-HSD), following nitrate exposure. This decrease goes in parallel with marked reduction in epididymal sperm count and weight of testis and epididymis in nitrate exposed rats. Meanwhile, a significantly elevated nitric oxide (NO) level, concomitantly with a reduction in reduced glutathione (GSH) and its metabolizing enzyme, γ-glutamyl transferase (γ-GT) was demonstrated. The study also showed a significant reduction in testicular enzymes, acid phosphatase (ACP) and alkaline phosphatase (ALP), but serum ACP and ALP showed significant increase. Consequently, marked histopathological changes in the testis characterized by degenerative lesions in spermatogenic cells, as well as Leydig cells degeneration have been demonstrated in nitrate exposed rats. On the other hand, intake of tumeric and curcumin tended to reduce these histopathological changes and also seemed to be potent in preventing changes in testicular enzymes, NO and GSH levels, as well as serum androgens (T and DHEA), indicating that the two agents posses potent androgenic activity. Thus, tumeric and curcumin could be used as natural agents in controlling various reproductive disorders.

Key words: reproductive disorders, nitric oxide, 3β-hydroxysteroid dehydrogenase, dehydroepiandrosterone, glutathione.

1. Introduction

Nitrate water pollution is a problem in surface and ground water in many farm land areas. The most important sources of nitrate pollution in the environment are the application of excessive or inappropriate concentrations of nitrogen fertilizers in agriculture. Livestock and poultry production, as well as sewage treatment also contribute nitrogenous wastes to the soil and water (Stoate et al., 2001).

World Health Organization (WHO, 1985) and European Union (Roux, 1995) recommended the maximal admissible nitrate level in drinking water at 44.3 and 50 mg/L, respectively. The human intoxication with nitrates is produced with exposure levels >50 mg/L through drinking water. In some European countries, private wells in rural areas have elevated nitrate concentrations reaching 10–15 times the recommended level (European Environment Agency, 2003). Levels of nitrate exceeding WHO guideline were also reported in private well-water in countries like China, Turkey, and Mexico (WHO, 2003), as well as in Egypt (El-Wakf et al., 2009).

Nitrate itself is not toxic where it is stable inert compound that cannot be metabolized by human enzymes. However, the nitrate reducing activity of gut bacteria may convert nitrate into nitrite and other bioactive nitrogen compounds that may affect human health (Ward et al., 2005). In evaluating health effects of nitrate, several cases of methemoglobinemia (in which nitrate binds with hemoglobin to form methemoglobin) were reported in children who drank water containing nitrate at concentrations higher than the maximum permissible by WHO (Avery, 1999). An association between maternal ingestion of nitrate from drinking water and developmental effects in their infants was also established. These effects include, CNS anomalies, intra-uterine growth restriction, cardiac defects, pre-term delivery and spontaneous abortion. Besides, consumption of water with high nitrate levels was associated with thyroid hypertrophy, increased blood pressure and acute respiratory tract infections in human (Aly et al., 2009).

Other studies have also shown reproductive toxicity associated with high nitrate levels in drinking water in many vertebrates (Manassaram et al., 2007 and Aly et al., 2009). In accordance with this, nitrate exposure inhibits gonodal testosterone...
synthesis in rodents and bulls and is also associated with gonadal atrophy, altered sperm morphology and decreased sperm count in rodents (Pant and Srivastava, 2002). Thus, nitrate could pose a direct threat to the conservation and restoration of vertebrate populations and the ecosystems they depend on for survival.

Recently, much attention has focused on the role of natural food additives or spices in various disorders reflecting alteration in cellular function and structure. There is now a growing body of evidence to support the concept that spices, such as Curcuma longa have medicinal properties and may not only alleviate symptoms, but also help to prevent diseases. Curcuma longa, which belongs to the Zingiberaceae family, is a rhizomatous perennial herb distributed throughout tropical and subtropical regions of the world, being widely cultivated in Asiatic countries, mainly India and China. Its rhizomes are oblong, ovate, pyriform, often short-branched and they are widely used as household remedy (Eigner and Scholz, 1999). As a powder called turmeric, it has been in continuous use for its flavoring, as a spice in both vegetarian and non-vegetarian food preparations and it also has digestive properties. Current traditional medicine in Indian and other countries claims the use of tumeric powder against biliary disorders, anorexia, cough, diabetic wounds, hepatic disorder, rheumatism and sinusitis (Ammon et al., 1992). Curcumin, a yellow pigment derived from the rhizomes of turmeric, has a wide array of pharmacological and biological activities. Curcumin has been claimed to be a potential anti-inflammatory agent (Balasubramanyam et al., 2003), with phyttonutrient and bioprotective properties. Curcumin has also shown to alleviate various forms of male reproductive disorders in experimental animals and thus to enhance fertility (Ilbey et al., 2009 and Noorafshan et al., 2009). Based on these observations, the present study was carried out to investigate the efficacy of curcumin compared to tumeric in reducing nitrate-related reproductive toxicity. This was evaluated based on levels of androgens, activity of testicular enzymes and levels of nitric oxide (as a marker of nitrate toxicity), as well as histopathological changes of testis.

