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Taurine reverses sodium fluoride-mediated increase in inflammation, caspase-3 activity and oxidative damage along the brain-pituitary-gonadal axis in male rats

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Abstract

Excessive exposure to fluoride is associated with male reproductive dysfunction in humans and animals. Taurine (2-aminoethane sulfonic acid) is a free intracellular β -amino acid with antioxidant, anti-inflammatory and neuroprotective properties. However, the effect of taurine on fluoride-induced reproductive toxicity has not been reported. The present study investigated the influence of taurine on sodium fluoride (NaF)-induced functional changes along the brain-pituitary-gonadal axis in male rats. NaF was administered singly in drinking water at 15 mg/L alone or orally co-administered by gavage with taurine at 100 and 200 mg/kg body weight for 45 consecutive days. Results showed that taurine significantly prevented NaF-induced increase in oxidative stress indices as well as augmented antioxidant enzymes activities and glutathione level in the brain, testes and epididymis of the treated rats. Moreover, taurine reversed NaF-induced elevation in inflammatory biomarkers and caspase-3 activity as well as histological damage in the brain, testes and epididymis of the treated rats. The significant reversal of NaF-induced decreases in testosterone level and testicular activities of acid phosphatase, alkaline phosphatase and lactate dehydrogenase by taurine was accompanied by enhancement in sperm functional characteristics in the treated rats. Taurine may be a possible chemopreventive candidate against reproductive dysfunction resulting from fluoride exposure.

Keywords: Sodium fluoride, Taurine, Reproductive toxicity, Antioxidant, Anti-inflammatory.

Introduction

Fluorides of many metals and non-metals are used industrially for drinking water fluoridation, aluminum production and in dental preparations (Lu et al. 2000). The permissible limit of fluoride in drinking water ranged from 0.7 to 1.0 mg/L (WHO 2004). However, people in certain parts of the world including Africa, Asia and the Eastern Mediterranean could be exposed to contaminated ground water with fluoride concentration reaching up to 20 mg/L (WHO 2006). Fluoride was detected at 96.8 mg/L in industrial wastewater (Ding et al. 1998) and up to 3000-5000 mg/L in extreme cases (Wu et al. 2006).

The scientific understanding of the association between environmental exposure to fluoride and the declining human fertility rates is growing. Epidemiological studies indicated that environmental exposure to fluoride was associated with male infertility and low birth rates in endemic areas of fluorosis (Long et al. 2009; Ortiz-Perez et al. 2003). Furthermore, numerous clinical investigations and animal studies revealed that fluoride is a testicular toxicant that disrupts reproductive hormones levels, induces structural and functional defects in sperm and consequently reduced fertility (Long et al. 2009; Lu et al. 2014; Sun et al. 2014; Dong et al. 2016). Indeed, the three important processes namely the spermatogenesis, capacitation and acrosome reaction which spermatogonia undergoes to finally fertilize an oocyte have been reported to be impaired by excessive exposure to fluoride (Long et al. 2009). The biochemical mechanisms of fluoride mediated testicular toxicity have been shown to involve induction of oxidative stress, inflammatory response and apoptosis (Barbier et al. 2010; Zhang et al. 2013; Wei et al. 2016). Hence, a suitable intervention strategy for fluoride-induced toxicity would involve the suppression and/or inhibition of oxidative stress, inflammation and apoptosis using antioxidants.

Taurine (2-aminoethane sulfonic acid) is a free intracellular β -amino acid which is well reported to elicit beneficial health effects via multiple actions on cellular functions (Huxtable 1992; Das et al. 2012). Cellular biosynthesis of taurine occurs in the liver via the cysteine sulfonic acid pathway while it is exogenously obtained from dietary sources (Huxtable 1992; De Luca et al. 2015). Besides its nutraceutical role, taurine reportedly exerts robust pharmacological actions via modulation of signaling pathways and targets (De Luca et al. 2015). Although no data on the systemic toxicity of taurine is available till date, 3 g per day of taurine for 4 months produced no obvious signs of toxicity during human trials (Shao and Hathcock 2008). Thus, suggesting its relative safety as a chemoprotective agent. Unfortunately, there is no published animal study investigating the safety and toxicological aspects of acute, subchronic or chronic oral taurine treatment. The antioxidant, anti-inflammatory, nephroprotective and neuroprotective activities of taurine have been reported (Aruoma et al. 1988; Das et al. 2012; Adedara et al. 2016). However, there is dearth of scientific report on the influence of taurine on fluoride-induced testicular toxicity.

