Use of Tumeric and Curcumin to Alleviate Adverse Reproductive Outcomes of Water Nitrate Pollution in Male Rats

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Abstract: The present study was carried out to examine adverse reproductive effects of water nitrate pollution in male rats and the use of whole plant, tumeric (*Curcuma longa*) and its active component, curcumin in alleviating these effects. Nitrate pollution was achieved in rats via $NaNO_3$ intake in drinking water at a dose of 550 mg/L for period of four months. Tumeric plant was given as powder in diet (1% w/w), while curcumin was given orally at dose of 20 mg/kg b.w . Nitrate exposed rats showed significant elevations in total lipid (TL), total cholesterol (TC), triglycerides (TGs) and phospholipids (PLs) in serum and testis, but significant reduction in total protein, RNA and DNA contents was recorded. Also, a reduction in epididymal sperm number, weights of testis and epididymis and male sex hormones [testosterone (T) & dehydroepiandrosterone (DHEA)], as well as testicular 3β-hydroxysteroid dehydrogenase (3β-HSD) was observed following nitrate exposure. Meanwhile, the results showed marked reduction in the testicular antioxidant components, glutathione (GSH), superoxide dismutase (SOD) and γ-glutamyl transpeptidase (γ-GT), along with elevation in the level of nitric oxide (NO), lipid peroxidation (MDA) and protein carbonyl, indicating induction of oxidative stress in testis of nitrate exposed rats . On the other hand, the use of turmeric and curcumin appeared to be effective in reducing nitrate-induced reproductive changes, as evidenced by normalized NO, lipid peroxidation, protein carbonyl and lipid profile, as well as antioxidant components, total protein, DNA, RNA, male hormones and sperm number. The results thus suggested that tumeric and curcumin could be useful in treatment of male infertility , with oligospermia, reduced male sex hormones and other adverse reproductive outcomes.

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1. Introduction:

Contamination of aquatic ecosystems by nitrate has become an increasing global concern with respect to the health of humans and wildlife **(Guillette and Edwards, 2005)**. Nitrate contamination occurs through human activities in agricultural and urban areas **(Rouse *et al.*, 1999)**.The health risks of nitrate exposure have been widely evaluated in several vertebrates, including humans **(Fewtrell, 2004)**, livestock **(El Bahri *et al.*, 1997)**, domestic fowls (**Atef *et al*., 1991**), free-living birds (**Ley, 1986**), fishes (**Williams and Eddy, 1989**) and amphibians **(Marco *et al.*, 1999)**. In mammals, exposure to nitrate produces retarded growth, pathologic changes in liver, kidneys and small intestine, changes in blood and plasma biochemistry and reduced immune response (**Zaki *et al.*, 2004**).

Apart from the above described effects, nitrate causes toxic effects on male reproductive activity, through disrupting gonadal function and steroid synthesis pathways (**Guillette and Edwards, 2005; Aly *et al.*, 2009**). The postulated mechanisms of nitrate toxicity is through generating reactive oxygen and nitrogen species, such as hydrogen peroxide (H_2O_2), peroxynitrite ($ONOO^-$) and superoxide anion (O_2^-), which disturb the balance between pro-oxidants and antioxidants in favor of the former, resulting in oxidative stress **(Ahsan *et al.*, 2003**).

In recent years, much attention has been focused on the use of herbal medicines and their derivatives in healing different ailments related to oxidative stress **(Lee and Park, 2003**). The rhizomatous herb, *Curcuma longa* (turmeric) has been widely used as a spice and coloring agent in many foods. Consumption of turmeric has been associated with various beneficial effects on human health through protection against inflammatory, apoptotic and oxidative processes **(Ammon and Wahl, 1991)**. Curcumin, is the main active component of turmeric. It is a yellow phenolic pigment derived from the rhizome of turmeric which has shown to possess a broad spectrum of biological and pharmacological activities. Curcumin has been claimed to be a potential anti-inflammatory, antineoplastic and antimutagenic agent **(Naik *et al.*, 2004)**. It has also shown to be a powerful antioxidant through inhibiting generation of reactive oxygen

species (ROS) both *in vitro* and *in vivo* **(Joe and Lo-Kesh, 1994)**.

