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ARTICLE

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^{-1} alone or orally co-administered by gavage with taurine at 100 and 200 mg·(kg body mass)$^{-1}$ 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 of sperm functional characteristics in the treated rats. Taurine may be a possible chemopreventive candidate against reproductive dysfunction resulting from fluoride exposure.

Key words: sodium fluoride, taurine, reproductive toxicity, antioxidant, anti-inflammatory.

Résumé : L'exposition excessive au fluor est associée à un dysfonctionnement de la fonction reproductive masculine chez l'humain et les animaux. La taurine (acide 2-aminoéthane sulfonique) est un acide aminé β libre disposant de propriétés anti-oxydantes, anti-inflammatoires et neuroprotectrices. Cependant, l'effet de la taurine sur la toxicité reproductive en réaction au fluor n'a pas été rapporté dans la littérature. Dans les présents travaux, nous avons étudié l'influence de la taurine sur les variations fonctionnelles au long de l'axe hypophyso-gonadique cérébral que provoque le fluorure de sodium (NaF) chez des rats de sexe masculin. Pendant 45 jours consécutifs, nous avons administré du NaF à 15 mg·L^{-1} d'eau à boire seul ou en association avec l'administration par gavage de taurine à 100 et à 200 mg·(kg de poids corporel)$^{-1}$. Les résultats ont montré que la taurine pouvait nettement prévenir les augmentations des indices de stress oxydatif provoqués par le NaF, ainsi que l'augmentation de l'activité des enzymes antioxydants et des taux de glutathion dans le cerveau, les testicules et l'épididyme des rats traités. De plus, la taurine permettait d'inverser la hausse des biomarqueurs de l'inflammation et de l'activité de la caspase-3 ainsi que l'augmentation des lésions histologiques dans le cerveau, les testicules et l'épididyme des rats traités. Le NaF permettait d'inverser de façon marquée la diminution des taux testiculaires de testostérone et de l'activité de la phosphatase acide, de la phosphatase alcaline et de la déshydrogénase lactique testiculaires, ce qui était accompagné d'une amélioration des caractéristiques fonctionnelles des spermatozoïdes chez les rats traités. En somme, la taurine constitue un candidat biochimique possible contre le dysfonctionnement reproducteur provoqué par l'exposition au fluor. [Traduit par la Rédaction]

Mots-clés : fluorure de sodium, taurine, toxicité reproductive, anti-oxydant, anti-inflammatoire.

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Introduction

Fluorides of many metals and nonmetals are used industrially in drinking water treatment, aluminum production, and dental preparations (Lu et al. 2000). The permissible limit of fluoride in drinking water ranges from 0.7 to 1.0 mg·L^{-1} (World Health Organization 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^{-1} (World Health Organization 2006). Fluoride has been detected at 96.8 mg·L^{-1} in industrial wastewater (Ding et al. 1998) and up to 3000–5000 mg·L^{-1} in extreme cases (Wu et al. 2006).

The scientific understanding of the association between environmental exposure to fluoride and declining human fertility rates is growing. Epidemiological studies have indicated that environmental exposure to fluoride is associated with male infertility and low birth rates in endemic areas of fluorosis (Long et al. 2009; Ortiz-Pérez et al. 2003). Furthermore, numerous clinical investigations and animal studies have revealed that fluoride is a testicular toxicant that disrupts reproductive hormone levels, induces structural and functional defects in sperm, and consequently reduces fertility (Long et al. 2009; Lu et al. 2014; Sun et al. 2014; Dong et al. 2016). Indeed, the three important processes that

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spermatogonia undergo to finally fertilize an oocyte — namely spermatogenesis, capacitation, and the acrosome reaction — 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 known 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 exogenously it can be 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 have been available to date, 3 $g \cdot day^{-1}$ 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. 2017). However, there is a dearth of scientific reports 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 the brain–pituitary–gonadal axis in the rat. This was achieved by measuring plasma hormone levels, sperm functional characteristics, antioxidant status, and marker enzymes of testicular function along with performing histological analyses of the brain, testes, and epididymis of the experimental animals.

Materials and methods

Chemicals

Taurine, sodium fluoride (NaF), thiobarbituric acid, trichloroacetic acid, epinephrine, glutathione, hydrogen peroxide (H_2O_2), 5′,5′-dithio-bis-2-nitrobenzoic acid (DNTB), and 1-chloro-2,4-dinitrobenzene (CDNB) were purchased from Sigma Chemical Co. (St. Louis, Missouri, USA). The remaining reagents were of analytical grade and were procured from British Drug Houses Ltd. (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 light : 12 h dark cycle, fed rat chow, and given drinking water ad libitum in their home cages for a week before the experiments commenced. 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 Sciences (NAS) and published by the National Institutes of Health.

Experimental protocol

The rats were randomly assigned to 5 groups of 12 rats each and were treated for 45 consecutive days as follows: group I rats received drinking water alone and served as control; group II rats were exposed to NaF in drinking water at 15 $mg \cdot L^{-1}$ alone; group III rats were orally administered taurine at a dose of 200 $mg \cdot kg^{-1}$ using drinking water as vehicle; group IV rats were co-administered NaF and taurine at 100 $mg \cdot kg^{-1}$ (TAU 1); group V rats were co-administered NaF and taurine at 200 $mg \cdot kg^{-1}$ (TAU 2).