2. Material and Methods

Animals:

This study was performed on male Wistar albino rats (Rattus rattus), initially weighing 170-180 g. Rats were obtained from the Institute of Ophthalmic Disease Research, Cairo, Egypt. They were housed in stainless steel cages at a well ventilated animal house. Rats were permitted adequate standard diet and given water ad libitum for one week of adaptation period prior to the experimental work.

2.2. Diet:

The control group was fed a standard diet consisting of protein 21%, fat 3.2% and fibers 3.44%, according to the Nutrient Requirements of Laboratory Animals (1995). In the turmeric group, the standard diet was supplemented with 1g tumeric for each 100 g diet (1% w/w) and mixed with little distilled water, then the mixture was made into pellets form (Yasni et al., 1993) and dried in open air. Tumeric was purchased from a local market at Mansoura city, Egypt.

2.3. Chemicals

Sodium nitrate (NaNO₃) was purchased from El-gomhoria Company, Egypt. Curcumin, dehydroepiandrosterone, NAD, glycerol, EDTA, potassium phosphate and dimethylsulphoxide (DMSO) were purchased from Sigma Company for Chemicals, Egypt. All other reagents are of analytical grade and purchased from local suppliers.

2.4. Experimental design

After two weeks of adaptation, rats were randomly divided into seven groups of six animals each. The first was considered as control group in which animals received normal laboratory diet (NLD) without supplementation. The second group, was fed NLD and received 5% DMSO (5ml DMSO dissolved in 95 ml water) orally with a gastric tube at a dose of 0.1ml/100g b.w. The third group, was fed powdered tumeric mixed with NLD as pellets at dose of 1% (w/w) (Pulla Reddy and Lokesh, 1993). In fourth group, rats were fed NLD and received curcumin orally at dose of 20 mg/kg b.w. (Sinha et al., 1974) dissolved in 5% DMSO solution. Rats of fifth group were fed NLD and received sodium nitrate (NaNO₃) in drinking water at dose of 550 mg/L to provide average daily dose of approximately 49.40 mg/kg b.w. Rats in the sixth and seventh groups were supplied with sodium nitrate plus tumeric or curcumin at the same route and doses, as described in the above groups. Rats were administrated their respective doses daily for four months, except for curcumin which was given on every alternate day (Sinha et al., 1974). The applied nitrate concentration in drinking water was chosen based on the recent experimental study carried by El-Wakil et al., 2009.

2.5. Blood and tissue sampling

At the end of the study period, all rats were fasted overnight. At 8.00 in the morning, animals were sacrificed under ether anesthesia. Blood
samples were collected in clean dry centrifuge tubes. Sera were separated by centrifugation at 855 g for 10 minutes and then quickly frozen at -20°C for further biochemical analysis. Immediately after collecting blood, the abdomen was exposed, dissected by longitudinal incision and the two testes and epididymes from each rat were removed, weighed and their relative weights were calculated as the ratio of organs weight to animal body weight (mg/100g b.w.). Then the right testis was homogenized for biochemical measurements, while the left was fixed in 10% neutral formaldehyde for histological study.

2.6. Preparation of testis homogenate:
A portion of the right testis was weighed and homogenized in cold distilled water using teflon homogenizer, centrifuged for 10 min at 855 g and the resultant supernatant was used for analyzing biochemical parameters, except for [3β-hydroxysteroid dehydrogenase, 3β-HSD and nitric oxide, NO]. Another portion from the right testis was weighed and homogenized at 4°C, in 20% spectroscopic grade glycerol containing 5 mmol potassium phosphate and 1 mmol EDTA at tissue concentration of (100 mg/ml), then centrifuged at 10,000 g for 30 min at 4°C. The supernatant was taken for the assay of 3β-HSD activity (Talalay, 1962). The remaining portion from right testis was weighed and homogenized with phosphate buffer solution (PH 7.4) and centrifuged at 10,000 g for 20 minutes and supernatant was separated for NO analysis (Montgomery and Dymock, 1961).