Therefore, the present study aimed to investigate whether the use of tumeric and curcumin could alleviate adverse reproductive outcomes, in particular those related to oxidative stress that produced in male rats by prolonged nitrate exposure.

2. Material and Methods:

1. 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. Diet

The control group was fed a standard diet consisting from protein 21%, fat 3.2% and fibers 3.44%, according to **the Nutrient Requirements of Laboratory Animals (1995)**. In the tumeric group, the standard diet was supplemented with 1g tumeric for each 100 g diet and mixed with little distilled water, and 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.

3. Chemicals

Sodium nitrate ($NaNO_3$) 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

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 DMSO (5%) as vehicle 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 DMSO (5%). Rats of **fifth group** were fed NLD and received sodium nitrate ($NaNO_3$) in drinking water at dose of 550 mg/L to provide average daily dose of approximately 49.4 mg/kg b.wt. Rats in **the sixth** and **seventh groups** were supplied with sodium nitrate plus tumeric or curcumin at the same way 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 for evaluating nitrate toxicity **(El-Wakf *et al.,* 2009)**.

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 epididymis 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 one of the two testes was homogenized for biochemical measurements.

4. Preparation of testis homogenate:

A portion of the testis was weighed and homogenized in cold distilled water using tephlon 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 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 and 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 testis was weighed and homogenized with phosphate buffer solution (PH 7.4), then centrifuged at 10,000 g for 20 minutes and the supernatant was separated for NO analysis **(Montgomery and Dymock, 1961)**.

2.5. Biochemical Assessment:

5.1. Measurment 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.

5.2. Determination of 3β-HSD 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 dehydroepiendosterone 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**).

5.3. Determination of reduced glutathione:

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

5.4. Determination of superoxide dismutase:

Superoxide dismutase activity (SOD) was assayed by the procedure of **Nishikimi *et al.* (1972)**. The assay relies on the ability of the enzyme to inhibit phenazine methosulphate mediated reaction of nitroblue tetrazollium dye.

5.5. Determination of malondialdehyde level:

The level of malondialdehyde (MDA) (the end product of lipid peroxidation) in testis homogenate was determined as thiobarbituric acid reactive substance (TBARS) according to a modified method of **Ohkawa *et al.* (1982)**.

5.6. Determination of protein carbonyl content:

Protein carbonyl content was measured by forming labeled protein hydrazone derivatives using 2,4-dinitrophenylhydrazide (**Smith *et al.*, 1991**).

5.7. Determination of nucleic acids (DNA and RNA):

The extraction procedure was carried out according to that described by **Melmed *et al.* (1976)**.

DNA content was estimated using the method of **Dische and Schwarz (1977)**, while RNA content was determined using the method of **Thoresen *et al.* (1983)**.

2.5.8. Biochemical parameters measured using kits supplied by Bio-diagnostic Company, Mansoura, Egypt on the basis of the following methods:

Total protein (TP) **(Gornal *et al.*, 1949),** total cholesterol (TC) (**Allain *et al.*, 1974**), triglycerides (TGs) **(Fassati and Prencipe, 1982),** total lipids **(Zolliner and Kisch, 1962)**, phospholipids (PLs) **(Connerty *et al.*, 1961)**, γ-glutamyl transferase (γ-GT) **(Szasz, 1969)** and nitric oxide (NO) **(Montgomery and Dmock, 1961)**.

