The Reproductive Toxicity Assessment of Potassium Nitrate in Sprague Dawley Rats

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ABSTRACT: Epidemiologic evidence of a direct exposure-response relationship between drinking water nitrate level and adverse reproductive effects is still unclear. Present study was conducted to determine dose-dependent effects of potassium nitrate (KNO₃) on male reproductive function viz. Organ weights, spermatogenesis, daily sperm production rate (DSP), Leydig cell (LC) morphology, epididymal sperm counts (ESC) and steroidogenic enzymes activity, e.g. Δ⁵,3β-Hydroxysteroid dehydrogenase (Δ⁵,3β-HSD) and 17β-Hydroxysteroid dehydrogenase (17β-HSD) in rats treated with 45, 90 and 135 mg/kg body weight for 15 days. Results showed no significant differences in water/food intake, body and reproductive organ weights in treated rats except significant decrease in prostate weight at 135mg/kg KNO₃ as compared to controls. Testis showed 40±8.9% abnormalcy exhibiting significant decrease in tubular/LC diameter, spermatocytes/spermatids number, DSP, ESC, Δ⁵,3β-HSD and 17β-HSD activity at 135mg/kg dose level than lower doses used. Results indicate testicular cytotoxicity by inhibition of spermatogenesis and steroidogenesis at 135mg/kg dose of KNO₃ in rats.

KEYWORDS: Potassium nitrate, Spermatogenesis, Steroidogenesis, Sperm counts, Rat.

I. INTRODUCTION

The haphazard use of nitrogenous fertilizers in agricultural activities and its contamination by human/animal organic waste on large-scale has led to increased concentration of nitrate in drinking water, food as well as in air [1, 2]. Nitrate, nitrite and ammonia are naturally occurring molecules in the environment and are the products of oxidation of nitrogen by microorganisms in plants tissues, soil and water [3, 4]. Nitrate is a salt of nitric acid, with an ion composed of one nitrogen and three oxygen atoms (NO₃⁻), and the nitrite (NO₂⁻), a salt of nitrous acid) can be formed in nitrate-containing and oxygen-poor water within galvanized steel pipes in the water distribution systems [2]. Ingestion of dietary (inorganic) nitrate elevates circulating and tissue level of nitrite via bioconversion in the entero-salivary circulation and also causes dose-dependent elevation in plasma nitrite and cGMP concentration with a consequent decrease in blood pressure in healthy volunteers. Post hoc analysis demonstrated a sex difference in sensitivity to nitrate supplementation depending on resting baseline blood pressure and plasma nitrite concentration, whereby blood pressure is decreased in male volunteers with higher baseline blood pressure and lower plasma nitrite concentration but not in female volunteers [5, 6]. The dietary nitrate is reduced to nitrite which is converted to nitric oxide (NO) in acidic gastric juice and then NO in the stomach plays an important role in the protection of gastric mucosa from hazardous stress also [7]. Recent epidemiologic data have been suggested an association between developmental effects in offspring and the maternal ingestion of nitrate from drinking water, but a definite conclusion on the cause and effect relationship have not been yet drawn [4]. Previous studies have shown that the high levels of nitrate in water leads to increased the incidence of gastric cancer [8, 9], pulmonary lesions, blood disorders, retarded growth in children [10-13], hypertrophy of thyroid/thyroid disorders in rat and human [14-17], pregnancy- related disorders in rats, depression, muscle tremors in goats, sexual disorders in sheep [18], and reproductivedevelopmental effects [3, 19, 20, 21]. Rats...