Taurine supplementation at dosages ranging from 500 to 2000 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 previously published data (Chattopadhyay et al. 2011; Adedara et al. 2017). Twenty-four hours after the last exposure, the final body masses of the rats were measured, and blood was collected from the retro-orbital venous plexus using heparin-containing tubes before rats were sacrificed by cervical dislocation. Plasma samples were separated from blood cells by centrifugation at 3000g for 10 min. The plasma samples were subsequently kept frozen at −20 °C until the determination of hormone concentrations using an enzyme-linked immunosorbent assay (ELISA) strip reader (Robonik India Private Ltd., Mumbai, India). The brain, testes, and epididymis 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 × organ mass (g)/body mass (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 luteinizing hormone (LH) (RPN 2562, Amersham, UK), follicle-stimulating hormone (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 5α-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 releasing sperm onto a sterile, clean glass slide. Subsequently, the sperm was diluted with 2.9% sodium citrate dehydrate solution that had been prewarmed to 37 °C, mixed carefully, and covered with a coverslip (24 mm × 24 mm). Sperm motility was evaluated by examining a minimum of 10 microscopic fields under a phase contrast microscope at 200× magnification. Sperm motility was obtained by scoring the number of all progressive sperm, followed by the nonprogressive and then 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 methodology (World Health Organization 1999). Briefly, the sperm obtained by mincing the cauda epididymis in normal saline was filtered using a nylon mesh. Subsequently, an aliquot of 5 μL of the sperm was mixed with 95 μL of diluent (0.35% formalin containing 5% $NaHCO_3$ and 0.25% trypan blue). After 10 μL of the diluted sperm was placed on the hemocytometer, the sperm were allowed to sediment by standing for 5 min in a humid chamber before they were counted using the improved Neubauer chamber (1/10 m depth; LABART, Munich, Germany) with a light microscope at 400×.

Evaluation of sperm morphological abnormalities and viability assay

Sperm morphological abnormality was determined by smearing a portion of the sperm suspension that had been placed on a

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glass slide with another slide followed by staining with a reagent containing 0.2 g eosin and 0.6 g fast green FCF dissolved in distilled water and ethanol in a ratio of 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 mmol·L^{-1} Tris–HCl buffer (pH 7.4) containing 1.15% potassium chloride. Subsequently, the homogenates were centrifuged at 12 000g for 15 min at 4 °C, and the resulting supernatants were used for the biochemical estimations. Protein concentration was assayed using the method of Bradford (1976). H_2O_2 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 to the method of Misra and Fridovich (1972). Catalase (CAT) activity was determined using H_2O_2 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) that are based on the hydrolysis of *p*-nitrophenyl phosphate in acid and alkaline medium, respectively. Lactate dehydrogenase-X (LDH-X) activity was determined according to an established method that is based on the inter-conversion of lactate and pyruvate (Vassault 1983).

Evaluation of pro-inflammatory biomarker concentrations

Myeloperoxidase (MPO) activity was determined according to the method described by Granell et al. (2003) (expressed as μmol H_2O_2·L^{-1}·min^{-1}·(mg protein)$^{-1}$). 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 a standard and expressed as μmol nitrites·L^{-1}·(mg protein)$^{-1}$ according to Green et al. (1982). The concentrations of tumor necrosis factor (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 a 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 a 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 using a microtome to produce 4–5 μm sections, fixed on the slides, and stained with hematoxylin and eosin (H&E). All slides were coded prior to examination with a light microscope (Olympus CH; Olympus, Tokyo, Japan) and photographed by pathologists using a Sony DSC-W 30 Cyber-shot (Sony, Tokyo, Japan).

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

		OSI		
	Body mass gain (g)	Brain	Testes	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

Note: NaF (15 mg·L^{-1} in drinking water); TAU, taurine; TAU 1, 100 mg·kg^{-1} taurine; TAU 2, 200 mg·kg^{-1} taurine. Values are means ± SD of 12 rats per group.

Histological analysis of the testes and cauda epididymis was performed according to standardized procedure (Hess 1990) with slight modifications. Semiquantitative evaluation of histological alterations in the testes included 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 as follows: 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 to compare the experimental groups followed by a Dunnett test for post hoc evaluation using GraphPad Prism 5 software (Version 4; GraphPad Software, La Jolla, California, USA). Results with $p < 0.05$ were considered significant.

Results

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

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

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

Oxidative stress indices in the brain, testes, and epididymis of control rats and those treated with NaF alone or in combination with taurine are depicted in Figs. 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 a 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 the brain, testes, and epididymis of the treated rats 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

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Fig. 1. Effects of taurine on hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) levels in the brain, testes, and epididymis of sodium fluoride (NaF)-exposed rats. NaF (15 mg·L^{-1} in drinking water); TAU, taurine; TAU 1, 100 mg·kg^{-1} taurine; TAU 2, 200 mg·kg^{-1} taurine. Each bar represents the mean ± standard deviation of 12 rats per group. Different lowercase letters represent statistically significant differences between groups (a: values differ significantly from control ($p < 0.05$); b: values differ significantly from NaF alone ($p < 0.05$)). [Colour online.]