6. 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:

As shown from the present findings, administration of tumeric rhizome powder or curcumin each alone exhibited significant increase in GSH content and SOD activity **(Table 3)** but did not exhibit significant changes in other tested parameters in comparison to control animals, indicating their non toxic effects at applied doses **(Tables 1,2,3,4)**. However chronic nitrate exposure via drinking water at dose 550 mg/L tended to exhibit significant decreases in epididymal sperm number, serum levels of testosterone and dehydroepiandrosterone (DHEA), as well as absolute and relative weights of testis and epididymis compared with control group **(Table 1)**. In addition, nitrate exposed rats exhibited significant decrease in serum and testis total protein. While, serum and testis lipid profile (TL, TC ,TGs and PLs) were significantly elevated **(Table 2)**. Besides, the activities of 3β-HSD, γ-GT and SOD, as well as GSH content were significantly reduced, whereas MDA, protein carbonyl and NO levels were significantly elevated in testis of nitrate exposed group comparing with the control animals **(Table 3)**. Meanwhile**,** nitrate exposed rats exhibited significant decreases in testis DNA and RNA contents **(Table 4)**.

On the other hand, co-administration of tumeric and curcumin (each alone) with nitrate helped to reduce nitrate-related reproductive disorders, as evidenced by the increased sperm number, serum male hormones, weights of sex organs and testicular 3β-HSD, SOD, γ-GT, GSH, as well as serum and testis total protein and testis DNA and RNA contents. This goes in parallel with marked reduction in testicular NO, MDA and protein carbonyl levels, as well as serum and testis lipid fractions, indicating reproductive benefits of applying tumeric powder and its active component curcumin **(Tables 1,2,3,4)**. Although the observed action was more apparent with curcumin than tumeric, the differences between them were generally not statistically significant.

Table 1: Effect of tumeric and curcumin on sperm number, testis and epididymis weights and male sex hormones, testosterone (T) and dehydroepiandrosterone (DHEA) in nitrate exposed male rats

	Control	DMSO	Tum	Cur	Nitrate	Tum+Nitrate	Cur+Nitrate
Epididymal sperm count (10^4)/g	5.06±0.23	5.07±0.42	5.06±0.13	5.49±0.44	2.15±0.25[a]	3.67±0.27[a,b]	4.45±0.21[c]
Absolute testis weight (g)	1.58±0.04	1.51±0.01	1.54±0.05	1.59±0.05	1.32±0.02[a]	1.50 ± 003[b]	1.51±0.04[c]
Relative testis weight	0.56±0.07	0.51±0.02	0.50±0.08	0.56±0.02	0.45±0.02[a]	0.47±0.02[a]	0.56±002[c,e]
Absolute epididymis	0.57±0.03	0.56±0.01	0.56±0.06	0.55±0.01	0.44±0.02[a]	0.54±0.04[b]	0.55±0.02[c]
Relative epididymis	0.19±0.008	0.18±0.006	0.18±0.005	0.19±0.01	0.15±0.009[a]	0.18±0.005[b]	0.19±0.004[c]
Testostertone (ng/ml)	5.08±0.35	4.73±0.55	4.97±0.37	5.83 ± 0.08	1.58±0.17[a]	3.63±0.35[b]	4.49±0.25[c]
DHEA (ng/ml)	5.90±0.33	5.50±0.52	5.96±0.49	6.18±0.19	3.57±0.25[a]	4.34±0.16[a]	5.69±0.23[c]

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).

Table 2: Effect of tumeric and curcumin on serum and testis total protein and lipid profile in nitrate exposed male rats