2.7. Biochemical assessment:
2.7.1. Measurement of male hormones:
Serum testosterone (T) and Dehydroepiandrosterone (DHEA) levels were estimated using kits supplied by Rock Diagnostics GmbH, D-68298 according to the methods of Tietz (1995) and Longcope (1996), respectively.

2.7.2. Determination of 3β-hydroxysteroid dehydrogenase activity in testis:
The supernatant at a volume of 1 mL was mixed with 1 mL of 100 μM of sodium pyrophosphate buffer, pH=8.9, 40 μL of ethanol containing 30 mg of dehydroepiandrosterone and 960 μL of 25 mg% of bovine serum albumin (BSA) making the total incubation mixture of 3 mL. Enzyme activity was measured after addition of 100 μL of 0.5 μM nicotinamide adenine dinucleotide (NAD) to the tissue supernatant mixture in a spectrophotometer cuvette at 340 nm against a blank (without NAD). One unit of enzyme activity was the amount causing a change in absorbance of 0.001 /min at 340 nm (Talalay, 1962).

2.7.3. Biochemical parameters measured using kits supplied by Bio-diagnostic company, Mansoura, Egypt on the basis of the following methods:
Acid phosphatase (ACP) activity (Kind and King, 1954), alkaline phosphatase (ALP) activity (Belfield and Goldberg, 1971), γ-glutamyl transferase (γ-GT) activity (Szasz, 1969) and nitric oxide (NO) level (Montgomery and Dymock, 1961).

2.7.4. Determination of reduced glutathione:
Reduced glutathione was assessed in testicular homogenate based on the method was adopted by (Prins and Losse, 1969). It depends on the precipitation of protein using tungstate/sulfuric acid solution and the formation of yellow color after reaction with 5,5dithiobis-2-nitrobenzoic acid (DTNB) and the absorbance was determined within 30-60 Sec at 412 nm against the blank.

2.8. Assessment of sperm count:
Epididymal sperm count was done by homogenizing the epididymis in 5 ml of normal saline. Counting was then done using the counting chamber in the haemocytometer (Adeeko and Dada, 1998). The total number of sperm per gram of epididymis was then calculated.

2.9. Histopathological examination of the testis:
The fixed testes were dehydrated through the ascending series of ethyl alcohol, cleared in xylene, and infiltrated and embedded in paraffin wax. Transverse sections of testis were cut at thickness of 5 μm and stained with Harris's alum hematoxyline and counter stained with eosin according to (Mallory, 1938).

2.10. Statistical analysis:
All data are represented as means±SE. One way analysis of variance (One-way ANOVA) followed by Least Significant Difference (LSD) test was used to determine differences among means of investigated groups. The differences were considered to be statistically significant at P<0.05 (Snedecor and Cochran, 1982).

3. Results
3.1. Testosterone and dehydroepiandrosterone (DHEA) levels, sperm count and weights of testis and epididymis in nitrate exposed male rats.
Chronic nitrate exposure via drinking water at dose 550 mg/L tended to exhibit significant decreases in serum levels of testosterone (T) and dehydroepiandrosterone (DHEA), as well as
epididymal sperm number and absolute and relative weights of testis and epididymis compared with control group. No such changes were observed in these tested parameters in tumeric or curcumin treated groups. Besides, co-administration of tumeric or curcumin with nitrate restored reduction in the above mentioned parameters, except the relative testis weight and DHEA level in (Tum+Nitrate) group which still significantly lowered comparing to the control group (Table 1).