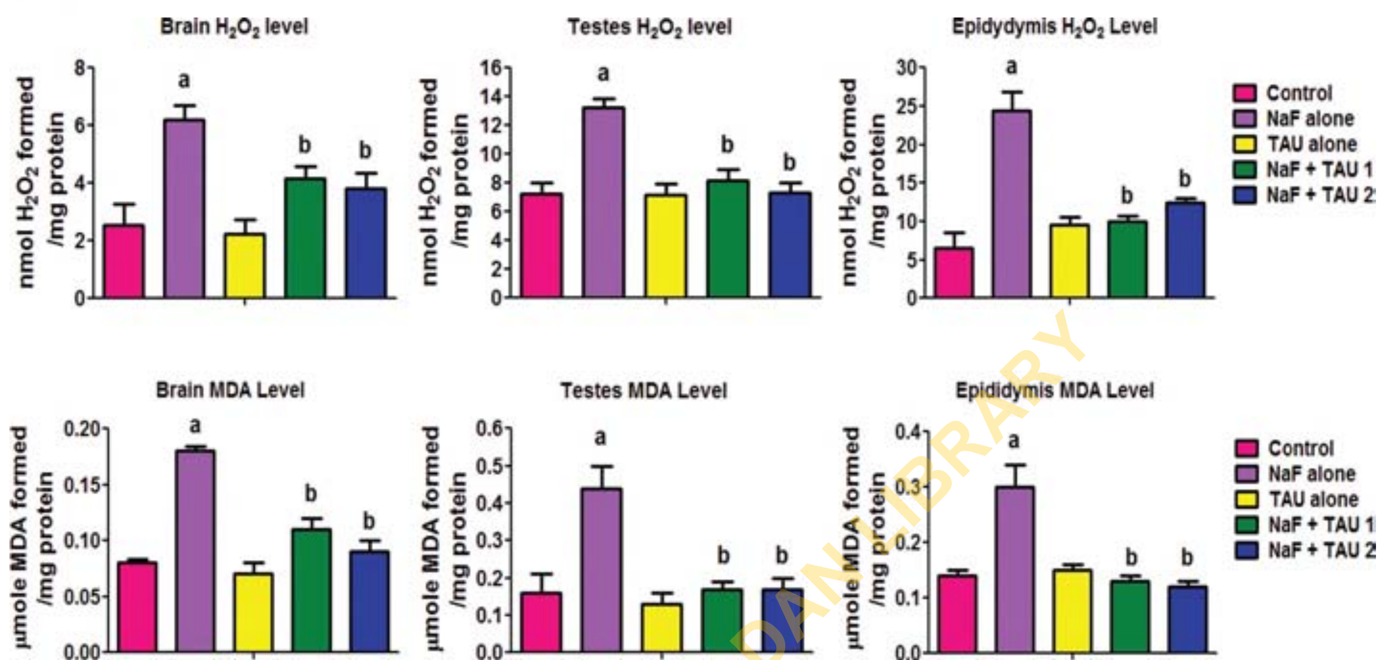

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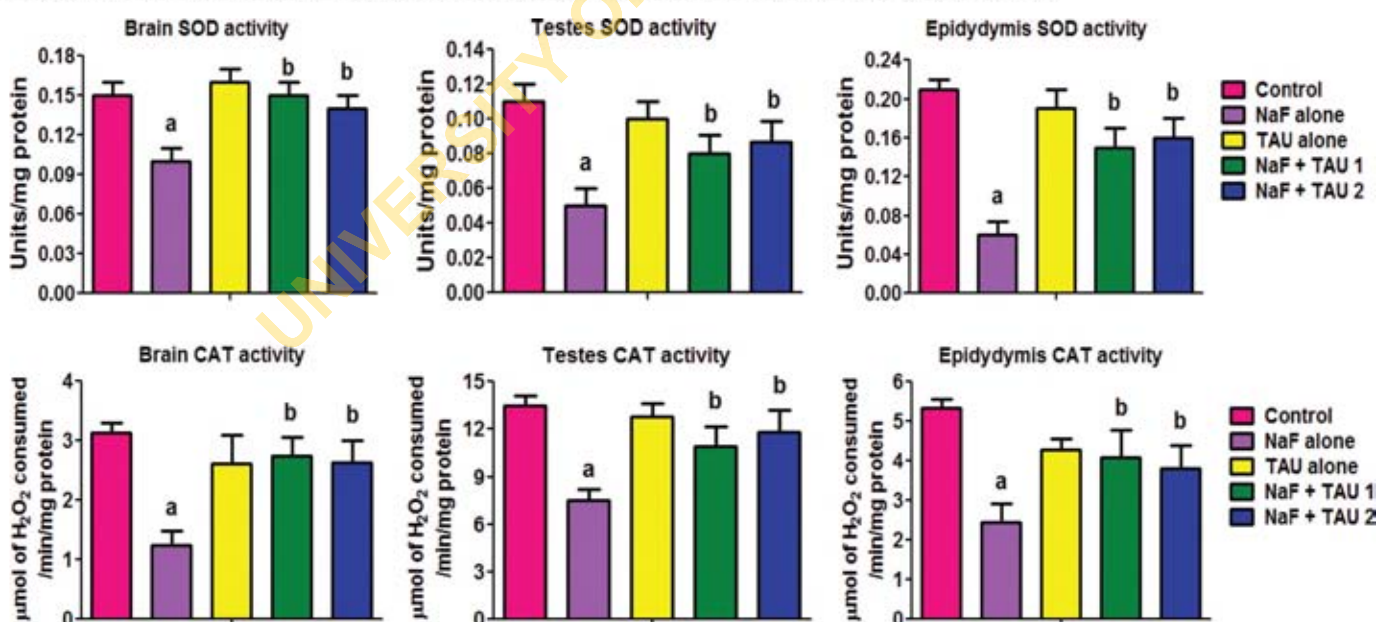
Fig. 2. Effects of taurine on superoxide dismutase (SOD) and catalase (CAT) activities in the brain, testes, and epididymis of sodium fluoride (NaF)-exposed rats. NaF (15 mg·L^{-1} in drinking water); TAU, taurine; TAU 1, 100 mg·kg^{-1} taurine; TAU 2, 200 mg·kg^{-1} taurine. Each bar represents the mean ± standard deviation of 12 rats per group. Different lowercase letters represent statistically significant differences between groups (a: values differ significantly from control ($p < 0.05$); b: values differ significantly from NaF alone ($p < 0.05$)). [Colour online.]



brain, testes, and epididymis to normalcy in fluoride-treated rats compared with the control.