given 400 ppm sodium nitrite or sodium nitrate in drinking water for 6 months exerted immunosuppressive effect [22]. Nitrate has also shown mutagenic, embryotoxic and carcinogenic effects in experimental animals as well as in human [13, 23]. Several cases of methemoglobinemia have been reported in infants in the United States using water containing nitrate at levels higher than the current maximum contaminant level (MCL) of 45 ppm (mg/liter) NO₃ or 10 ppm nitrate–nitrogen, but none of the cases reported at lower doses than 45mg/L [10, 13, 24, 25-27]. The toxicity of nitrate to humans is due to endogenous reduction of nitrate to nitrite, which in turn may react with secondary amine to yield N-nitrosoamines, several of which are potent carcinogens in animals [28, 29]. Analysis of gastric contents after consumption of meals containing nitrate indicated that volatile nitrosamines may be formed under similar conditions in vivo [20]. Animal studies have shown some indication that nitrate, nitrite, and N-nitroso compounds may traverse the placenta and affect the foetus in utero [30, 31], and induce abortion in experimental animals [13, 24, 32], but showed no teratogenic effects. The fertility and gestation rates of sheep fed potassium nitrate were considerably lower (by 36% at 5 mg/kg body weight per day and 33% at 10 mg/kg body weight per day) and causes 32% decrease in number of oestrous sheep by 20mg/kg KNO₃ than in the control group [33] without affecting cyclic sexual behaviour. Existing animal experimental data have shown that reproductive toxicity is associated with high exposure levels to nitrate or nitrite, which are not likely to be encountered in drinking water. It includes spermatotoxicity in mice [34] and bulls [35] and inhibition of testosterone production and testicular size in male rat [36]. But, no consistent antiandrogenic effects were observed with nitrate exposure during pre-natal period and post natal development of dams [21]. The dose-dependent increase in weight and volume of the thyroid gland and decreased levels of triiodothyronine (T₃) and thyroxine (T₄) in rats and human exposed to nitrate in the drinking water have been reported at 150 and 500 mg/l of nitrate [15-17; 37]. The numerous endocrine disrupting chemicals, including certain anti-androgenic phthalates have been shown to decrease the anogenital distance (AGD) and impair steroidogenesis in foetal and neonatal male rats at critical periods of sexual development leading to malformations in the male reproductive system and reduced sperm count later in life [38-45]. However, information about nitrate toxicity is fragmentary and no definite conclusions can be drawn due to lack of pertinent male reproductive toxicity data in the literature. The present study was conducted to determine the dose-dependent effects of KNO₃ to which the human population is exposed to nitrate in the drinking water in certain parts of India as well as at its higher dose level, on the male reproductive organs function viz. organs weights, quantitative analysis of testicular spermatogenesis, DSP, epididymal sperm count, key testicular androgenic enzymes activity eg. Δ₅, 3β-hydroxysteroid dehydrogenase (Δ₅, 3β-HSD) and 17β-hydroxysteroid Dehydrogenase (17β-HSD) and Leydig cell morphology in adult Sprague Dawley rats.