		Control	DMSO	Tum	Cur	Nitrate	Tum+Nitrate	Cur+Nitrate
Serum	Total protein (g/dl)	7.59±0.58	7.12±0.25	8.54±0.30	8.35±0.20	5.52±0.50[a]	7.00±0.16	7.39±0.17[c]
	Total Lipid (mg/dl)	425.14 ±19.06	433.98±11.71	424.51±26.59	376.76±23.70	671.61±39.58[a]	520.26±11.87[b]	438.45± 23.85[c]
	T. Cholesterol (mg/dl)	56.83 ± 2.49	57.05±2.45	57.56± 0.76	55.51±1.29	96.73±3.97[a]	74.45 ±5.91[a,b]	59.16 ±1.43[c,e]
	Triglycerides (mg/dl)	61.09±1.81	62.11±4.35	61.26±2.58	53.4 ± 1.51	79.02 ±2.19[a]	71.28 ± 2.90	61.24±2.35[c]
	Phospholipids (mg/dl)	7.82±0.25	8.08±0.33	7.88±0.26	7.45±0.23	14.21 ±0.40[a]	9.40 ±0.20[a,b]	7.50 ±0.52[c,e]
Testis	Total Protein (mg/g)	3.62±0.30	3.23±0.40	3.47±0.30	3.54±0.27	2.10±0.11[a]	2.83±0.12	3.30±0.12[c]
	Total Lipid (mg/g)	101.11±4.48	96.29±3.22	91.20±3.33	81.31±5.25	158.94±10.57[a]	128.24±7.28[b]	115.51±5.49[c]
	T.Cholesterol (mg/g)	27.44 ±2.06	27.88±1.99	27.79± 0.47	25.58 ± 0.38	37.12±2.69[a]	31.37±0.83	28.11±0.70[c]
	Triglycerides (mg/g)	34.10±0.76	34.31±2.65	34.09 ±0.91	31.94±1.42	43.35±1.68[a]	35.38± 0.87[b]	31.84 ± 1.50[c]
	Phospholipids (mg/g)	10.28± 0.36	10±0.36	9.46±0.72	8.42 ± 0.54	17.97 ± 0.4[a]	12.13 ±0.21[b]	11.31 ±0.10[c]

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).

Table 3: Effect of tumeric and curcumin on 3β-hydroxysteroid dehydrogenase and oxidative stress markers in testis of nitrate exposed male rats

	Control	DMSO	Tum	Cur	Nitrate	Tum+Nitrate	Cur+Nitrate
3β-HSD (U/mg)	9.95±0.26	9.6±0.15	9.95±0.25	10.83 ±0.43	7.86±0.16[a]	8.97±0.13[b]	9.68±0.13[c]
MDA (nmol/g)	18.88±0.59	19.80±0.55	18.74±0.58	16.98±0.59	23.93±1.65 [a]	20.09 ±0.62 [b]	19.35±0.47 [c]
Protein carbonyl (µmol/g)	0.18±0.007	0.18±0.006	0.17±0.006	0.16±0.006	0.24 ±0.007	0.19±0.005 [b]	0.18±0.006 [c]
GSH(mg/g)	5.18± 0.07	5.01±0.07	6.28±0.16 [a]	6.95±0.22[a]	2.71±0.41 [a]	5.1± 0.37 [b]	5.17±0.05 [c]
γ-GT(U/g)	8.87±0.80	8.93±0.64	8.76±0.87	9.31± 0.74	3.23± 0.30 [a]	7.01±0.93 [b]	7.46± 0.76[c]
SOD (U/g)	18.13±0.19	17.38±0.18	19.01±0.24[a]	19.21±0.04 [a]	14.61±0.15 [a]	17.35±0.16 [b]	17.9±0.26 [c]
NO(µmol /g)	16.21±0.34	16.01±0.92	14.99±1.02	14.82±0.36	48.24±3.14[a]	24.72±0.84 [a,b]	23.98±0.95[a,c]

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).

Table 4: Effect of tumeric and curcumin on DNA and RNA contents in testis of nitrate exposed male rats

	Control	DMSO	Tum	Cur	Nitrate	Tum+Nitrat	Cur+Nitrate
DNA (mg/g)	40.71±1.11	38.65±0.78	38.87±1.42	42.54±1.71	29.35±1.09[a]	33.28±0.91	41.67±3.37 [c,e]
RNA (mg/g)	33.92 ± 0.97	32.58 ±0.92	33.61±0.47	34.50±0.94	25.50± 0.85 [a]	27.83±1.11[a]	33.42± 0.82 [c,e]

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).