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

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

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Fig. 3. Effects of taurine on glutathione-S-transferase (GST) and glutathione peroxidase (GPx) activities in the brain, testes, and epididymis of sodium fluoride (NaF)-exposed rats. NaF (15 mg·L⁻¹ in drinking water); TAU, taurine; TAU 1, 100 mg·kg⁻¹ taurine; TAU 2, 200 mg·kg taurine⁻¹. Each bar represents the mean ± standard deviation of 12 rats per group. Different lowercase letters represent statistically significant differences between groups (a: values differ significantly from control ($p < 0.05$); b: values differ significantly from NaF alone ($p < 0.05$)). [Colour online.]

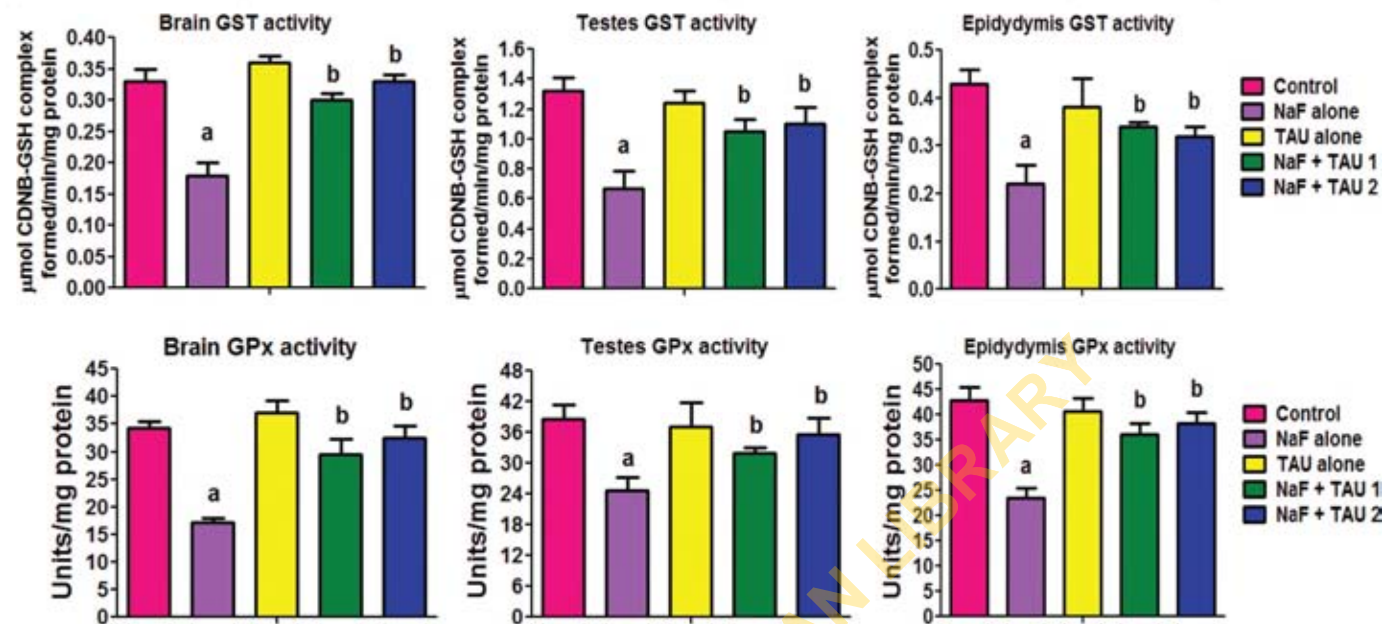

Fig. 4. Effects of taurine on glutathione (GSH) level and myeloperoxidase (MPO) activity in the brain, testes, and epididymis of sodium fluoride (NaF)-exposed rats. NaF (15 mg·L⁻¹ in drinking water); TAU, taurine; TAU 1, 100 mg·kg⁻¹ taurine; TAU 2, 200 mg·kg⁻¹ taurine. Each bar represents the mean ± standard deviation of 12 rats per group. Different lowercase letters represent statistically significant differences between groups (a: values differ significantly from control ($p < 0.05$); b: values differ significantly from NaF alone ($p < 0.05$)). [Colour online.]

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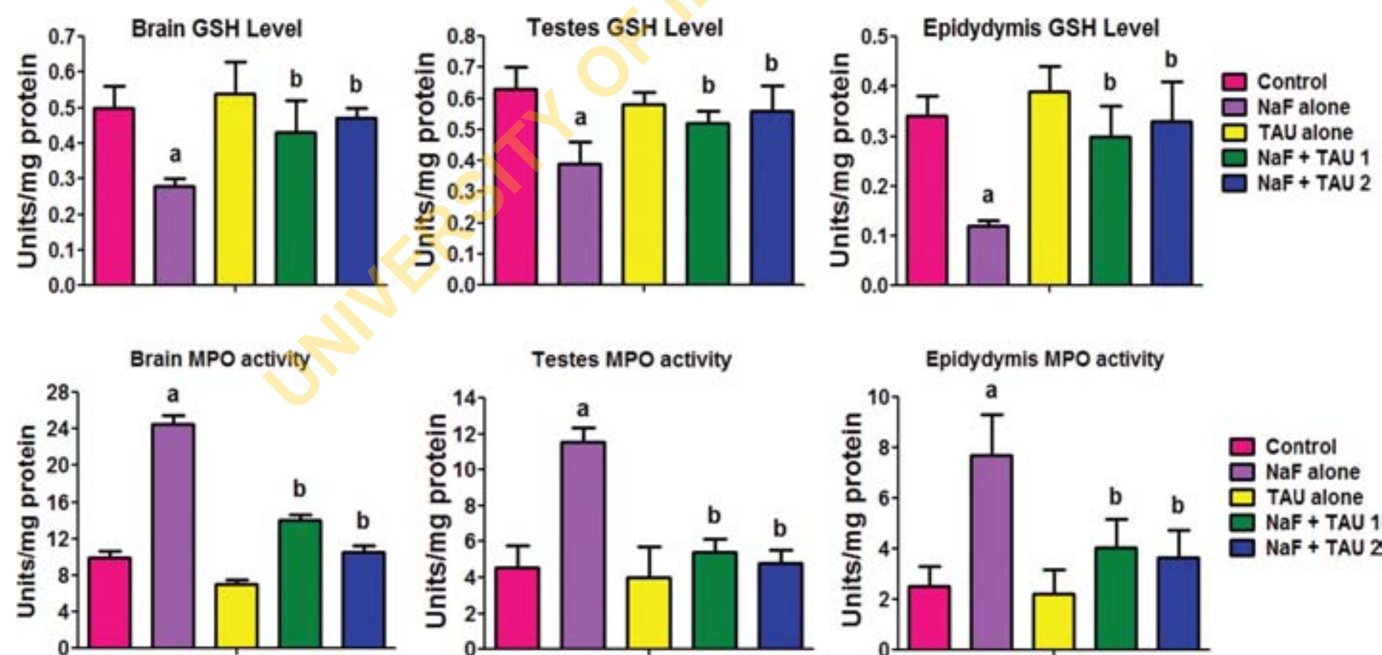