Thus, the aim of the present study was to evaluate the possible modulatory effects of taurine on fluoride-induced functional alterations in brain-pituitary-gonadal axis in rat. This was achieved by measuring the plasma hormone levels, sperm functional characteristics, antioxidant status and the marker enzymes of testicular function along with the histological analyses of the brain, testes and epididymides of the experimental animals.

Materials and methods

Chemicals

Taurine, sodium fluoride (NaF), thiobarbituric acid, trichloroacetic acid, epinephrine, glutathione, hydrogen peroxide, 5',5'-dithio-bis-2-nitrobenzoic acid (DNTB) and 1-chloro-2,4-dinitrobenzene (CDNB) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). The remaining reagents were of analytical grade and were procured from the British Drug Houses (Poole, Dorset, UK).

Animal model

Sixty adult male Wistar rats (8 weeks old; 140 ± 2 g) obtained from the Department of Biochemistry, University of Ibadan, Ibadan were used for this study. The animals were kept under standard laboratory conditions of a 12 h/12 h light/dark cycle, fed with rat chow and given drinking water *ad libitum* in their home cages for a week before the commencement of the experiment. Animal care and experimental protocols were executed according to the approved guidelines set by the University of Ibadan Ethical Committee, which is in agreement with the Guide for the Care and Use of Laboratory Animals' prepared by the National Academy of Science (NAS) and published by the National Institute of Health.

Experimental protocol

The rats were randomly assigned to five groups of twelve rats each and were treated for 45 consecutive days as follows:

Group I: The rats received drinking water alone and served as control.

Group II: The rats were exposed to NaF in drinking water at 15 mg/L alone.

Group III: The rats were orally administered taurine at a dose of 200 mg/kg using drinking water as vehicle.

Groups IV: The rats were co-administered with NaF and taurine at 100 mg/kg (TAU 1).

Groups V: The rats were co-administered with NaF and taurine at 200 mg/kg (TAU 2).

Taurine supplementation at dosages ranging from 500 mg to 2,000 mg have shown efficacy without toxicity and are well-tolerated in humans (Galloway et al. 2008; Rutherford et al. 2010; Balshaw et al. 2013). The doses of NaF and taurine used in the present study were selected based on the previously published data (Chattopadhyay et al. 2011; Adedara et al. 2017). Twenty-four hours after the last exposure, the final body weights of the rats were taken, blood collected from retro-orbital venous plexus using heparin containing tubes before they were sacrificed by cervical dislocation. Plasma samples were separated from blood cells by centrifugation at 3000 g for 10 min. The plasma samples were subsequently kept frozen at -20°C until the determination of hormones concentrations using ELISA strip reader (Robonik India Private Limited, Mumbai, India). The brain, testes and epididymides were carefully excised, weighed and subsequently processed for biochemical estimations and histological analysis. The organo-somatic index (OSI) of the brain, testes or epididymis was calculated using the formula, $OSI = 100 \times \text{organ weight (g)}/\text{body weight (g)}$.

Determination of plasma concentrations of pituitary and testicular hormones

The plasma concentrations of pituitary and testicular hormones were assayed using available commercial enzyme immunoassay kits specific for rats namely LH (RPN 2562, Amersham, UK), FSH (RPN 2560, Amersham, UK) and testosterone (EIA-5179, DRG Diagnostics GmbH, Marburg, Germany) as per the manufacturer's instruction. The sensitivity of LH was 0.08 ng at 90% whereas FSH sensitivity was 0.05 ng at 95%. The intra-assay coefficients of variations were 3.5% for LH and 3.8% for FSH. The sensitivity of the testosterone assay was 0.06 ng/mL with negligible cross-reactivity with other androgen derivatives like methyl testosterone, androstenedione and 5 α -dihydrotestosterone. The intra-assay coefficient of variation was 3.5%. Inter-assay variation was avoided by assaying all the samples on the same day.