II. MATERIALS AND METHODS

Chemicals: Chemicals including haematoxylin, eosin, NaCl, NaN₃, Triton X-100, KCl, potassium phosphate, sodium pyrophosphate, Na₂HPO₄, KH₂PO₄, D-glucose, testosterone, Dehydroepiandrosterone (DHEA), EDTA, BSA, NAD, NAD⁺ and Bradford reagent were purchased from Sigma Chemicals Company, USA. Potassium nitrate (98.5% pure) was procured from Merck Limited, Mumbai, India. All other chemicals of analytical grade were purchased locally.

Animals, treatment and tissue fixation: Adult male Sprague Dawley rats (180-200g Body weight) obtained from the Institute’s breeding colony were acclimatized to uniform husbandry conditions (22 ± 3°C, 12 h light: 12 h dark cycle) for 1 week prior to the experiment. Animals were fed with measured amount of food (a pellet diet supplied by Hindustan Lever Ltd., Bangalore) and water daily to access food and water intake. All rats were handled in accordance with the standard guide for the care and use of laboratory animals, and experimental protocol was approved by Institutional Animal Ethics Committee (IAEC).

48 adult male rats were randomly divided into four groups (Gr. I-IV) containing 12 animals in each group. Rats in Gr. I were given vehicle (10ml/kg distilled water) only and served as control. Animals in Gr. II, III and IV were administered orally with 45, 90 and 135 mg/kg body weight of potassium nitrate (dissolved in distilled water), respectively, for 15 days. Food intake, water intake, and gross behavioural changes were recorded daily for 1-2 hr after the drug
administration. Autopsy of animals from control and treated groups was done on day 16 after 15 days of treatment. The reproductive organs viz. testes, epididymis and accessory sex organs (ventral prostate and seminal vesicles) were dissected out, rinsed immediately in chilled saline, freed from connective tissues and blood clots, weighed and left side testes and epididymis fixed in Bouin’s fluid (24 h) for histology purpose. Tissues from right side were used for quantitation of daily sperm production rate in testis, biochemical estimation of enzymes (Δ^5-3β HSD and 17β-HSD) and epididymal sperm counts, etc.

**Histo-morphometry of testis and epididymis:** Bouin’s fixed testes and epididymis from control and treated groups of rats were dehydrated in graded series (50-100%) of ethanol, cleared in xylene, and infiltrated and embedded in paraffin wax (at 58°C). Serial cross sections (5 μm) were stained with haematoxylin and eosin. Measurements of testicular tubular diameter (20 tubules each rat with total of 100 tubules/group) and the nuclear diameter of Leydig cells (20 Leydig cells each rat with total of 100 cells/group) were carried out under Olympus Trinocular Microscope (Olympus BX 51, Japan) at x400 and x1000 (oil immersion) magnification respectively using Biovis Image Plus Software for image analysis and processing (Expert Vision Labs Pvt. Ltd. Mumbai, India).

**Quantitative analysis of spermatogenesis:** Quantitative analysis of spermatogenesis was carried out as per method of Srivastava et al. [46] based on nuclear morphology of germ cells and Sertoli cells. The number of germ and Sertoli cell nuclei were counted in at least 20 round seminiferous tubules selected randomly from at least 2-3 cross sections in each rat. The number of germ cells. viz. A-spermatogonia, non-pachytene (pre-leptotene, leptotene and zygotene) and pachytene spermatocytes, round and elongated spermatids were assessed in each group of 5 rats at 400X magnification under Olympus Trinocular Microscope (Olympus, BX 51, Japan). The cell counts for different germ cell types were corrected by Abercrombie’s formula [47].

**Estimation of daily sperm production (DSP):** Measurement of DSP in testicular homogenates was carried out as per method of Amann et al. [48]. Briefly, testicular parenchyma was cut into small pieces, placed in 0.25 mol/l sucrose solution (pH 7.5) and homogenized in a fluid containing 150 mM NaCl/l, 3.8 mM NaN3/l, and 0.05% (v/v) Triton X-100 using an Ultra Turrax® (Janke and Kunkel, Staufen, Germany). The homogenates were further diluted with the same medium, and homogenization-resistant spermatid nuclei in steps 17-19 of stages IV–VIII of the spermatogenic cycle were counted in triplicate under the Olympus microscope using a Neubauer’s haemocytometer [49]. DSP per gram testicular parenchyma was calculated by dividing the number of spermatid nuclei with the product of weight of parenchyma and time divisor of 6.3 days reported for rat [49]. The values are represented as mean ± se for six animals in each group.

**Epididymal sperm count:** Cauda epididymis was excised surgically, cut into small pieces in suspension medium containing 140 mmol NaCl, 0.3 mmol KCl, 0.8 mmol Na2HPO4, 0.2 mmol KH2PO4 and 1.5 mmol D-glucose (pH adjusted to 7.3 by adding 0.1 (N) NaOH) and sperms were collected by centrifugation at 100 × g for 2 min. The resultant precipitate was resuspended in fresh suspension medium, diluted and placed on both sides of Neubauer’s hemocytometer. The counting of number of sperms was carried out in four chambers of hemocytometer in duplicate in each rat under Olympus Trinocular microscope at x100 magnification. The values were expressed as an average of total counts of sperm number per ml of suspension [46, 50].