Taurine prevents fluoride-induced suppression of testosterone concentration and marker enzymes of testicular function in rats

The influence of taurine co-administration on circulatory hormone concentrations and marker enzymes of testicular function in fluoride-treated rats is depicted in Fig. 6. There were no treatment-related effects on LH, FSH, and testosterone levels in rats exposed to taurine alone 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-X, were significantly decreased in fluoride-treated rats compared with control. However, co-administration of taurine significantly ameliorated fluoride-induced testicular toxicity by restoring the activities of ACP, ALP, and LDH-X toward normalcy in the treated rats.

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Fig. 5. Effects of taurine on nitric oxide (NO) and tumor necrosis factor (TNF)-α levels in the brain, testes, and epididymis of sodium fluoride (NaF)-exposed rats. NaF (15 mg·L^{-1} in drinking water); TAU, taurine; TAU 1, 100 mg·kg^{-1} taurine; TAU 2, 200 mg·kg^{-1} taurine. Each bar represents the mean ± standard deviation of 12 rats per group. Different lowercase letters represent statistically significant differences between groups (a: values differ significantly from control (p < 0.05); b: values differ significantly from NaF alone (p < 0.05)). [Colour online.]

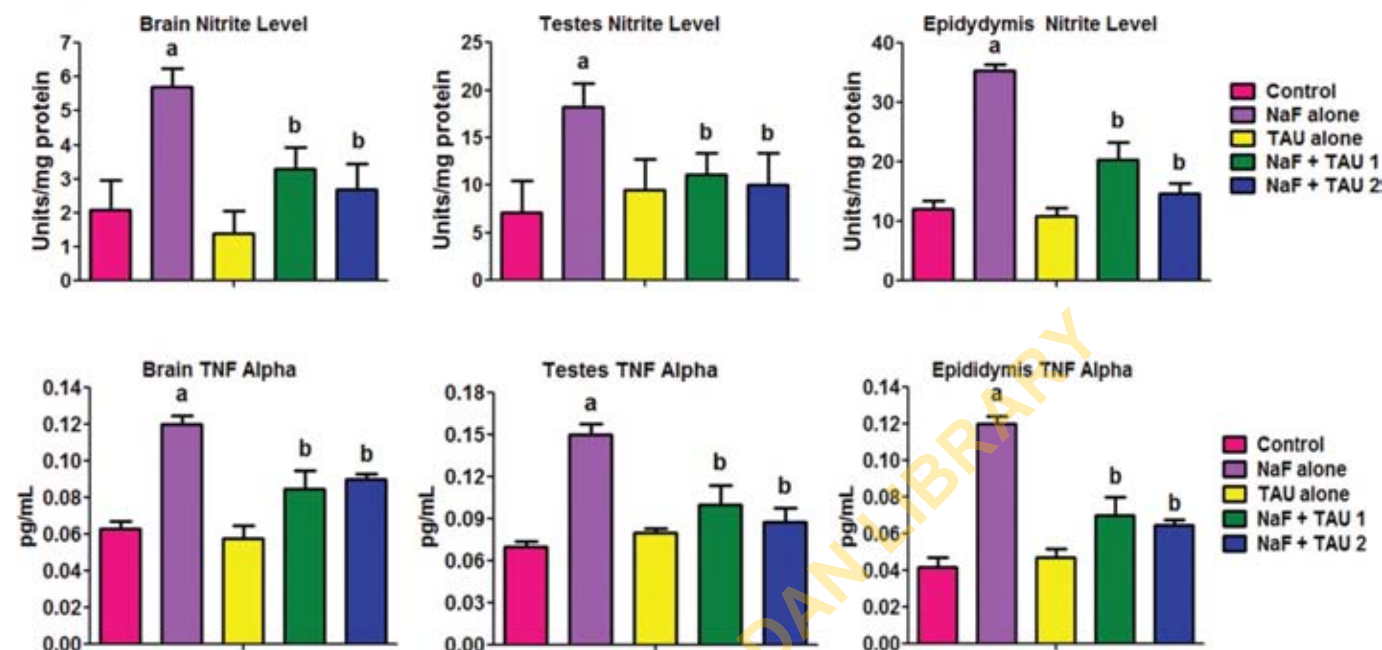

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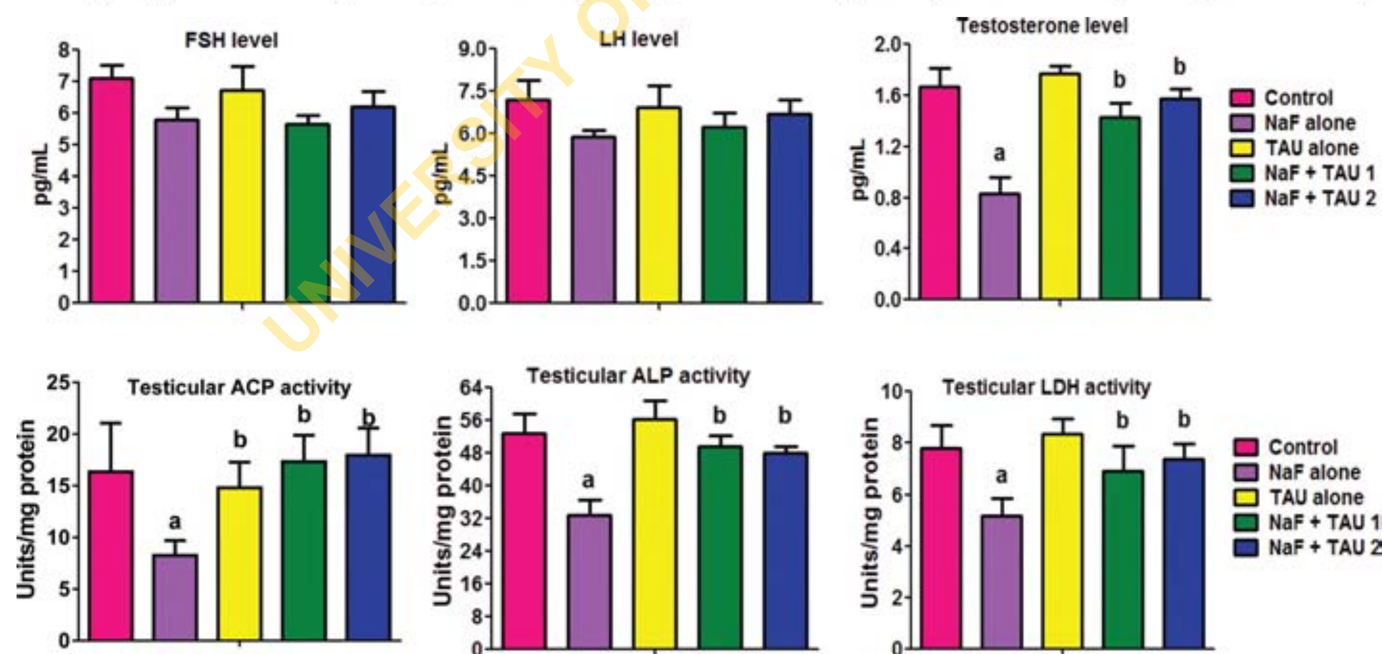