Assessment of sperm progressive motility

The sperm progressive motility was assessed according to the method of Zemjanis (1970). Briefly, epididymal sperm was obtained by cutting the cauda epididymis with surgical blades and released onto a sterile clean glass slide. Subsequently, the sperm was diluted with 2.9% sodium citrate dehydrate solution which had been pre-warmed to 37°C, mixed carefully and covered with a coverslip (24 x 24 mm). The sperm motility was evaluated by examining a minimum of ten microscopic fields under a phase contrast microscope at 200x magnification. Sperm motility was obtained by scoring the number of all progressive sperm, followed by the non-progressive and then the immotile sperm in the same field. The data were expressed as percentage of sperm progressive motility.

Evaluation of epididymal sperm count

The epididymal sperm count was determined according to established method (WHO 1999). Briefly, the sperm obtained by mincing the cauda epididymis in normal saline was filtered using a nylon mesh. Subsequently, an aliquot of 5 μ L of the sperm was mixed with 95 μ L of diluent (0.35% formalin containing 5% NaHCO_3 and 0.25% trypan blue). Ten microlitres of the diluted sperm was placed on the hemocytometer, allowed to sediment by standing for 5 minutes in a humid chamber before they were counted using the improved Neubauer chamber (Deep 1/ 10 m; LABART, Munich, Germany) with a light microscope at 400x.

Evaluation of sperm morphological abnormalities and viability assay

Sperm morphological abnormality was determined by smearing a portion of the sperm suspension placed on a glass slide with another slide followed by staining with a reagent containing 0.2 g eosin and 0.6 g fast green dissolved in distilled water and ethanol in a ratio of two to one (2:1). A total of 400 sperm cells from each rat were examined for morphological aberration. Sperm viability was evaluated by staining smeared slides with 1% eosin and 5% nigrosine in 3% sodium citrate dehydrate solution according to Wells and Awa (1970).

Biochemical Assays

The post-mitochondrial fractions of the brain, testes and epididymis of the rats were obtained by homogenizing the tissues separately in 50 mM Tris-HCl buffer (pH 7.4) containing 1.15% potassium chloride. Subsequently, the homogenates were centrifuged at 12,000 g for 15 min at 4°C and the supernatants obtained were used for the biochemical estimations. Protein

concentration was assayed using the method of Bradford (1976). Hydrogen peroxide generation was determined using the method of Wolff (1994). Lipid peroxidation was quantified as malondialdehyde (MDA) according to the method described by Farombi et al (2000). Activity of superoxide dismutase (SOD) was determined according the method of Misra and Fridovich (1972). Catalase (CAT) activity was determined using hydrogen peroxide as substrate according to the method of Clairborne (1995). The level of reduced glutathione (GSH) was determined according to the method described by Jollow et al. (1974). Glutathione-S-transferase (GST) activity was determined according to the method of Habig et al. (1974). Glutathione peroxidase (GPx) activity was determined according to the method of Rotruck et al. (1973).

Evaluation of activities of marker enzymes of testicular function

Activities of acid phosphatase (ACP) and alkaline phosphatase (ALP) were determined in the testes supernatant according to established methods (Shen and Lee 1984; Abd El Tawab et al. 2014), which is based on the hydrolysis of p-nitrophenyl-phosphate in acid and alkaline medium, respectively. Lactate dehydrogenase-X (LDH-X) activity was determined according to established method which is based on the inter-conversion of lactate and pyruvate (Vassault 1983).

Evaluation of pro-inflammatory biomarkers concentrations

Myeloperoxidase (MPO) activity was determined according to the method described by Granell et al. (2003). Myeloperoxidase activity was expressed as $\mu\text{M H}_2\text{O}_2/\text{min}/\text{mg protein}$. Nitric oxide (NO) level was determined by quantifying the nitrites content, the stable end products of nitric oxide. The nitrites content was obtained using a sodium nitrite curve as standard and expressed

as μM of nitrites/mg protein according to Green et al. (1982). The concentrations of tumor necrosis factor alpha (TNF- α) was determined using commercially available ELISA kits (ABCAM PLC, UK).

Determination of caspase-3 activity

Caspase-3 activity was determined using commercially available ELISA kits (Elabscience Biotechnology Company, Beijing, China) with the aid of DNM 9602 Microplate Reader (China) in accordance with the procedure described in the assay manual.