4. Discussion:

Nitrate water pollution has recently begun to receive attention for its ability to disrupt male gonadal functions and steroid synthesis pathways in many verterbrates **(Guillette and Edwards, 2005)**. Various studies confirmed this and further added that nitrate exposure is associated with gonadal atrophy, altered sperm morphology, decreased sperm count and reduced sperm motility in rats **(Aly *et al.*, 2009; Yarube *et al.*, 2009)**. In this line, the present study exhibited decreased epididymal sperm number and lowered testis and epididymis weights in nitrate exposed rats. Also nitrate exposure led to reduction in the level of male sex hormones, testosterone and DHEA along with decreased activity of testicular 3β-hydroxysteroid dehydrogenase (3β-HSD), the key enzyme in the process of steroidogenesis **(Durdi, 2002)**. Thus, indicating that nitrate can inhibit Leydig cells steroidogenesis and suppress male reproductive activity. Several causes are suggested. One of them may be related to the metabolic changes occurring in response to nitrate exposure. This is clearly emphasized by the increased testicular lipids (TL, TC, TGs, PLs), as shown in the present study and others reported by **Bassuny *et al.* (2004) and Kostogrys *et al.* (2006).** In accord, **Chowdhury *et al.* (1990)** evidenced that changes in testicular lipid profile were strongly correlated to testicular degeneration, histological and biochemical disturbances. The etiology may be ascribed to the fact that lipids, in particular cholesterol is the precursor of male sex hormones and thus increased testicular

cholesterol may result due to impaired utilization in steroidogenesis, **(El-Sweedy *et al.*, 2007),** associated with impaired testicular activity. In this regard, 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 concentrations below **(Eskiocak *et al.*, 2005)** or above **(Tajtakova *et al.*, 2006)** the WHO nitrate standard of 50 mg/L. It was found that the composition and transport of lipoproteins are seriously disturbed in human thyroid diseases. According to **Duntas (2002) and Luboshitzky (2002),** sub-clinical hypothyroidism characterized by decreased fT4 and increased TSH concentrations is associated with elevated total cholesterol levels, increased LDL, and lowered HDL concentrations. Also, serum triacylglycerol concentrations were significantly elevated which could be related to a reduced removal rate of triacyglycerols from plasma in case of hypothyroidism **(Duntas, 2002)**. In support, **Luboshitzky (2002)** found that the development of hypertriglyceridemia was associated with subclinical hypothyrodism in humans. As a result, these lipid changes collectively form testicular lipid accumulation which was mediated by decreased testosterone and DHEA among nitrate exposed animals. In other words, this form of testicular lipid accumulation seemed to be related to lowered androgenicity, mostly observed following nitrate exposure. Therefore, the present finding of increased testicular lipids can be considered as an indicator for impaired steroidogenesis process and testicular function.

