Biomedicine & Pharmacotherapy 102 (2018) 739–748

Contents lists available at ScienceDirect

Biomedicine & Pharmacotherapy

journal homepage: www.elsevier.com/locate/biopha

Ebselen can Protect Male Reproductive Organs and Male Fertility from Manganese Toxicity: Structural and Bioanalytical Approach in a Rat Model

Amany Tharwat Mohammed[a,*], Lamiaa L.M. Ebraheim[b], Mohamed M.M. Metwally[c]

[a] Department of Forensic Medicine and Toxicology, Faculty of Veterinary Medicine, Zagazig University, Zagazig, 44511, Egypt
[b] Department of Histology and Cytology, Faculty of Veterinary Medicine, Zagazig University, Zagazig, 44511, Egypt
[c] Department of Pathology, Faculty of Veterinary Medicine, Zagazig University, Zagazig, 44511, Egypt

ARTICLE INFO

Keywords:
Ebselen
Manganese
Toxicity
Rat
Testis
Seminal vesicle

ABSTRACT

Ebselen (EBS) is a versatile compound that can protect the cellular components from oxidative and free radical-mediated damage. In the present study, we investigated the protective effect of EBS against manganese (Mn) toxicity on male reproductive organs. Thirty-two male rats were assigned into four groups, namely, negative control, EBS (15 mg/kg body weight (bw), as a single protective IP injection), $MnCl_2$ (50 mg/kg bw, orally for 30 consecutive days), and EBS + $MnCl_2$ (as mentioned before). The results showed that EBS ameliorated the alterations caused by $MnCl_2$ in the testicular, epididymal, and seminal vesicle tissues. $MnCl_2$ increased the sperm abnormalities, decreased gonadosomatic index (GSI), sperm motility, and sperm count. Further, it reduced the serum levels of testosterone (T) and luteinizing hormone (LH). The elevated levels of malondialdehyde (MDA), nitric oxide (NO), and 8-OH-2′-deoxyguanosine (8-OHdG) and decreased the levels of superoxide dismutase (SOD), glutathione (GSH), and catalase (CAT) upon exposure to $MnCl_2$ indicated a disturbance in the activities of the testicular antioxidant enzymes and indices. Histologically, $MnCl_2$ decreased the diameter of seminiferous tubules (ST), the height of germinal epithelium, number of spermatogonia/ST, spermatocytes/ST, spermatids/ST, and Leydig cells/intertubular area (IA). Chemoprotection with EBS successfully mitigated most of the above-mentioned parameters concluding that EBS could be used as a useful prophylactic therapy whenever Mn toxicity is involved.

1. Introduction

EBS (2-phenyl-1, 2-benzisoselenazol-3 (2 H)-one) is a lipid-soluble, organo-selenium compound that is used as a synthetic antioxidant and possesses unique glutathione peroxidase-like activity [1]. EBS also displays a wide spectrum of therapeutic uses that include anti-inflammatory [2], anti-thrombotic [3], anti-lipoperoxidative [4], and anti-atherosclerotic activities [5,6]. It acts as an antagonist for the ROS generated during DNA and cytotoxic damages [7]. Another extraordinary property is that it is a potent scavenger of peroxynitrite, hydroperoxide as well as hydroperoxides of membrane-bound phospholipids. This is attributed to a multitude of mechanisms by which it inhibits the free radical-induced injury, coupled with its lack of side effects, good blood-brain barrier permeability, and rapid absorption following intraperitoneal administration [8]. EBS appears to be a promising drug in clinical trials against stroke, reperfusion injury with anti-atherosclerotic [9], and nephroprotective effects [10].