Histological examination

Brain samples were fixed with 10% neutral-buffered formalin whereas testes and cauda epididymis samples were fixed with Bouin's solution and processed for histology according to standardized procedure (Bancroft and Gamble 2008). Briefly, the fixed tissues were dehydrated using increasing concentrations of alcohol, cleared by xylene and embedded in paraffin wax. The tissues were subsequently cut to produce 4–5 μm sections using a microtome, fixed on the slides and stained with hematoxylin and eosin (H & E). All slides were coded prior to examination with light microscope (Olympus CH; Olympus, Tokyo, Japan) and photographed using Sony DSC-W 30 Cyber-shot (Sony, Tokyo, Japan) by pathologists.

Histological analysis of the testes and cauda epididymis was performed according to standardized procedure (Hess 1990) with slight modifications. Semi-quantitative evaluation of the histological alterations in the testes includes the following scoring: (0), minimal changes, <5% of tubules affected; (1), slight changes, 5–25% tubules affected; (2), moderate changes, 25–50% tubules affected; (3), marked changes, 50–75% tubules affected; (4), severe changes, >75%

tubules affected. The caudal epididymal grading and histopathological alterations were: (0) no observable effect; (1) slight changes, normal sperm concentration and 5–10 necrotic cells in the efferent ductules; (2) moderate changes, moderate decrease and 11–50 necrotic cells; (3) marked changes, marked decrease and >50 necrotic cells; (4) severe changes with a marked decrease in sperm concentration or azoospermia in the efferent ducts.

Statistical analyses

Statistical analyses were carried out using one-way analysis of variance (ANOVA) to compare the experimental groups followed by Dunnett's t-test for post hoc evaluation using GRAPHPAD PRISM 5 software (Version 4; GraphPad Software, La Jolla, California, USA). Values of $P < 0.05$ were considered significant.

Results

Body weight gain and organo-somatic indices of the brain, testes and epididymis of rats

The body weight gain and organo-somatic indices (OSI) of the brain, testes and epididymis of control and treatment groups are shown in **Table 1**. Following the exposure period, there were no treatment-related effects of taurine alone, NaF alone or in combination with taurine was observed on the OSI of the brain, testes and epididymis when compared with the control.

Taurine inhibited oxidative damage in the brain, testes and epididymis of fluoride-exposed rats

The oxidative stress indices in the brain, testes and epididymis of control rats and those treated with fluoride alone or in combination with taurine are depicted in **Figures 1-4**. There were no treatment-related effects on the antioxidant status and oxidative stress indices in rats exposed to taurine alone when compared with the control. However, administration of fluoride alone resulted in significant ($p < 0.05$) increase in the levels of H_2O_2 and MDA, a biomarker of lipid peroxidation in the brain, testes and epididymis of fluoride-treated rats when compared with the control. Besides, fluoride administration significantly decreased the activities of antioxidant enzymes, SOD, CAT, GPx and GST as well as GSH level in brain, testes and epididymis of the treated rats when compared with the control. Conversely, co-administration of taurine significantly decreased the levels of H_2O_2 and MDA and restored the antioxidant status of the brain, testes and epididymis to normalcy in fluoride-treated rats when compared with the control.

Taurine suppressed inflammatory biomarkers in the brain, testes and epididymis of fluoride-exposed rats

The influence of taurine co-administration on the inflammatory mediators in fluoride-treated rats are depicted in **Figures 4-5**. There were no treatment-related effects on the inflammatory biomarkers namely MPO, NO and TNF- α in rats exposed to taurine alone when compared with the control. Administration of fluoride alone significantly increased the MPO activity, NO and TNF- α levels in the brain, testes and epididymis of the treated rats when compared with the control. However, co-administration with taurine markedly suppressed these inflammatory mediators in fluoride-treated rats.

Taurine prevented fluoride-induced suppression of testosterone concentration and marker enzymes of testicular function in rats

The influence of taurine co-administration on circulatory hormones concentrations and marker enzymes of testicular function in fluoride-treated rats are depicted in **Figure 6**. There were no treatment-related effects on the LH, FSH and testosterone levels in rats exposed to taurine alone when compared with the control. Administration of fluoride alone significantly decreased the circulatory concentration of testosterone whereas it caused no statistically significant differences in the levels of LH and FSH in the treated rats. Co-administration of taurine significantly restored the testosterone concentration in the treated rats. Moreover, activities of marker enzymes of testicular function including ACP, ALP and LDH were significantly decreased in fluoride-treated rats when compared with control. However, co-administration of taurine significantly ameliorated fluoride-induced testicular toxicity by restoring the activities of ACP, ALP and LDH toward normalcy in the treated rats.