**Estimation of Δ^5, 3β- and 17β- Hydroxysteroid dehydrogenase activity:** The testicular Δ^5, 3β-Hydroxysteroid dehydrogenase (Δ^5, 3β-HSD) and 17β- Hydroxysteroid dehydrogenase (17β-HSD) enzyme activity was measured according to the methods described previously [51, 52]. The testis was encapsulated and tissues of each animal was homogenized in 15% glycerol containing 5 mmol potassium phosphate and 1 mmol EDTA at a tissue concentration of 100 mg/ml. The homogenates were centrifuged at 10,000 × g for 30 min at 4°C. For Δ^5, 3β-HSD enzyme activity, the supernatant (1 ml) was mixed with 1 ml of 100 μmol sodium pyrophosphate buffer (pH 8.9) and 30 μg of dehydroepiandrosterone (Sigma, USA) in 40 μl of ethanol and 960 μl of 25% BSA (Sigma, USA), making the incubation mixture to 3 ml. The enzyme activity was measured after addition of 0.5 μmol of NAD (Sigma, USA) to the
tissue supernatant mixture using a U2000 spectrophotometer (Hitachi, Japan) cuvette at 340 nm against a blank (without NAD). One unit of enzyme activity is the amount causing a change in absorbance of 0.001/min at 340 nm. The activity of testicular 17β-HSD was measured using the same supernatant prepared for the assay of Δ5, 3β-HSD (above). The supernatant (1 ml) was mixed with 1 ml of 440 μmol sodium pyroposphate buffer (pH 10.2), 40 μl of ethanol containing 0.3 μmol of testosterone (Sigma, USA) and 960 μl of 25% BSA (Sigma), making the incubation mixture a total of 3 ml. The enzyme activity was measured after addition of 1.1 μmol NAD (Sigma) to the tissue supernatant mixture in a U2000 spectrophotometer cuvette at 340 nm against a blank (without NAD). One unit of enzyme activity is equivalent to a change in absorbance of 0.001/min at 340 nm.

**Protein estimation:** Bradford Assay [53] was used to determine the total protein concentration of the samples from control and treated rats in thrice. Different dilutions (1 μg to 10 μg) of 1% BSA in different eppendorfs were made. 5 μl from each sample using eppendorf was taken in different wells of ELIZA plates. Then added 150 μl of Bradford reagent (Sigma Chem. Co., USA) to each well. Covered the plate with aluminium foil as Bradford reagent is light sensitive and read the O.D. at 595 nm with the help of µQuant ELISA reader. Plotted a Standard graph of protein estimation with different dilutions of BSA concentration and O.D. obtained. Testicular extracts (100 mg/ml) were prepared in homogenizing fluid, ultra-centrifuged at x10000 rpm for 30 minutes. Supernatant was separated. In ELIZA plate (96 well plates) 190 μl Bradford reagent+5 μl samples was added and read at 595 nm wavelength. O.D. was recorded and Concentrations of protein in testicular tissue was calculated using Standard BSA plot. Three readings per sample were taken.

**Statistical analysis:** Statistical analysis for significant differences between control and treated group of animals was done by Students’ 't' test and one-way ANOVA (one factor analysis of variance). Values with P < 0.05 were considered as significant. Values were expressed as Mean ± SE.

### III. RESULTS

**Body and reproductive organ weights:** Body weights did not show any significant change in rats treated with KNO3 at the doses of 45 mg/kg (Gr. II), 90 mg/kg (Gr. III) and 135 mg/kg (Gr. IV) for 15 days as compared to control (Gr. I) rats. The reproductive organ weights viz. testes, epididymis, prostate and seminal vesicles did not show any significant change in treated as compared to in control rats (Table I).

**Table I. Effect of p-nitrosodimethylaniline on body and reproductive organ weights in male rats after 15-days of treatment.**