Manganese (Mn) is an important trace element involved in many physiological processes [11]. However, recently, its rapid use has raised concerns about the possibilities of its toxicity beyond its physiological needs [12]. It is widely utilized in the form of numerous compound metals that are broadly employed in commercial products, industrial settings, fertilizers, fungicides, and maneb-adulterated food and livestock feeds, drinking water purified using potassium permanganate, and as an automobile anti-knocking additive in unleaded gasoline [13]. Furthermore, the administration of Mn-SOD mimetics (EUK–8) and I/V use of methcathinone, a euphoric stimulant [14], is considered to be the sources of pollution by Mn. Human and animal populations are primarily exposed to higher Mn levels through ingested food and secondarily through drinking water and inhalation of manganese dust, obtained from the soil or through leaching from pollutants [15]. Although Mn is a well-known neurotoxic element for nearly 175 years and is responsible for the Parkinson-like disease called manganism [16], yet little is known about its toxicity in male fertility and genital organs. The cytotoxic effects of elevated levels of Mn are mostly related to its potential to generate reactive oxygen species (ROS) and interference with iron metabolism [11]. Apart from its toxic effects on the nervous systems, few studies have reported its negative effects on the development

* Corresponding author.
E-mail address: atkhalil@zu.edu.eg (A.T. Mohammed).

https://doi.org/10.1016/j.biopha.2018.03.086
Received 19 November 2017; Received in revised form 14 March 2018; Accepted 14 March 2018

and the reproductive, hepatic, and immune systems [11,17,18]. Its reproductive toxicity is manifested by impotence, libido loss [19], accumulation of Mn in the testicular tissue [20], and testicular tissue alterations, mainly shrinkage of seminiferous tubules [21], and high level of apoptosis of the germinal epithelium [22] that varies with sex and age in males. In females, Mn toxicity was not found to be associated with any significant evidence of fertility impairments; however, reduction in the implantation rate was recorded [23]. In fetuses, fetotoxicity but not teratogenicity has been documented [24]. There are several reports, e.g., [25], which have documented the protective effects of EBS against the neurotoxicity of Mn. On the contrary, its protective effects against the reproductive toxicity of Mn are not well established. In this context, the present study assessed the protective effect of EBS against the influence of high exposure of Mn on the testes and seminal vesicles in male rats.

2. Materials and methods

2.1. Animals and experimental treatments

A total of 32 male Sprague-Dawley rats (8 weeks old, 200 g) obtained from the Laboratory Animal House, Faculty of Veterinary Medicine, Zagazig University, Egypt were used in the study. The animals were housed in plastic cages placed in a well-ventilated rat house, fed on rat chow and water ad libitum, and subjected to the natural photoperiod of 12-h light:12-h dark. All the animals received human care according to the criteria stated in the "Guide for the Care and Use of Laboratory Animals" in the scientific research prepared by the National Institutes of Health (NIH). All studies were approved by the Ethics of Animal Use in Research Committee (EAURC) at Zagazig University. The ethical regulations were followed in accordance with the national and institutional guidelines for the protection of animal welfare during the experiments. The rats were randomly assigned into four groups of eight rats each. The experimental design involved treatment of rats with EBS and $MnCl_2$ for 30 consecutive days as follows: Group 1, control rats (negative control group) exposed to normal saline; group 2, rats exposed to EBS (positive control group) in a single dose of 15 mg/kg body weight (bw) (0.6 mL from 15 mg/kg bw dissolved in 0.9% normal saline) via intraperitoneal injection [8]; group 3, rats exposed to $MnCl_2$ at a dose of 50 mg/kg bw [26] daily throughout the experiment orally via gastric tube; group 4, rats exposed to a mixture of the treatments as 15 mg/kg bw of EBS same as in group 2 as a protective single dose. Then after 3 h, rats were exposed to 50 mg/kg bw of $MnCl_2$, same as in group 3. The rats in all the groups were weighed at days 0, 7, 14, 21, and 30.

2.2. Sample collection

At the end of treatment period, rats were weighed followed by their sacrifice by cervical dislocation under light ether anesthesia. The rats were necropsied, and the blood was collected from retro-orbital venous plexus. The blood was then allowed to clot, and the serum was separated by centrifugation at 1000 × *g* for 10 min at 4 °C to conduct the hormonal analysis. Both testicles were dissected out and weighed using a digital balance, then gonadosomatic index (GSI) was calculated using the equation GSI = gonad weight/total body weight × 100% [27].