Beside these lipid changes, other studies indicated that some of nitrate testicular toxicity may be mediated by a reduction in testicular protein content, as evidenced by the present results and previous findings realized on adult male rats (**Zabulyte *et al.*, 2007**). The decrease in total protein concentration may result due to nitrate toxicity mediated through formation of nitric oxide or peroxynitrite, which oxidises proteins and lipoproteins (**Guzik *et al.*, 2000**), impairs liver metabolism (**Zraly *et al.*, 1997**) and kidney functions **(Pfeifer and Weber, 1979)**. The reduction in total protein in animals exposed to environmental pollutants could be attributed to changes in protein and free amino acid metabolism, such as reduced protein synthesis or increased proteolytic activity or degradation **(Yousef *et al.*, 2008)**. In other way, decreased protein content in response to nitrate exposure may be contributed to the harmful effect of its active metabolite nitrite. Various studies confirmed this hypothesis and further added that nitrite effect is reflected on the biosynthesis of protein (**Helal, 2001**). It was found that serum protein of rats are decreased due to the toxic effect of nitrite on the thyroid and adrenal glands that leads to block of protein synthesis, while fast breakdown occurs. This leads to an increase of free amino acids and to a decrease of protein turnover **(Yanni *et al.*, 1991)**. Also, it is clear that sodium nitrite decreases total serum protein and albumin mainly through its effects on the liver, either through the necrotic changes, especially of the plasma membrane **(Guler *et al.*, 1994)** or through inhibiting oxidative phosphorylation process and hence the availability of energy source for protein synthesis and other metabolic processes (**Anthony *et al.*, 1994**). At the same time, nitrite effect on the process of reabsorption in the kidney tubules and absorption of digested food materials can not be ignored. Accordingly, it can suggest that nitrate exposure leads to protein loss which contribute to some of testicular derangements being observed following nitrate exposure.

Apart from the role of metabolic changes, further studies have indicated that one of the most established mechanisms of nitrates toxicity is their ability to induce oxidative stress, through generating reactive oxygen species (ROS), including hydrogen peroxide (H_2O_2) and superoxide anione (O_2^-) (**Singhal *et al.*, 2001; Manassaram, 2006**). Moreover, it is now recognized that nitrate is the precursor of nitric oxide (NO) which in turn leads to tissue damage. The contribution of NO to tissue damage can be a direct effect mediated by NO itself **(Davis *et al.*, 2001)** or an indirect effect mediated by reactive nitrogen species, such as peroxynitrite ($ONOO^-$) produced by the interaction of NO with superoxide anions or oxygen (**Wink and Mitchell, 1998 and Davis *et al.*, 2001**). NO can interact with ROS to form $ONOO^-$, which is a powerful oxidant and cytotoxic agent and may play an important role in the cellular damage associated with overproduction of NO. Under simultaneous generation of NO and ROS, the cellular capabilities of antioxidant systems are suppressed **(de Pinto *et al.*, 2002**). The antioxidant systems in the body contain numerous enzymatic antioxidants, such as superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and catalase (CAT), while non-enzymatic substances, such as glutathione (GSH) are also employed to protect the body from oxidative stress **(Mruk *et al.*, 2002).** Oxidative stress refered to an imbalance between intracellular production of ROS and the cellular antioxidant defense mechanisms **(Ogur *et al.*, 2004**). In other words, oxidative stress is responsible for many deleterious effects in cell, including DNA damage **(Stohs *et al.*, 2001**). The obtained decrease in testicular DNA in nitrate exposed rats, as seen in the

present study and in other investigations (**Ramakrishnan *et al*., 2006**) is probably via generation of NO and other free radicals, as $ONOO^-$, one of the most DNA damaging agents (**Szabo´and Ohshima, 1997)**. In this case, nitrate-induced ROS and oxidative stress may also affect other biological molecules, such as proteins and phospholipids, causing protein modification and lipid peroxidation being related for membrane and tissue damage (**Vuillume, 1987)**. Spermatozoa are rich in polyunsaturated fatty acids, which are more liable for lipid peroxidation by ROS **(El-Sweedy *et al.,* 2007**). Lipid malondialdehyde (MDA) increases in most spermatogenic disturbances (**Sharma and Agarwal, 1996**). Increased testicular lipid peroxidation, as evidenced in the present study may thus represent a key event linked to reduced sperm number and further established over production of ROS in response to nitrate toxicity. Over production of ROS is evidenced also in the present study from increased testicular protein carbonyl (as index of protein oxidation) accompanied by depletion of antioxidant components, SOD, γ-GT and GSH. Thus, indicating that testicular antioxidant mechanisms fail to protect the over production of ROS which subsequently produces oxidative stress. Oxidative stress is an established major factor responsible for male infertility and high levels of ROS are detected in the semen samples of infertile males **(Sikka, 1996)**. Oxidative stress is also responsible for the deterioration of accessory sex organs **(Ochsendorf, 1999)**. In the present study, nitrate-induced decrease in sex organs weights and sperm count may indicate increased oxidative stress in response to nitrate toxicity and further strengthen the hypothesis that ROS play a key role in nitrate toxicity.