Fig. 6. Effects of taurine on circulatory concentrations of reproductive hormones and marker enzymes of testicular function in sodium fluoride (NaF)-exposed rats. NaF (15 mg·L^{-1} in drinking water); TAU, taurine; TAU 1, 100 mg·kg^{-1} taurine; TAU 2, 200 mg·kg^{-1} taurine. Each bar represents the mean ± standard deviation of 12 rats per group. Different lowercase letters represent statistically significant differences between groups (a: values differ significantly from control (p < 0.05); b: values differ significantly from NaF alone (p < 0.05)). [Colour online.]



Taurine improves sperm functional characteristics in fluoride-treated rats

The influence of taurine co-administration on sperm functional characteristics in fluoride-treated rats is depicted in Fig. 7. There were no treatment-related effects on sperm viability in any of the treatment groups. The percentage of sperm progressive motility and sperm count were significantly decreased in the fluoride-treated rats, whereas abnormal sperm with morphological defects were significantly increased. 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 compared with the group exposed to fluoride alone.

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Fig. 7. Effects of taurine on sperm functional characteristics in sodium fluoride (NaF)-exposed rats. NaF (15 mg·L⁻¹ in drinking water); TAU, taurine; TAU 1, 100 mg·kg⁻¹ taurine; TAU 2, 200 mg·kg⁻¹ taurine. Each bar represents the mean ± standard deviation of 12 rats per group. Different lowercase letters represent statistically significant differences between groups (a: values differ significantly from control ($p < 0.05$); b: values differ significantly from NaF alone ($p < 0.05$)). [Colour online.]