The right epididymis from each rat was dissected out and used for semen picture analysis. The right testis and seminal vesicles were kept in the plastic bags at −20 °C till the biochemical and residual analysis were performed. The left testis, epididymis, and seminal vesicles were fixed for the light and electron microscopic examinations.

2.3. Semen picture analysis

2.3.1. Sperm progressive motility assay

The progressive motility of sperm of the experimental rats was evaluated according to Zemjanis [28]. Briefly, epididymal sperms were obtained by cutting the caudal epididymis with surgical blades and putting onto a sterile clean glass slide. Subsequently, the sperms were diluted with 2.9% sodium citrate dehydrate solution that was prewarmed to 37 °C, mixed carefully, and covered with a 24 × 24 mm coverslip. The sperm motility was assessed by observing a minimum of 10 microscopic fields under a phase contrast microscope at 200 × magnification. The sperm motility was calculated by scoring the number of progressive sperms, followed by non-progressive and immotile sperms in the same field. The data were expressed as the percentage of sperm progressive motility.

2.3.2. Evaluation of epididymal sperm count

The epididymal sperm count of rats was obtained according to the method described in the [29]. Briefly, the sperms obtained by mincing the cauda epididymis in normal saline were filtered using a nylon mesh. Subsequently, an aliquot of 5 µL of the sperm suspension was mixed with 95 µL of diluent (0.35% formalin containing 5% $NaHCO_3$, and 0.25% trypan blue). Of the diluted sperms, 10 µL was placed on the hemocytometer, allowed to sediment by standing for 5 min in a humid chamber before the sperm were counted using the improved Neubauer (Deep 1/10 m; Labart; Munich, Germany) chamber with a light microscope at 400×.

2.3.3. Sperm morphological abnormalities and viability assay

A portion of the sperm suspension that was placed on a glass slide was smeared out with another slide and stained with a reagent containing 0.2 g eosin and 0.6 g fast green which was dissolved in distilled water and ethanol in a ratio of 2:1 for morphological examination. A total of 400 sperm cells from each rat were used for morphological examination. Sperm viability was determined by staining the smeared slide with 1% eosin and 5% nigrosin in 3% sodium citrate dehydrate solution according to Wells and Awa [30].

2.4. Hormonal analysis

Radioimmunoassays (RIAs) were used to analyze T, LH, and follicular stimulating hormone (FSH) in the serum samples using T–125I, LH–125I, and FSH–125I RIA kits (Beijing North Institute of Biological Technology, People's Republic of China), respectively, according to the manufacturer's protocol. Radioactivity was determined using an automatic gamma counter. All samples were run in duplicate in a single assay to avoid inter-assay variations.

2.5. Homogenate preparation

The tissues of testes and seminal vesicles were washed twice with a cold saline solution, placed in glass bottles, labeled, and stored in a deep freezer (−20 °C) until processing (maximum 10 h). After weighing, the tissue (1 g) was placed on dry ice, cut into small pieces with scissors and homogenized (2 min at 3000 × g) in five volumes (1:5, w/v) of ice-cold Tris-HCl buffer (50 mM, pH 7.4) using a glass Teflon homogenizer (Ultra-Turrax IKA T10 Basic; Staufen, Germany). All procedures for homogenate preparation were performed at 4 °C. After addition of butylhydroxytoluene (4 mL/mL), the tissue homogenate samples were used for estimating NO, MDA, SOD, CAT, GSH, and 8-OHdG.

2.6. Biochemical analysis

Tissue NO, MDA, and CAT concentrations were estimated using commercial rat ELISA kit (MyBioSource, China). Further, tissue SOD and GSH were estimated using the commercial rat ELISA kits (CUSABIO BIOTECH Co, Ltd; China). Lastly, 8-OHdG was estimated using the Rat tissue ELISA kit (Abnova CO, Taiwan).