Table 1: Effect of tumeric and curcumin on male hormones, testosterone and dehydroepiandrosterone(DHEA), sperm count and absolute and relative weights of testis and epididymis in nitrate exposed male rats.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>DMSO</th>
<th>Turn</th>
<th>Cur</th>
<th>Nitrate</th>
<th>Tum+Nitrate</th>
<th>Cur+Nitrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Testosterone (ng/ml)</td>
<td>5.08±0.35</td>
<td>4.73±0.55</td>
<td>4.97±0.37</td>
<td>5.83±0.08</td>
<td>1.58±0.17&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.63±0.35&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.49±0.25&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>DHEA (ng/ml)</td>
<td>5.90±0.33</td>
<td>5.50±0.52</td>
<td>5.96±0.49</td>
<td>6.18±0.19</td>
<td>3.57±0.25&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.34±0.16&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.69±0.23&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Epididymal sperm count (10&lt;sup&gt;4&lt;/sup&gt;/g)</td>
<td>5.06±0.23</td>
<td>5.07±0.42</td>
<td>5.06±0.13</td>
<td>5.49±0.44</td>
<td>2.15±0.25&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.67±0.27&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.45±0.21&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Absolute testis weight (g)</td>
<td>1.58±0.04</td>
<td>1.51±0.01</td>
<td>1.54±0.05</td>
<td>1.59±0.05</td>
<td>1.32±0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.50±0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.51±0.04&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Relative testis weight</td>
<td>0.56±0.07</td>
<td>0.51±0.02</td>
<td>0.56±0.08</td>
<td>0.56±0.02</td>
<td>0.45±0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.47±0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.56±0.02&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Absolute epididymis weight (g)</td>
<td>0.57±0.03</td>
<td>0.56±0.01</td>
<td>0.56±0.06</td>
<td>0.55±0.01</td>
<td>0.44±0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.54±0.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.55±0.02&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Relative epididymis weight</td>
<td>0.19±0.008</td>
<td>0.18±0.006</td>
<td>0.18±0.005</td>
<td>0.19±0.01</td>
<td>0.15±0.009&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.18±0.005&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.19±0.004&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are means±SE of 6 animals for each group. Tum=tumeric, Cur=curcumin, DMSO=dimethylsulphoxide. Values bearing superscript are significantly different by ANOVA at p<0.05.

Table 2: Effect of tumeric and curcumin on 3β-hydroxysteroid-dehydrogenase (3β-HSD), γ-glutamyl transferase(γ-GT), glutathione (GSH) and nitric oxide (NO) in testis of nitrate exposed male rats.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>DMSO</th>
<th>Turn</th>
<th>Cur</th>
<th>Nitrate</th>
<th>Tum+Nitrate</th>
<th>Cur+Nitrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>3β-HSD (U/mg)</td>
<td>9.95±0.26</td>
<td>9.6±0.15</td>
<td>9.95±0.25</td>
<td>10.83±0.43</td>
<td>7.86±0.16&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.97±0.13&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.68±0.13&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>γ-GT (U/g)</td>
<td>8.87±0.80</td>
<td>8.93±0.64</td>
<td>8.76±0.87</td>
<td>9.31±0.74</td>
<td>3.23±0.30&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.01±0.93&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.46±0.76&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>GSH (mg/g)</td>
<td>5.18±0.07</td>
<td>5.01±0.07</td>
<td>6.28±0.16&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.95±0.22&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.71±0.41&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.1±0.37&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.17±0.05&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>NO (umol/g)</td>
<td>16.21±0.34</td>
<td>16.01±0.92</td>
<td>4.99±1.02</td>
<td>14.82±0.36</td>
<td>48.24±3.14&lt;sup&gt;a&lt;/sup&gt;</td>
<td>24.72±0.84&lt;sup&gt;a&lt;/sup&gt;</td>
<td>23.98±0.95&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are means±SE of 6 animals for each group. Tum=tumeric, Cur=curcumin, DMSO=dimethylsulphoxide. Values bearing superscript are significantly different by ANOVA at p<0.05.

3.2. 3β-hydroxysteroid-dehydrogenase (3β-HSD), γ-glutamyl transferase(γ-GT), glutathione (GSH) and nitric oxide (NO).

Nitrate exposed rats exhibited significant decrease in the activity of testicular 3β-HSD and γ-GT, as well as GSH content. While, NO level was found to be significantly increased. Concomitant tumeric or curcumin supplementation with nitrate, ameliorated disturbances occurred in these biochemical parameters, particulary in Cur+Nitrate group. However, no changes were observed in the above mentioned parameters in animals treated with tumeric or curcumin each alone (Table 2).