<table>
<thead>
<tr>
<th>Groups/Treatment</th>
<th>Body weights</th>
<th>Reproductive organ weight (mg)</th>
<th>Epididymis</th>
<th>Vasculal</th>
<th>Prostate</th>
<th>Seminal vesicles</th>
</tr>
</thead>
<tbody>
<tr>
<td>I Control</td>
<td>341 ± 5</td>
<td>1.225 ± 0.025</td>
<td>0.680 ± 0.038</td>
<td>0.457 ± 0.037</td>
<td>1.833 ± 0.134</td>
<td></td>
</tr>
<tr>
<td>II. KNO3 (45 mg/kg)</td>
<td>341.5 ± 0.25</td>
<td>1.230 ± 0.053</td>
<td>0.670 ± 0.045</td>
<td>0.506 ± 0.042</td>
<td>1.830 ± 0.130</td>
<td></td>
</tr>
<tr>
<td>III. KNO3 (90 mg/kg)</td>
<td>340.5 ± 0.20</td>
<td>1.254 ± 0.058</td>
<td>0.630 ± 0.071</td>
<td>0.570 ± 0.093</td>
<td>1.850 ± 0.140</td>
<td></td>
</tr>
<tr>
<td>IV. KNO3 (135 mg/kg)</td>
<td>340 ± 5.25</td>
<td>1.260 ± 0.080</td>
<td>0.640 ± 0.092</td>
<td>0.560 ± 0.084</td>
<td>1.870 ± 0.098</td>
<td></td>
</tr>
</tbody>
</table>

Values expressed mean ± SE, n=10 number of animals. Significance between treated vs. control: *p<.05, **p<.01, ***p<.001, NS = Non significant.

**Histo-morphometry of testis:** In control rats, testicular seminiferous epithelium showed all germ cell types, eg. Spermatogonia, Non-pachytene (preleptotene, leptotene, zygotene) and pachytene primary spermatocytes, spermatids (round and elongated) and spermatiating spermatozoa. The Sertoli and Leydig cells showed normal morphology. In rats treated with 45 and 90 mg/kg, all germ cells types and Sertoli cells in the seminiferous epithelium, and interstitial Leydig cells showed normalcy. In contrast, rats treated with 135 mg/kg dose of KNO3 caused seminiferous epithelial...
destruction in 40±8.9% testicular tubules (Abnormal tubules). The degenerative changes in abnormal tubules were marked by cytoplasmic vacuolization, multinuclear giant cell formation, desquamation and degeneration of germ cells into tubule lumen and disintegration of spermatozoa. Some of the testicular tubules exhibited complete destruction of germ cells up to primary spermatocytes stage and presence of only the spermatogoniae and Sertoli cells was observed. However, 60±8.9% testicular tubules showed normal seminiferous epithelium exhibiting all germ cell types at 135mg/kg dose. The seminiferous tubular diameter and diameter of Leydig cells nuclei showed significant reduction (p<0.001) in abnormal tubules as compared to normal tubules/controls. The Leydig cells between affected tubules displayed hyperplasia with increased leucocytes infiltration at 135 mg/kg dose of KNO₃ (Table 2, Fig. 1 A-F).

Quantitation of spermatogenesis: Quantitative analysis of spermatogenesis carried out in rats treated with 45 and 90 mg/kg of KNO₃ did not show any significant difference in the number of all germ cell types and Sertoli cell number when compared with control rats. However, in rats treated with 135mg/kg dose of KNO₃, showed significant decrease in number of primary spermatocytes (non-pachytene and pachytene) and spermatids (round and elongated) in normal as well as in abnormal tubules as compared to controls (Table 2, Fig. 2).
Estimation of daily sperm production (DSP): Estimation of daily sperm production rate in testicular homogenates of rats treated with 45 or 90 mg/kg of KNO₃ for 15 days showed no significant change as compared to controls. However, a significant decrease (p<0.02) in DSP rate was observed in rats treated with higher dose (135 mg/kg/day) of KNO₃ as compared to control rats (Fig. 3 A).

Epididymal sperm counts: Cauda epididymal sperm counts in rats treated with 45 and 90 mg/kg KNO₃ did not show any significant decrease but, exhibited a significant decrease (p<0.05) in number of sperms at 135 mg/kg dose as compared to controls (Fig. 3 B).
Effects of potassium nitrate on enzymatic analysis: There was a significant decrease in testicular estimation of steroidogenic enzymes, viz. Δ5-3β-HSD (Fig. 4 A) and 17β-HSD (Fig. 4 B) activity in rats treated with 135mg/kg dose of KNO3 for 15 days as compared to control rats. Whereas, in rats treated with lower doses (45 and 90 mg/kg) of KNO3 did not show any significant change in both the enzymatic activity.
Food and water Intake: There was no difference in the uptake of water and food consumption in control and treated animals during the entire treatment period (Fig. 5 A, B).