Taurine improves sperm functional characteristics in fluoride-treated rats

The influence of taurine co-administration on sperm functional characteristics in fluoride-treated rats are depicted in **Figure 7**. There were no treatment-related effects on the sperm viability in all the treatment groups. The percentage of sperm progressive motility and sperm count were significantly decreased whereas abnormal sperm with morphological defects were significantly increased in the fluoride-treated rats. The major abnormalities observed in the fluoride-treated rats were tailless heads, curved mid-pieces and bent mid-pieces. However, the sperm functional

parameters were restored to near control levels in rats co-treated with taurine when compared with the group exposed to fluoride alone.

Taurine suppressed fluoride-mediated activation of caspase-3 activity in the brain, testes and epididymis of rats

The influence of taurine administration on the caspase-3 activity in the brain, testes and epididymis of fluoride-treated rats are depicted in **Figure 8**. There were no treatment-related effects on the caspase-3 activity in rats exposed to taurine alone when compared with the control. Administration of fluoride alone significantly increased caspase-3 activity in the brain, testes and epididymis of fluoride-treated rats when compared with control. However, co-administration of taurine was effective in decreasing caspase-3 activity significantly to near normal in fluoride-treated rats.

Taurine ameliorates histological alterations in brain, testes and epididymis of fluoride-treated rats

The representative photomicrographs of brain, testes and cauda epididymis from the control and treatment groups are shown in **Figure 9**. Microscopic examination revealed normal architecture of the brain, testes and epididymis in control (B1, T1 and E1) and rats treated with taurine alone (B3, T3 and E3). The brain of fluoride-treated rats (B2) showed focal area of vacuolation (red arrow), satelliosis (yellow notched arrow) and congestion of the microcirculation (black notched

arrow). The fluoride-treated testes (T2) showed disorganization of spermatogenic cells (blue arrow) and shedding of sperm cells within the lumen (black arrow) of the seminiferous tubules. Epididymis from fluoride-treated rats (E2) showed hyperplasia of epithelial cells lining the duct of epididymis (black arrow) and lumen containing scanty sperm cells (red notched arrow). However, the brain, testes and epididymis of rats co-treated with taurine at 100 mg/kg (B4, T4 and E4) and 200 mg/kg (B5, T5 and E5) appeared structurally and functionally normal.

The magnitude of lesions that occurred in the testes and epididymis was evaluated with a semi-quantitative grading system and the result presented in **Table 2**. Administration of fluoride alone significantly increased histopathology lesions in the testes and epididymis of the treated rats when compared with control. The alterations observed in the testes were not stage dependent. However, co-administration of taurine was effective in decreasing histopathology lesions significantly to near normal in fluoride-treated rats.

Discussion

Fluoride represents a dangerous environmental and industrial contaminant which has generated widespread public health concerns due to its association with several human diseases including adverse reproductive outcomes (Freni et al. 1994; Susheela and Jethanandani 1996; Ortiz-Perez et al. 2003). The present study showed that while the body weight gain and the OSI of the brain, testes and epididymis appeared not to be affected in fluoride-treated rats, the noxious effects of fluoride exposure were evident in the spermiogram, biochemical and histological analyses. The present investigation demonstrates, for the first time, the efficacy of taurine in abrogating

fluoride-induced sperm toxicity, endocrine suppression, oxidative stress, inflammation and histological damage in the brain, testes and epididymis of Wistar rats.

Excessive ROS production results in peroxidative breakdown of the phospholipids components of cellular membranes to produce MDA, a lipid peroxidation product and consequently the propagation of cellular injury. The elevated levels of H_2O_2 and MDA observed in the brain, testes and epididymis of fluoride-treated rats in the present investigation corroborate previous studies on the role of oxidative stress in fluoride-induced toxicity (Shivarajashankara et al. 2001; Ghosh et al. 2002; Izquierdo-Vega et al. 2008). The normalization of H_2O_2 and MDA levels following taurine co-treatment is related to its antioxidant and anti-peroxidative properties as previously demonstrated (Das et al. 2012). Taurine reportedly interacts with cell membranes to stabilize them against oxidative injuries (Green et al. 1998).