3.3. Serum and testis acid phosphatase (ACP) and alkaline phosphatase (ALP).

The activities of ACP and ALP were significantly elevated in serum, while reduced in testis of nitrate exposed group, comparing with the control animals. On the other hand, the rat groups administrated tumeric or curcumin concomitant with nitrate showed significant improvement in ACP and ALP activities, comparing with rats of nitrate group. Mean while, curcumin exhibited the greater improvement for both ACP and ALP comparing with tumeric. Tumeric or curcumin supplementation each alone did not exhibit significant changes in any of tested parameters in comparison to control animals, indicating their non toxic effects at applied doses (Table 3).
Table 3: Effect of turmeric and curcumin on serum and testis enzymes, acid phosphatase (ACP) and alkaline phosphatase (ALP) in nitrate exposed male rats.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>DMSO</th>
<th>Turn</th>
<th>Cur</th>
<th>Nitrate</th>
<th>Turn+Nitrate</th>
<th>Cur+Nitrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum ACP (U/L)</td>
<td>41.23 ± 2.65</td>
<td>44.45 ± 2.17</td>
<td>42.14 ± 0.95</td>
<td>37.39 ± 2.86</td>
<td>71.41 ± 3.15</td>
<td>53.25 ± 0.97</td>
<td>41.36 ± 1.22</td>
</tr>
<tr>
<td>Serum ALP (U/L)</td>
<td>102.37 ± 1.70</td>
<td>103.95 ± 2.47</td>
<td>104.44 ± 2.56</td>
<td>98.97 ± 3.22</td>
<td>230.10 ± 10.56</td>
<td>126.33 ± 1.71</td>
<td>106.19 ± 1.86</td>
</tr>
<tr>
<td>Testis ACP (U/g)</td>
<td>2.79 ± 0.11</td>
<td>2.75 ± 0.12</td>
<td>2.74 ± 0.21</td>
<td>2.96 ± 0.21</td>
<td>1.44 ± 0.20</td>
<td>2.17 ± 0.12</td>
<td>2.43 ± 0.10</td>
</tr>
<tr>
<td>Testis ALP (U/g)</td>
<td>31.15 ± 0.80</td>
<td>30.71 ± 0.94</td>
<td>29.64 ± 0.85</td>
<td>34.21 ± 1.13</td>
<td>18.50 ± 0.57</td>
<td>25.20 ± 0.61</td>
<td>28.98 ± 1.38</td>
</tr>
</tbody>
</table>

Values are means±SE of 6 animals for each group. Tum=tumeric, Cur= curcumin, DMSO= dimethylsulphoxide. Values bearing superscript are significantly different by ANOVA at p< 0.05.

a: when compared different groups with control.  b: when compared (Tum+Nitrate) with nitrate.
c: when compared (Cur+Nitrate) with nitrate.  e: when compared (Cur+Nitrate) with (Tum+Nitrate).

3.4. Histopathological examination:

Normal testicular architecture observed in testis from control group (Fig.1) and the group which received the vehicle (Fig.2), as well as the groups administered tumeric or curcumin, each alone (Figs.3&4, respectively). Nitrate exposure at dose 550 mg/L exhibited irregular and diminished seminiferous tubules with moderate tubular necrosis and Leydig cells (LC) degeneration. Focal disorganization of germ cells, as well as sloughed and degenerated sperms (SP) were also seen. Other changes were seen in the testis, including reduction of spermatogenic cells (SPGS) and irregular distribution with severe degradation of spermatocytes, resulting in spermatogenesis arrest (Fig.5). Intake of tumeric or curcumin, each alone with nitrate showed marked recovery, where the tubules appeared with almost regular distribution of spermatocytes and increased number of mature sperms in the central lumen compared to nitrated treated rats. (Figs. 6&7, respectively).

Plate 1: Normal testicular architecture was observed in testes from control group (Fig.1). Rats treated with vehicle displayed normal spermatogenesis and normal interstitial tissue (Fig.2). Administration of tumeric or curcumin each alone showed normal testicular histology and normal spermatogenesis with increased sperm (SP) count (Figs.3&4, respectively). Nitrate exposure at dose 550 mg/L resulted in histopathological changes included vaculation (V), diminished seminiferous tubules and Leydig cells degeneration (LC) with arrested spermatogenesis process (Fig.5). Oral intake of tumeric or curcumin, each alone with nitrate showed significant recovery, where the tubules were comparable to those in control groups, with normal testicular morphology and recovered Leydig cell (LC). (Figs. 6&7, respectively). H&E stain x200.
4. Discussion