Toxicity study: The animals remained apparently normal during entire treatment period, except for development of dullness in 135 mg/kg KNO₃ treated animals.

IV. DISCUSSION

Present study was conducted to determine dose-dependent effects of KNO₃ on male reproductive organs with special emphasis on testicular toxicity. Results of the present study did not show any significant changes in the body weights and reproductive organ weights in rats treated with KNO₃ at 45, 90 and 135 mg/kg doses for 15 days, except for a significant decrease (p<0.02) in weight of ventral prostate gland at 135 mg/kg dose. Previous study with KNO₃ in mice has also been shown no any significant difference in body and reproductive organ weights at higher dose of 900 ppm in mice [34, 54]. The morphometric analysis of testes carried out in the present study showed 40±8.9% abnormal testicular tubules (with incomplete stages of spermatogenesis) and 60±8.9% Normal tubules (with complete spermatogenesis depicting all germ cell types) only at 135 mg/kg dose of KNO₃. The quantitative analysis of spermatogenesis in treated rats showed a significant inhibition of primary spermatocytes viz. non-pachytene (p<0.05-0.001) and pachytene (p<0.005-0.001), and spermatids (round and elongated in steps 1-19 of spermiogenesis, p<0.001) in both normal and abnormal tubules only at 135 mg/kg dose level as compared to controls (Table 2). In contrast, the number of spermatogonia-A and Sertoli cells were observed to be unchanged than in control rats. The inhibition of germ cells in higher dose (135 mg/kg) of KNO₃ was also associated with decreased daily sperm production rate in testicular homogenates as well as cauda epididymal sperm counts in treated rats. The suppression of spermatogenic germ cells was also accompanied by extensive cytotoxicity in seminiferous epithelium depicting cytoplasmic vacuolization, giant cell formation, pyknosis of nuclei, desquamation and dissolution of germ cells in tubule lumen and disintegration of spermatozoa in abnormal tubules of treated (135 mg/kg) rats. Further, KNO₃ treatment also caused
significant decrease (p<0.001) in testicular tubular diameters (in both normal and abnormal tubules) at 135 mg/kg dose level as compared to controls. However, in rats treated with 45 and 90 mg/kg doses of KNO₃, indicated no significant changes as compared to in controls. Our results, are in agreement with the previously reported studies that indicate no any detectable histopathological change at lower doses (90, 200, 500 and 700 ppm) of KNO₃ in mice, but revealed moderate atrophy with disturbed spermatogenesis, prominent interstitial space at 900-ppm congestion (Pant and Srivastava, 2002) and spermatotoxicity in rat [29] and bulls [35]. Results of the study also showed significant inhibition of androgenic function evidenced by reduced size and morphology of Leydig cells and significant decline (p<0.02) in accessory sex organ (prostate) weight at 135 mg/kg KNO₃ treatment for 15 days as compared to controls. Existing reported studies have been shown the inhibition of testosterone production and testicular size in nitrate and nitrite exposure via drinking water [36]. It has been also reported that KNO₃ treatment causes an increase in weight of thyroid gland but, decreased thyroxin hormone levels [15, 16]. In contrast, lower doses of sodium nitrate did not inhibit testosterone level in wister rats [29]. In pregnant dams, nitrate and nitrite exposure from days 7 to 21 of gestation at 17.5, 50, 450 and 900 mg/l did not affect the testosterone, progesterone and thyroxin levels and shown no histopathological changes during sexual development of male foetuses [21]. Further, studies on testosterone deprivation have been shown to suppress plasma LH, nuclear morphology of Leydig cells and germ cells, while testosterone supplementation restores normal function of Leydig cells and spermatogenesis indicating testosterone dependent cellular sensitivity of Leydig cells [55-58]. The anti-androgenic phthalates exposure to prenatal period have been also demonstrated to show impairment of steroidogenesis in foetal and neonatal male rats leading to malformation in male reproductive system and