Nowadays, trends on applying nutritional antioxidants in ailments related to oxidative stress have gained immense interest. Herbal plants such as tumeric are known to exert their health effects by scavenging free radicals and modulating antioxidant defense system. In the present study, the use of tumeric or its active component, curcumin counteracted nitrate-induced testicular toxicity, however, curcumin was more effective than tumeric. The presence of tumeric or curcumin with sodium nitrate increased epididymal sperm number, weights of sex organs, male sex hormones, serum and testis total protein and nucleic acids, as well as antioxidant components, along with decreased lipid profile, MDA, protein carbonyl and NO levels. With respect to tumeric, several reports have linked its protective action to anti-inflammatory and anti-infectious activities of this plant **(Srinivas, 1992)**. These effects, taken together, improved fertility and testicular performance, through controlling both lipoperoxidation and NO production, which simultaneously affect sperm motility (**Romeo *et al.,* 2003**). The role of turmeric in testicular protection may be referred also to its anti-oxidant property **(Mohanty *et al*., 2006)**. **Padmaja and Raju (2004)** showed that treatment of Curcuma longa ameliorated selenium-induced damage in wistar rat lens by reducing lipid peroxidation. The supplementation also enhanced antioxidant systemes, SOD and CAT and tended to delay opacities formation in the lens. The anti-oxidant activity of tumeric could be mainly related to its active ingredient, curcumin **(Adams *et al*., 1994)**. Curcumin, is a yellow phenolic compound, naturally present in the rhizome of tumeric, which has been claimed to exhibit protective effect against oxidative damage **(Salama and El-Bahr, 2007)**. In the present study, the protective effect of curcumin on the testis may be explained by the fact that it prevents cellular damage occurring as a result of oxidative stress in spermatogenic cells of seminiferous tubules and Leydig cells of the stroma **(Aly *et al.,* 2009)**. It was also found that curcumin supplementation had prevented chromium-induced decrease in weight of accessory sex organs due to normal serum testosterone level **(Chandra *et al.*, 2007)**. Moreover, curcumin administration to male Wistar rats was able to ameliorate lindane-induced reproductive toxicity in pretreatment, post treatment and combination groups **(Sharma and Singh, 2010).** Additionally, it was demonstrated that curcumin exerts its protective effect by modulating lipid peroxidation and augmenting antioxidant defense system **(Kalpana and Menon, 2004)**. More specifically, curcumin significantly decreased the levels of free radicals and this protective effect was attributed to its free radical scavenging activity, induction of detoxification enzymes and providing protection against degenerative diseases **(Manikandana *et al.,* 2004)**. Furthermore, **Halliwell and Gutteridge (2002)** suggested that treatment with curcumin reduced oxidative damage, probably through its capacity to quickly and efficiently scavenge lipid peroxyl radicals before they attack membrane lipids. Results of **Tirkey *et al*. (2005)** indicated that curcumin improved renal GSH levels in arsenite-treated rats. The presence of curcumin with sodium arsenite alleviated its toxicity and ameliorated SOD and CAT levels **(Yousef *et al.,* 2008**). **Dinkova-Kostova and Talalay (1999)** reported that the protective effects of curcumin as an antioxidant resides mainly on its phenolic contents (two orthomethoxylated phenols) besides the β-diketone moiety **(Masuda *et al.,* 1999)**, which provide the free radical trapping capacity of curcumin. Moreover, curcumin bears the potential to inhibit the expression of inducible NO synthase

(*i*NOS) **(Camacho-Barquero *et al.*, 2007**), the enzyme responsible for production of high amounts of NO from L-arginine **(Bogdan *et al*., 1998)**. Thus, curcumin could inhibit NO production in testis which disturbs steriodogenesis and testicular function.