The endocrine-disrupting action of various chemical contaminants have been a recent focus of much studies (Guillette and Crain, 2000 and Mclachlan, 2001). These studies have investigated chemicals released from industrial activities, sewage treatment works, refuse dumps, confined animal feed operation and agriculture fields. Endocrine disrupting chemicals affect an organism’s physiology through a number of mechanisms. They may mimic naturally occurring hormones, act as hormone receptor agonists or antagonists or alter the enzymes responsible for hormone synthesis and degradation (Gray et al., 2001). Using these definitions, chemicals as nitrates have been defined as endocrine disrupting contaminants. Nitrate is a global pollutant of most aquatic systems (Guillette and Edwards, 2005) and have been reported to be toxic in human and animals (Avery, 1999).

Several studies have observed alterations in various endocrine parameters associated with high nitrate exposure (Sampat, 2000; Guillette and Edwards, 2005). As part of these studies, nitrates have shown to cause depressed iodide uptake with subsequent alterations in thyroid gland morphology and function in human, domesticated mammals and fish (Guillette and Edwards, 2005). Additional studies using rodents or in vitro cell culture (mouse Leydig tumor cells MLTC-1) indicated to inhibition of androgen synthesis from mammalian testicular Leydig cells in vitro and androgen synthesis in vivo following nitrate exposure (Panesar, 1999). Epidemiologic studies also indicated that nitrate/nitroglycerin therapy in human influenced circulating androgen concentrations, as well as blood pressure (Panesar and Chan, 2000).

Further examinations provided evidence that high nitrate exposure tended to cause marked reproductive toxicity, characterized by loss of testicular and epididymal weights with reduced sperm count in male rats (Aly et al., 2009). It was also reported that oral administration of nitrate to bulls suppressed Leydig cell activity and reduced sperm motility during and particularly after the administration period (Zraily et al., 1997). In addition, a number of occupational studies indicated that nitrate exposure has a potential reproductive toxicity in males associated with atrophy of testis (Pant and Srivastava, 2002) and reduced reproductive hormones, testosterone (T) and dehydroepiandrosterone (DHEA), indicating a negative linear relationship between male sex hormones and total nitrogen concentration. In support, the present findings indicated decreased male hormones, testosterone and dehydroepiandrosterone, along with decline in epididymal sperm count in nitrate exposed rats. As male fertility correlated with sperm count (Aly et al., 2009), the results may reflect an overall reduction in this vital process. In addition, nitrate exposure significantly decreased testis and epididymis weights. In mature rats, these organs have already attained development, and their main function is production and maturation of spermatozoa, as well as synthesis of hormones (Dixit et al., 1988). Usually, a decrease in their weights is observed in animals with low testosterone level (Li et al., 2001), as observed in the present study. Further, the present study showed marked histopathological changes, including damaged spermatogenic cells (spermatogonia, spermatocytes and spermatozoa), as well as Leydig cells degeneration with arrest of spermatogenesis process. These findings are consistent with previous reports which, in all, showed reduced sperm count and inhibited androgen production with nitrate intake (Aly et al., 2009 and Guillette and Edwards, 2005). Several mechanisms are suggested, however the most common one is via nitrate conversion to nitric oxide (NO) (Panesar and Chan, 2000). Nitrate when ingested can be converted into nitrite via the gut. Gut bacteria have nitrite reductase activity that can generate NO from nitrite locally (Duncan et al., 1995). Besides, NO is readily generated from L-arginine by three isoforms of the enzyme NO synthase (NOS): endothelial NOS, neuronal NOS, and inducible NOS (iNOS) (MacMicking et al., 1997). The latter is responsible for production of sustained high levels of NO and is often considered the primary perpetrator of autotoxicity under cellular stressful conditions (Bogdan, 1998). It is generally thought that excessive NO production due to elevated expression of iNOS can cause cytotoxic effects on surrounding cells (Ilbey et al., 2009). The process of iNOS expression is often activated by a variety of transcriptional factors, as nuclear factor kappa B (NF-KB) that transduces environmental stimuli to nucleus for increased generation of this enzyme (Gilad et al., 1998).