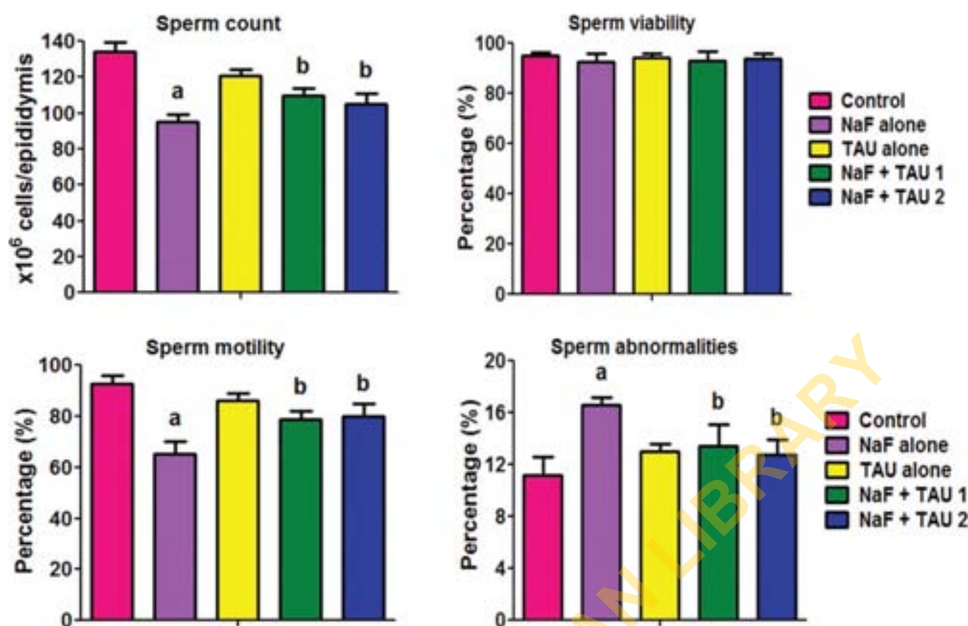

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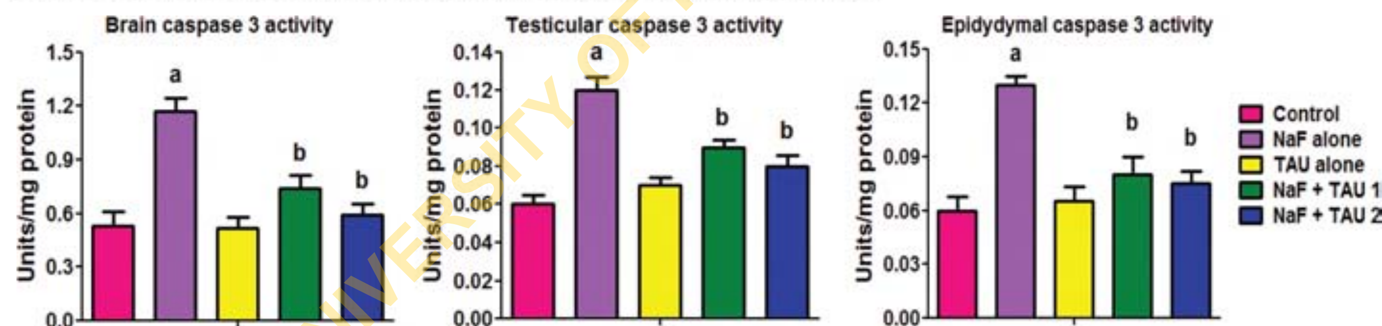
Fig. 8. Effects of taurine on caspase-3 activity in the brain, testes, and epididymis of sodium fluoride (NaF)-exposed rats. NaF (15 mg·L⁻¹ in drinking water); TAU, taurine; TAU 1, 100 mg·kg⁻¹ taurine; TAU 2, 200 mg·kg⁻¹ taurine. Each bar represents the mean ± standard deviation of 12 rats per group. Different lowercase letters represent statistically significant differences between groups (a: values differ significantly from control ($p < 0.05$); b: values differ significantly from NaF alone ($p < 0.05$)). [Colour online.]



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

The influence of taurine administration on caspase-3 activity in the brain, testes, and epididymis of fluoride-treated rats is depicted in Fig. 8. There were no treatment-related effects on caspase-3 activity in rats exposed to taurine alone compared with the control. Administration of fluoride alone significantly increased caspase-3 activity in the brain, testes, and epididymis of fluoride-treated rats compared with the 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 the brain, testes, and epididymis of fluoride-treated rats

Representative photomicrographs of brain, testes, and cauda epididymis from the control and treatment groups are shown in Fig. 9. Microscopic examination revealed normal architecture of the brain, testes, and epididymis in control rats (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), satellitosis (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 the 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⁻¹ (B4, T4, and E4) and 200 mg·kg⁻¹ (B5, T5, and E5) appeared structurally and functionally normal.

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

Fig. 9. Representative photomicrographs of the brain (B), testes (T), and cauda epididymis (E) of control and sodium fluoride (NaF)-exposed rats. The control group (B1, T1, and E1) showing normal architecture of the brain, testes, and epididymis, respectively. NaF-treated rats (B2, T2, and E2) showing histopathological alterations, specifically focal area of vacuolation (red arrow), satellitosis (yellow notched arrow), and congestion of the microcirculation (black notched arrow) in the 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 the epididymis (black arrow) and lumen containing scanty sperm cells (red notched arrow). The investigated organs from rats administered taurine alone (B3, T3, and E3) and those co-treated with taurine at 100 mg·kg^{-1} (B4, T4, and E4) and 200 mg·kg^{-1} appeared structurally and functionally normal (B5, T5, and E5). [Colour online.]