In parallel, curcumin attenuates the toxic effect of nitrate on the serum and testis protein content, as well as testicular DNA and RNA contents. Curcumin increased the level of total protein, perhaps due to stimulation of protein synthesis. Also, tumeric could enhance this decrease in total protein, but curcumin was more potent. This is confirmed by the present results showing increased testicular protein content in animals treated with curcumin than tumeric, which in turn may be related to the more increased DNA and RNA content in testis with curcumin administration. In particular, the free radical trapping capacity of curcumin **(Masuda *et al.*, 1999),** possibly prevents the reactive oxygen species from acting on DNA **(Srinivas *et al*., 1992)**. So, curcumin could repair DNA damage caused by nitrate, but tumeric had non significant role in repairing this damage, as evidenced in this study, thus indicating the major role of curcumin in this context.

The elevated testicular total cholesterol induced by nitrate; mainly due to impaired utilization in steroidogenesis; seemed to be also corrected by turmeric and curcumin administration. The hypocholesterolemic effect of curcumin can probably be explained by its effect on the stimulation of bile acid and biliary cholesterol secretion and enhanced excretion of bile acids and cholesterol in feces (**Srinivasan and Sambaiah, 1991)**. These results are in agreement with **Soudamini *et al.* (1992)** who found that oral administration of curcumin significantly lowered the serum cholesterol level. Also, **Pari and Amali (2005)** found that curcumin significantly decreased cholesterol and triglycerides. Curcumin could act in several ways to lower plasma LDL-bound cholesterol. First, uptake of cholesterol in the gastrointestinal tract could be inhibited; second, LDL-cholesterol (LDL-C) could be eliminated from the blood via LDL receptor; and finally, the activity of cholesterol-degrading enzymes, mainly cholesterol-7-hydroxylase could be increased (**Peschel *et al*., 2007**). Moreover, **Akila *et al.* (1998)** showed that curcumin reduced cholesterol and increased HDL-C, indicating that curcumin may enhance cholesterol mobilizing from extrahepatic tissues to the liver where it is catabolised. The relatively low absorption efficiency of curcumin goes in addition to this hypothesis, since the much greater curcumin concentration in the gut than in the blood makes an effect of curcumin on cholesterol absorption some what more plausible than an effect on cholesterol synthesis (**Arafa, 2005**). In this context, new lines of investigation are being also studied regarding the hypolipidemic effect of curcumin and its structurally related compounds (curcuminoids) that comprise the phenolic yellowish pigment of tumeric (**Asia and Miyazawa, 2001**). One possible mechanism by which curcuminoids lower the TG levels in rats, is through multiple inductions of intra- and extracellular fatty acid catabolism and utilization pathways (e. g., induction of fatty acid ß-oxidation and TG hydrolysis), with metabolites of absorbed curcuminoids serving as ligands that can activate peroxisome proliferator–activated receptor (PPARs), being reported to regulate gene expression of a variety of lipid metabolizing enzymes (**Schoonjans *et al*., 1996**). Therefore, PPARs are proposed to play a role in signaling system that controls lipid homeostasis (**Forman *et al.*, 1997**). Curcuminoids serving as ligands for PPARs that inturn displays hypolipidemic activities. An effect which seems to be responsible for the present findings of lowered lipid contents in both serum and testis following tumeric or curcumin administration, suggesting that tumeric and curcumin could modulate nitrate reproductive toxicity through hypolipidemic action.

In conclusion, the present study demonstrated that tumeric and curcumin; in particular, are effective in reducing nitrate reproductive toxicity, by normalizing sperm number, weights of sex organs, male sex hormones and other reproductive disorders. Thus, tumeric and curcumin could provide a viable food based approach for enhancing male fertility.

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