Moreover, the decrease in SOD and CAT activities in the brain, testes and epididymis following exposure to fluoride indicates enzyme inhibition and consequently increasing the steady-state level of oxidants (superoxide radicals and H_2O_2) and hydroxyl radical generation via Haber–Weiss reaction in the brain, testes and epididymis. Glutathione is a reducing agent which plays an essential role in the maintenance of intracellular redox status, antioxidant enzymes functions and xenobiotic detoxification in the aqueous phase of cellular systems (Rana et al. 2002). The marked decrease in the level of GSH and activities of GSH-dependent enzymes namely GPx and GST in the brain, testes and epididymis of the fluoride-treated rats indicates enzyme activity inhibition and depletion of GSH which could lead to oxidative damage. The remarkable reversal of the diminution in these antioxidants in rats co-treated with taurine supports previous study that taurine alleviates oxidative damage by enhancing antioxidant enzymes activities (Das et al. 2008).

Furthermore, the present investigation showed that fluoride administration elevated the levels of inflammatory biomarkers in the brain, testes and epididymis of the treated rats. TNF- α is a pro-inflammatory mediator which initiates and regulates cytokine cascade during an inflammatory response (Chen et al. 2003). Elevated TNF- α level reportedly up-regulates inducible nitric oxide synthase (iNOS) to increase NO production (Nussler et al. 1994) which subsequently elicits detrimental effects by reacting with superoxide anion to generate reactive nitrogen species (RNS) (Cavicchi et al. 2000). Persistent inflammation in the brain, testes and epididymis of the fluoride-treated rats could activate MPO activity, an enzyme which utilizes hydrogen peroxides to produce hypochlorite and ROS and consequently exacerbates oxidative injuries in the tissues. Interestingly, taurine suppressed the levels of TNF- α and NO as well as MPO activity in brain, testes and epididymis of the treated rats. Thus, indicating an anti-inflammatory mechanism of taurine in the prevention of fluoride-induced toxicity in rats.

The gonadotropins (i.e. FSH and LH) regulation of testosterone secretion by Leydig cells is required for the growth, maintenance of structural morphology of the seminiferous tubule and spermatogenesis (Sharpe 1994). The suppression of circulatory testosterone level without altering FSH and LH levels by fluoride in the treated rats represents another plausible mechanism of fluoride toxicity. Although increased oxidative stress has been shown to cause malfunctioning of hypothalamus which subsequently reduced gonadotropins production by the pituitary (Muthuvel et al. 2006), the lack of effect of NaF exposure on gonadotrophins despite the obvious oxidative damage in this study signifies that NaF-induced oxidative damage was not sufficient to impair hypothalamus function. However, the reduction in the testosterone level is correlated with the testicular spermatogenic dysfunction. Epididymal sperm count is considered as one of the most sensitive tests for evaluating spermatogenesis because it provides the outcome

of all the stages of meiosis, spermiogenesis, and transition in the epididymis (Chandra et al. 2007). The decrease in the sperm count and progressive motility with increased sperm abnormality observed in fluoride-treated rats in this study are attributable to induction of oxidative stress and reduced bioavailability of testosterone in the treated rats. However, the restoration of the hormone homeostasis and spermiogram following taurine co-administration demonstrated ameliorative role of taurine in fluoride-induced sperm toxicity and endocrine suppression in rats.

Some testicular enzymes are fundamental in keeping germ cell growing normally and their activities are tightly associated with spermatogenesis and are thus considered as key endpoints in assessing testicular toxicity (Hodgen and Sherins 1973). The present study evaluated the toxic effects of fluoride on testes biochemical metabolism by determining the testicular activities of ACP, ALP and LDH. In testis, ACP is mostly associated with the denaturation of seminiferous epithelium and phagocytosis of Sertoli cells whereas ALP is associated with transportation of nutrients to spermatogenic cells for proliferation and differentiation. The decrease in the testicular activities of ACP and ALP following fluoride exposure indicates impairment in testicular nutrients transportation, energy metabolism and spermatogenic cells division. Moreover, LDH which is usually distributed in the seminiferous tubules and germ cells is associated with maturation of spermatogenic cells and energy metabolism of sperm. The decrease in testicular LDH activity of fluoride-treated rats indicates its interference with maturation and energy metabolism of sperm and spermatogenic cells. Conversely, rats co-administered with taurine exhibited a significant increase in marker enzymes of testicular function in comparison to fluoride-treated rats, thus indicating chemoprotective effects of taurine against fluoride-induced gonadal toxicity in rats.