Nitric oxide is a potent cellular signal, used in a wide variety of regulatory physiological pathways (Nohl et al., 2001). In the testis, NO could be involved in the regulation of steroidogenesis, vasodilation, peristalsis, permeability of seminiferous tubules and development/apoptosis of germ cells (El-Gohary et al., 1999). Increased generation of NO and other reactive oxides is considered to be disruptive to cellular functions. Multiple mechanisms could be involved depending on the tissue, physiological state of the organism and environment. In particular, increased NO has the potential to reduce sperm numbers via apoptosis of...
spermatogonia, spermatocytes and spermatids (Lue et al., 2003 and Di Meglio et al., 2004). Other research indicated a correlation between seminal plasma NO level and sperm quality, where increased NO causes decreased semen concentration that could be related to direct inhibition of mitochondrial respiration and reduced ATP levels in cells. Decreased ATP content or production might result in insufficient energy and poor sperm motility because approximately 90% of energy is produced as ATP (Weinberg et al., 1995). Therefore, the present findings of decreased sperm count, with degeneration of spermatogenic cells could be attributed to the increased NO generation in the testis of nitrate exposed rats.

Other established actions of NO have been observed on Leydig cell steroidogenesis in humans, laboratory species and cell cultures (Guillette and Edwards, 2005). However, the effect of NO on steroidogenesis could be considered biphasic, resulting in stimulation at low concentration and inhibition at high concentration (Valenti et al., 1999). The inhibitory action of NO on steroidogenesis could occur via several mechanisms. In the first case, NO could act on Leydig cell steroidogenesis through the dilatation of testis microvasculature, as NO is a biogenic messenger that induces vasodilation and hypotension with subsequent hypoperfusion, edema and testicular dysfunction associated with reduced androgen production (Ilbey et al., 2009). Additionally it was proposed that NO causes inhibition of LH synthesis/secretion by the gonadotrope-releasing cells of hypophysis (Ceccatelli et al., 1993), which in turn may decrease testosterone synthesis.

Other roles played by NO will be briefly discussed as follows: NO released in the testis could alter expression and activity of steroidogenic regulatory protein (StAR). StAR transports free cholesterol (the basic steroid hormone precursor) to the inner mitochondrial membrane (Stocco et al., 1996), the site of P450 side-chain cleavage enzyme (P450scc) that cleaves cholesterol to form pregnenolone. The latter is the first steroid hormone in steroidogenic pathway, which is then converted via the action of 3β-hydroxysteroid dehydrogenase (3β-HSD) into progesterone and other steroid hormones including androgens (Panesar and Chan, 2000). NO possesses the ability to bind with the heme moiety of the mitochondrial enzyme P450scc, leading to inhibited enzyme activity (Stocco et al., 1996) and it also tended to inhibit the enzymatic action of 3β-HSD (Danielson, 2002). There is thus indication that nitrate reproductive toxicity might be a consequence of NO induced steroid hormone deficiency, through inhibiting enzymes; P450scc and 3β-HSD occurring in mitochondria. On the basis of these observations, the present findings of increased NO production, with reduced testicular activity of 3β-HSD seemed to be a causal factor for inhibited androgen production and impaired reproductive functions accompanying nitrate exposure. However, the nitrate inhibition to other testicular enzymes, γ-glutamyl transferase (γ-GT), alkaline phosphatase (ALP) and acid phosphatase (ACP), as evidenced in the present study may indicate alternative mechanisms for nitrate-mediated reproductive disorders. Of note is that γ-GT is a testicular marker of Sertoli cell function in the rat (Krueger et al., 1974). The specific activity of γ-GT increased markedly just before the onset of primary spermatocyte formation, and the enzyme levels remained constant during the completion of spermatogene sis (Hodgen and Sherins, 1973). It is also involved in transferring the glutamyl moiety (necessary for GSH synthesis) across the plasma membrane to preserve intracellular GSH level and homeostasis of oxidative stress (Yokoyama, 2007). A decrease in γ-GT activity as shown in this study may suggest incidence of oxidative stress being postulated as a risk factor for depressed spermatogenesis and induction of testicular degenerative lesions following nitrate exposure. Acid phosphatase, is an enzyme of lysosomal origin being detectable in all germinal cells, and its specific activity increases with the development of spermatocytes (Males and Turkington, 1971). It has been used as a marker of testicular activity. The alteration in the enzyme activity may lead to destruction of seminiferous epithelium and loss of germinal elements, resulting in reduction of the number of spermatids associated with decrease in the daily sperm production in the testis (Selvakumar et al., 2006). Activities of free lysosomal enzymes have been shown to rise when testicular steroidogenesis is increased (Mathur and Chattapadhyay, 1982). A decrease in the acid phosphatase in the free state would thus reflect decreased testicular steroidogenesis in the treated-rats and this may lead to reduced androgen production. Meanwhile, alkaline phosphatase (ALP) catalyzes the hydrolysis of monophosphate esters at an alkaline pH. ALP is predominantly a plasma membrane enzyme, localized to the absorptive or secretory surface of the cell (Hoffmann and Solter, 1999). It was reported that ALP helps in spermatogenesis, intermediate carbohydrate metabolism and plays a role in the synthesis of testicular hormones (Chowdhury and Mukherjea, 1976). Any interference in this enzyme might thus lead to biochemical impairment and testicular lesions (Yousef et al., 2008). It was suggested that the increase in the activity of this enzyme in plasma and
the decrease in different tissues might be due to the increased permeability of plasma membrane or cellular necrosis (Rahman et al., 2000). So, the present increase in ALP activity in the serum and decrease in testis might be due to necrosis of testis in response to nitrate exposure. Such findings and others obtained in this study showing decreased sperm number and inhibited testicular enzyme, 3β-HSD, together with induction of histopathological changes in testis of nitrate exposed rats, could suggest that nitrate has the potential to adversely affect testicular function and structure. Therefore, natural components of dietary origin known to possess therapeutic properties may ameliorate these testicular alterations. In particular, several studies have investigated the biological activities of diets rich in spices, such as turmeric and further indicated its importance in preventing and treating wide range of diseases.