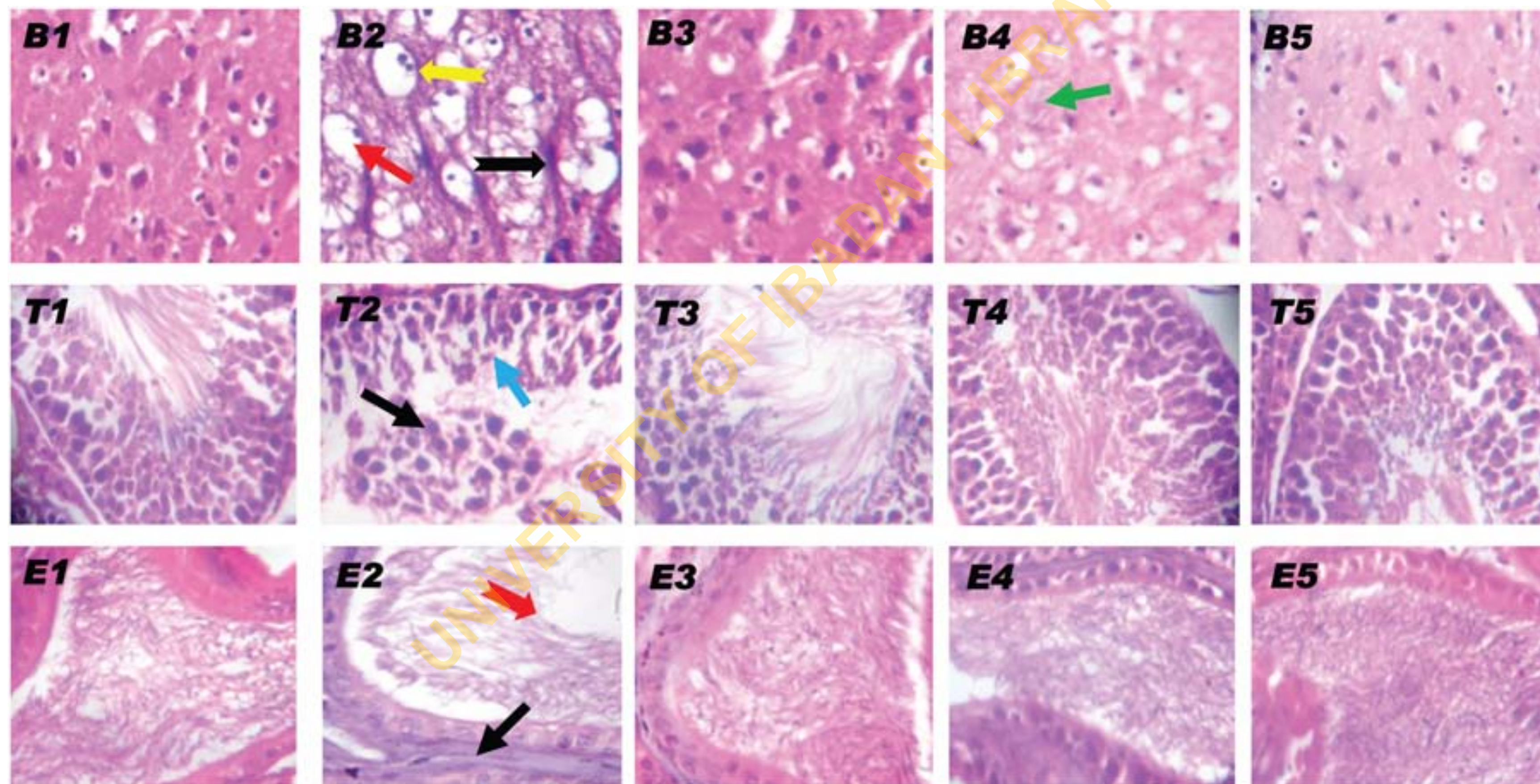


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Table 2. Influence of taurine on histological lesion score in testes and epididymis of rats treated with sodium fluoride (NaF) for 45 consecutive days.

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

Note: NaF (15 mg·L^{-1} in drinking water); TAU, Taurine; TAU 1, 100 mg·kg^{-1} taurine; TAU 2, 200 mg·kg^{-1} taurine. Each bar represents the mean ± SD of 12 rats per group. Different lowercase letters indicate a statistically significant difference between groups (a: values differ significantly from control ($p < 0.05$); b: values differ significantly from treatment with NaF alone ($p < 0.05$)).

Discussion

Fluoride is a dangerous environmental and industrial contaminant that has generated widespread public health concerns owing to its association with several human diseases, including adverse reproductive outcomes (Freni 1994; Susheela and Jethanandani 1996; Ortiz-Pérez et al. 2003). The present study showed that while the body mass 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 as well as 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 reactive oxygen species production results in peroxidative breakdown of the phospholipid 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 an increase in the steady-state level of oxidants (superoxide radicals and H_2O_2) and hydroxyl radical generation via the Haber–Weiss reaction in the brain, testes, and epididymis. GSH is a reducing agent that 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 a previous study showing 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 that initiates and regulates the cytokine cascade during an inflammatory response (Chen et al. 2003). Elevated TNF-α levels reportedly upregulate 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 (Cavicchi et al. 2000). Persistent inflammation in the brain, testes, and epididymis of the fluoride-treated rats could activate MPO activity, an enzyme that utilizes H_2O_2 to produce hypochlorite and reactive oxygen species and consequently exacerbates oxidative injuries in the tissues. Interestingly, taurine suppressed the levels of TNF-α and NO as well as MPO activity in the 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.

Gonadotropin (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 the circulatory testosterone level without an alteration in 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 the hypothalamus, which subsequently reduces gonadotropin 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 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 demonstrates the ameliorative role of taurine in fluoride-induced sperm toxicity and endocrine suppression in rats.

Some testicular enzymes are fundamental in maintaining normal germ cell growth, and their activities are tightly associated with spermatogenesis and are thus considered key endpoints in assessing testicular toxicity (Hodgen and Sherins 1973). The present study evaluated the toxic effects of fluoride on the biochemical metabolism of testes by determining the testicular activities of ACP, ALP, and LDH-X. In testes, 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 nutrient transportation, energy metabolism, and spermatogenic cell division. Moreover, LDH-X, 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-X activity of fluoride-treated rats indicates its interference with maturation and energy metabolism of sperm and spermatogenic cells. Conversely, rats co-administered taurine exhibited a significant increase in marker enzymes of testicular function in comparison to fluoride-treated rats, thus indicating the chemoprotective effects of taurine against fluoride-induced gonadal toxicity in rats.

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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 the brain, testes, and epididymis of fluoride-treated rats indicates induction of apoptotic cell death. The apparent decrease in 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 the observed biochemical results. The histo-architectures of the brain, testes, and epididymis of the fluoride-treated rats showed marked lesions characterized by a focal area of vacuolation, satellitosis, 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 the epididymis with lumen containing inadequate sperm cells. The ability of taurine to maintain tissue morphology somewhat similar to that of the control signifies chemoprotection related to taurine's antioxidant and anti-apoptosis activities.

Based on the data from this study, we conclude that taurine confers reproductive health benefits to 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, thereby augmenting the antioxidant status, testosterone level, and sperm characteristics with an 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 declare that there is no conflict of interest associated with this work.

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