Caspase-3 which belongs to a family of aspartate-specific cysteine proteases is the key executioner caspase regulating the apoptosis cascade (Zhuang et al. 2000). The significant increase in caspase-3 activity in brain, testes and epididymis of fluoride-treated rats indicates induction of apoptotic cell death. The apparent decrease in the caspase-3 activity following co-administration of taurine is indicative of taurine effectiveness in suppressing caspase-3 activity in the treated rats. Histologically, the degenerative conditions observed in the brain, testes and epididymis of fluoride-treated rats corroborate with the observed biochemical results. The histo-architectures of the brain, testes and epididymis of the fluoride-treated rats showed marked lesions characterized by focal area of vacuolation, sateliosis and congestion of the microcirculation of the brain, disorganization of spermatogenic cells and shedding of sperm cells within the lumen of the seminiferous tubules as well as hyperplasia of epithelial cells lining the duct of epididymis with lumen containing inadequate sperm cells. The ability of taurine to maintain the morphology of these tissues somewhat similar to the control signifies chemoprotection which is related to its antioxidant and anti-apoptosis activities.

Based on the data from this study, it is concluded that taurine confers reproductive health benefits by multiple targets in fluoride-treated rats. The protective effects exerted by taurine were not dose-dependent. Taurine co-administration efficiently ameliorated fluoride mediated damages by suppressing pro-inflammatory mediators, augmenting the antioxidant status, testosterone level and sperm characteristics with improvement in the histo-architecture of the brain, testes and epididymis in fluoride-treated rats. Therefore, taurine may be a possible supplement in enhancing the reproductive health of men who are exposed to fluoride.

Conflict of interest

The authors have no conflicts of interest to declare.

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Legend to figures

Figure 1: Effects of taurine on H₂O₂ and LPO levels in brain, testes and epididymis of NaF-exposed rats. NaF (15 mg/L in drinking water); TAU, Taurine; TAU 1, 100 mg/kg taurine; TAU 2, 200 mg/kg taurine. Each bar represents mean $\pm$ SD of 12 rats per group. a: Values differ significantly from control ($p < 0.05$). b: Values differ significantly from NaF alone.

Figure 2: Effects of taurine on SOD and CAT activities in brain, testes and epididymis of NaF-exposed rats. NaF (15 mg/L in drinking water); TAU, Taurine; TAU 1, 100 mg/kg taurine; TAU 2, 200 mg/kg taurine. Each bar represents mean $\pm$ SD of 12 rats per group. a: Values differ significantly from control ($p < 0.05$). b: Values differ significantly from NaF alone.

Figure 3: Effects of taurine on GST and GPx activities in brain, testes and epididymis of NaF-exposed rats. NaF (15 mg/L in drinking water); TAU, Taurine; TAU 1, 100 mg/kg taurine; TAU 2, 200 mg/kg taurine. Each bar represents mean $\pm$ SD of 12 rats per group. a: Values differ significantly from control ($p < 0.05$). b: Values differ significantly from NaF alone.

Figure 4: Effects of taurine on GSH level and MPO activity in brain, testes and epididymis of NaF-exposed rats. NaF (15 mg/L in drinking water); TAU, Taurine; TAU 1, 100 mg/kg taurine; TAU 2, 200 mg/kg taurine. Each bar represents mean $\pm$ SD of 12 rats per group. a: Values differ significantly from control ($p < 0.05$). b: Values differ significantly from NaF alone.

Figure 5: Effects of taurine on NO and TNF- α levels in brain, testes and epididymis of NaF-exposed rats. NaF (15 mg/L in drinking water); TAU, Taurine; TAU 1, 100 mg/kg taurine; TAU 2, 200 mg/kg taurine. Each bar represents mean $\pm$ SD of 12 rats per group. a: Values differ significantly from control ($p < 0.05$). b: Values differ significantly from NaF alone.