In the present study, administration of turmeric powder in diet to nitrate exposed rats exhibited potential action in reducing reproductive disorders caused by nitrate exposure. This was evidenced mainly in terms of improving androgens, testicular enzymes, NO and GSH levels, which may contribute in all to improved testicular status indicated by normalized sperm number, epididymal and testicular weights and testicular structure. Such an effect was also observed following curcumin administration, however it seemed to be more potent than turmeric. As support for the present findings, it was explained that turmeric benefits is attributed to its active component, curcumin (Khanna, 1999), thus the separate use of curcumin seemed to have advantage over the use of the whole plant.

From the previous studies, it appears that the beneficial properties of administering such two spices may be a function of two separate mechanisms: (1) inhibition of excess NO production and (2) protection against inflammatory and apoptotic activities. Tumeric, in particular, has been shown to exhibit anti-inflammatory and anti-infectious activities and also to control NO production, which, in all, play a role in improving fertility, testicular performance and sperm motility (Romeo et al., 2003). The role of turmeric in testicular protection may also be referred to its anti-apoptotic property (Nonn et al., 2007). The chemoprotective properties of curcumin have also been extensively investigated which linked to its anti-inflammatory activity (Camacho-Barquero et al., 2007). Moreover, curcumin bears the potential to inhibit the expression of iNOS through inhibition of NF-kB expression (Ilbey et al., 2009). So, curcumin could inhibit NO production which disturbs steriodogenesis. Therefore, the present findings that administration of tumeric or curcumin have relieved the increase in NO and reduction of androgens could indicate that the two used agents offer the ability to stimulate androgen production that in turn attenuated reproductive disorders caused by nitrate exposure. In addition, curcumin or turmeric decreased the induction of ACP and ALP of the nitrate exposed rats. These results are in a good accordance with that obtained by Pal et al. (2005) who proved that the presence of curcumin with hepatotoxic drug ameliorated increased transaminases and phosphatases levels to the normal level. It could be thus suggested that the leakage of enzymes because of testis injury is prevented by the testis cell membrane stabilizing action of curcumin (Yousef et al., 2008).

In conclusion: nitrate exposure leads to reduced androgens production with resulting reproductive disorders. However, tumeric or curcumin; in particular; appeared to be effective in reducing reproductive disorders probably through their androgenic activity. Thus, indicating the potential reproductive benefits of tumeric and curcumin.

**Corresponding author**

Azza M. El-Wakf
Zoology Department, Faculty of Science, Mansoura University, Mansoura, Egypt
dr_azzaelwakf@yahoo.com

**References**


Bogdan, C. (1998): The multiplex function of nitric...