reduction in the sperm count in later life [38-40]. Results of the present study on biochemical estimation of key steroidogenic enzymes, Δ⁵ 3β-HSD and 17β-HSD activity [59-61] in testicular tissue, indicated significant decrease in KNO₃-treated rats at 135 mg/kg dose for 15 days. Similar decrease in testicular 17β-HSD activity along with an increase in glutamil transeptidase activity in KNO₃-treated mice has been reported at higher dose level previously [34, 62, 63]. The inhibition of Δ⁵, 3β-HSD and 17β-HSD activity in testis may indicate inhibition of steroidogenesis in KNO₃-treated rats at 135 mg/kg dose level only. It was also accompanied with decreased nuclear diameter of Leydig cells, androgen-dependant primary spermatocytes (nonpachytene and pachytene) and spermatids (round and elongated) as well as deduction in weight of prostate gland at higher dose level rather than its lower doses. The inhibition of steroidogenesis and spermatogenesis may be due to the direct effect of nitrate or its metabolites or due to low levels of plasma gonadotrophins (plasma FSH and LH) responsible for regulating its activity in testis. It has been demonstrated that the nitrate interferes with the normal physiology of Sertoli cells which may affect the development of androgen-dependant germ cells (viz. spermatocytes, spermatids) and increases sperm abnormality as well as declines sperm counts [34] by 900ppm KNO₃ treatment for 35 days in mice [34], rats [29] and Bulls [35]. This loss of sperm function has been reported to be associated with excessive free radical generation in sperm and seminal plasma [64]. Our results at 135 mg/kg dose of KNO₃ have been also shown a significant decrease in testicular daily sperm production, germ cell population and epididymal sperm counts. It indicate that exposure to high concentration of nitrate could adversely effect the development of germ cells and as evidenced by decline spermaticogenic germ cells, steroidogenic enzyme activity, Leydig cell size and histopathology may indicate testosterone inhibition [65]. Reported studies with other compounds like carbaryl, carbofuran, arercnic chromium, lead, benzene, chloroform, phenol and trichloroethylene [66], other toxicants like lupeol acetate [67], intragastric perfusion of waste [68], benzene exposure [68-70] as well as other pollutants/ chemical agents as well as estrogen/antiestrogens/androgens have been shown to cause depletion of primary spermatocytes, spermatids and spermatotoxicity including inhibition of semen quality, sperm counts, sperm motility, total spermatozoa/ejaculates, daily sperm production, acrosomal dysgenesis, nuclear malformations, mating desire/ability, sperm quality and Leydig cell function associated with decreased serum concentrations of LH and Testosterone secretion and the activity of marker testicular enzymes associated with specific cell types and histopathological parameters [54, 55, 71-78]. Though the exact mechanism of action of KNO₃ treatment is still not clear, but, it may cause a reproductive toxicity only at higher dose level, which is not likely to be encountered in drinking water in human. Further, chronic administration of KNO₃ is needed to examine the adverse effects if any and to explore its exact mechanism of action more precisely by keeping in view the continuous exposure to human beings in actual prevailing condition.
The present study demonstrates the antispermatogenic effects of KNO₃ at 135mg/kg dose level for 15 days treatment in rats than that of its lower doses (45 and 90 mg/kg), which did not show any effect of testicular spermatogenesis. Inhibition of testicular spermatogenesis was evident by depletion of androgen-dependent meiotic primary spermatocytes (non-pachytene and pachytene) and post-meiotic spermatids (round and elongated), inhibition of testicular tubular diameter, DSP, key steroidogenic enzymes (Δ⁴-3β-HSD and 17β-HSD) activity, nuclear diameter of Leydig cells, epididymal sperm count and accessory sex organ (ventral prostate) weight at 135 mg/kg dose level, may indicate antispermatogenic and anti-androgenic effects of KNO₃ at 135 mg/kg dose level in rat.

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