Figure 6: Effects of taurine on circulatory concentrations of reproductive hormones and marker enzymes of testicular function in NaF-exposed rats. NaF (15 mg/L in drinking water); TAU, Taurine; TAU 1, 100 mg/kg taurine; TAU 2, 200 mg/kg taurine. Each bar represents mean $\pm$ SD of 12 rats per group. a: Values differ significantly from control ($p < 0.05$). b: Values differ significantly from NaF alone.

Figure 7: Effects of taurine on sperm functional characteristics in NaF-exposed rats. NaF (15 mg/L in drinking water); TAU, Taurine; TAU 1, 100 mg/kg taurine; TAU 2, 200 mg/kg taurine. Each bar represents mean $\pm$ SD of 12 rats per group. a: Values differ significantly from control ($p < 0.05$). b: Values differ significantly from NaF alone.

Figure 8: Effects of taurine on caspase-3 activity in brain, testes and epididymis of NaF-exposed rats. NaF (15 mg/L in drinking water); TAU, Taurine; TAU 1, 100 mg/kg taurine; TAU 2, 200 mg/kg taurine. Each bar represents mean $\pm$ SD of 12 rats per group. a: Values differ significantly from control ($p < 0.05$). b: Values differ significantly from NaF alone.

Figure 9: Representative photomicrographs of the brain (B), testes (T) and cauda epididymis (E). The control group (B1, T1 and E1) showing normal architecture of brain, testes and epididymis, respectively. NaF-treated rats (B2, T2 and E2) showing histopathological alterations specifically focal area of vacuolation (red arrow), satelliosis (yellow notched arrow) and congestion of the microcirculation (black notched arrow) in brain; disorganization of spermatogenic cells (blue arrow) and shedding of sperm cells within the lumen (black arrow) of the seminiferous tubules with hyperplasia of epithelial cells lining the duct of epididymis (black arrow) and lumen containing scanty sperm cells (red notched arrow). The investigated organs from rats administered with taurine alone (B3, T3 and E3) and those co-treated with taurine at

100 mg/kg (B4, T4 and E4) and 200 mg/kg appeared structurally and functionally normal (B5, T5 and E5).

Table 1: Influence of taurine on body weight gain and organo-somatic index (OSI) of the brain, testes and epididymis in fluoride-exposed rats.

	Body weight gain (g)	OSI of the brain	OSI of the testes	OSI of the epididymis
Control	84.58 ± 6.26	0.86 ± 0.03	1.13 ± 0.08	0.11 ± 0.02
NaF alone	83.24 ± 5.14	0.85 ± 0.07	1.14 ± 0.02	0.10 ± 0.01
TAU alone	79.15 ± 5.33	0.86 ± 0.05	1.21 ± 0.01	0.12 ± 0.02
NaF + TAU 1	72.37 ± 8.51	0.87 ± 0.07	1.19 ± 0.01	0.13 ± 0.04
NaF + TAU 2	75.18 ± 6.58	0.84 ± 0.06	1.23 ± 0.02	0.12 ± 0.02

NaF (15 mg/L in drinking water); TAU, Taurine; TAU 1, 100 mg/kg taurine; TAU 2, 200 mg/kg taurine. Values are mean ± SD of 12 rats per group.

Table 2: Influence of taurine on histological lesions score in testes and epididymis of rats treated with NaF for 45 consecutive days

	Histological Lesions Score	
	Testes	Epididymis
Control	0.16 ± 0.02	0.13 ± 0.01
NaF alone	0.68 ± 0.01 ^a	0.45 ± 0.02 ^a
TAU alone	0.15 ± 0.01	0.11 ± 0.01
NaF + TAU 1	0.27 ± 0.04 ^b	0.24 ± 0.01 ^b
NaF + TAU 2	0.21 ± 0.03 ^b	0.18 ± 0.02 ^b

NaF (15 mg/L in drinking water); TAU, Taurine; TAU 1, 100 mg/kg taurine; TAU 2, 200 mg/kg taurine. Each bar represents mean ± SD of 12 rats per group. a: Values differ significantly from control (p < 0.05). b: Values differ significantly from NaF alone.

Brain H_2O_2 level

Testes H_2O_2 level

Epididymis H_2O_2 Level

Control
NaF alone
TAU alone
NaF + TAU 1
NaF + TAU 2

Brain MDA Level

Testes MDA Level

Epididymis MDA Level

Control
NaF alone
TAU alone
NaF + TAU 1
NaF + TAU 2

Figure 2

Figure 3

Figure 4