Biomedicine & Pharmacotherapy 96 (2017) 685–694

Contents lists available at ScienceDirect

Biomedicine & Pharmacotherapy

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

Original article

Protective role of epigallocatechin-3-gallate on arsenic induced testicular toxicity in Swiss albino mice

Pushpa Rani Guvvala[a,*], Janivara Parameswaraiah Ravindra[a], Chukkath Vijayan Rajani[b], Muniandy Sivaram[c], Sellappan Selvaraju[a]

[a] Reproductive Physiology Laboratory, Animal Physiology Division, ICAR-National Institute of Animal Nutrition and Physiology, Adugodi, Bengaluru,560030, India
[b] Veterinary Anatomy and Histology, Kerala Veterinary and Animal Sciences University, Pookode, 673576, India
[c] Southern Regional Station, ICAR-National Diary Research Institute, Bengaluru,560030, India

ARTICLE INFO

Keywords:
Arsenic
EGCG
Spermatogenesis
Spermatozoa function
Oxidative stress
Lipid peroxidation

ABSTRACT

Arsenic, often referred to as the king of poisons is carcinogenic in humans and animals. It affects multiorgan systems including reproduction. The present study was undertaken to explore the protective role of green tea compound, epigallocatechin-3-gallate (EGCG) on arsenic induced testicular toxicity in Swiss albino mice. Thirty two adult male mice were randomly assigned to four groups (n = 8). Group I served as control without test chemical. The group II received arsenic (200 ppm) through drinking water, group III received only EGCG (20 mg/kg b.wt., intraperitoneally, alternate days) and group IV was administered arsenic + EGCG for 40 days. Factorial experimental design was employed to assess the treatment effect. The EGCG restored arsenic induced decrements in epididymal sperm concentration, kinematic attributes (total motility, rapid, progressive motile, fast progressive, VSL, VAP, VCL, BCF, LIN, WOB, STR and Type A), structutal membrane integrity, functional membrane integrity and mitochondrial membrane potential. As evidenced by the histoarchitectural studies, the EGCG reversed the deleterious effects of arsenic on testicular malondialdehyde ($p < 0.05$) levels, reduced glutathione, antioxidative enzymes and spermatogenesis. Overall, the results suggest that EGCG reduces the testicular oxidative stress induced by arsenic poisoning and thereby protect the reproductive system.

1. Introduction

Arsenic is a naturally occurring metalloid element but anthropogenic activities have made it cosmopolitan in distribution and a major contaminant in several areas of the world. Globally more than 200 million people are at risk of being exposed and suffering with diseases related to arsenic toxicity, such as cancer [1]. Elucidating the adverse effects of arsenic on mammalian reproduction and development continue to be a tough challenge for researchers. Arsenic acts as an endocrine disruptor and thereby alters the functions of hypothalamic-pituitary-testicular axis and subsequently the hormonal levels. Arsenic compounds affect testicular functions, leading to decreased testicular weight, sperm concentration and functions apart from inhibiting testicular androgenesis. Chronic oral exposure to arsenic also induces the response to stress by increasing adrenocortical activity and finally affects sperm quality [2–4].

Arsenic intoxication has been reported to arrest spermatogenic stages in seminiferous tubules [5], disrupt spermatogenesis and cause degeneration of Leydig and Sertoli cells [4,6] or/and also induce male infertility by inhibition of androgen receptor transcriptional activity in Sertoli cells [7]. In addition, arsenic induces oxidative stress (OS) through binding to thiol groups of biomolecules in testicular cells and organelles leading to altered male reproductive functions [8,9]. Arsenic generates reactive oxygen species (ROS) by increasing lipid peroxidation (LPO) and decreasing the activities of antioxidant enzymes thereby causing OS in testes. The arsenic induced OS also impairs spermatogenesis through direct effect on the germinal compartment [6,10].

Studies indicated that antioxidants boost the defense mechanism to protect against arsenic toxicity [11]. For our study, we set criteria that the antioxidant should be a natural substance, easily available and combat/chelate with the arsenic and subsequently get eliminated from the body without any side effects. One such natural source of antioxidants, epigallocatechin-3-gallate (EGCG), a major green tea (*Camellia sinensis*) compound, known to be a powerful antioxidant has attracted a great extent attention due to its beneficial effects on health. Many investigations have shown that catechins contained more hydroxyl (OH) bases structurally, with higher potential for preventing damage by free radicals [12]. Polyphenolic compound EGCG is

* Corresponding author.
E-mail address: gprani2010@gmail.com (P.R. Guvvala).

http://dx.doi.org/10.1016/j.biopha.2017.09.151
Received 18 May 2017; Received in revised form 9 September 2017; Accepted 12 September 2017

anticarcinogenic [13,14], prevents damage to chromosomes caused by ROS [15] by inhibiting the side effects of hydrogen peroxide (H_2O_2), superoxide (O_2^-), hydroxyl radicals (HO·) and nitric oxide (NO) [16,17]. Green tea catechins are reported to have a prominent antioxidant activity than ascorbic acid and tocopherol [18].

A number of pioneering studies have demonstrated a possible involvement of EGCG in the intonation of certain testicular functions [19–21]. In murine, EGCG supplementation increased the plasma testosterone levels [22], protected the germ cells from OS [20] and prevented the sperm abnormalities [23]. Low doses of EGCG exerted benefits on sperm survival and metabolism [24]. EGCG protected testicular seminiferous tubules from reperfusion/ischemia induced injury by inhibiting germ cell apoptosis and inflammation [25]. However, the mechanisms underlying these beneficial effects of EGCG during arsenic induced testicular injury/pathologies are unknown.

To the best of our knowledge, no previous *in vivo* reproductive studies investigated the ameliorative role of EGCG on arsenic exposed animals. Thus, the objective of the study was to explore the protective role of phytochemical EGCG against arsenic induced reproductive toxicity in male Swiss albino mice.

2. Materials and methods

2.1. Animals and experimental design

Swiss albino mice were maintained at the experimental animal house facility, Indian Council for Agricultural Research-National Institute of Animal Nutrition and Physiology (ICAR-NIANP), Bengaluru. The animals were housed in polypropylene cages at 25 ± 2 °C under 12 h light/dark schedules with *ad libitum* supply of water and standard mice feed. The research study was conducted with the approval from Institutional Animal Ethics Committee (IAEC). The EGCG and arsenic (factors) were given at two different levels resulting into four treatment groups. The experimental design employed in this research was 2^2 factorial experiments. Thirty two healthy adult male mice weighing about 35 ± 3 g and aged 90 ± 4 days were randomly assigned to four groups (n = 8 each). EGCG preparation was made with the concentration of 20 mg/mL of 0.9% NaCl as vehicle. Group I served as control and received potable water and the vehicle intraperitoneally (i.p.), without test chemical. The treatment group II received 200 ppm arsenic through drinking water and the vehicle (i.p.), group III was treated with EGCG (20 mg/kg body weight), administered intraperitoneally on alternate days and the combination group *i.e.* group IV received both arsenic (200 ppm) and EGCG (20 mg/kg body weight) through the same routes. The duration of the treatment was 40 days. The EGCG dose selected for the present investigation was based on the earlier reports where in no adverse effects were observed on animals [22,26]. Arsenic dose was selected based on the dose response studies conducted in our laboratory [9]. Test chemicals, sodium arsenate dibasic heptahydrate (A6756, purity ≥98%) and epigallocatechin-3-gallate (E4143, purity ≥95%) were procured from Sigma–Aldrich, USA, and other chemicals were obtained from Himedia and Sisco Research Laboratories Pvt. Ltd., India.

2.2. Sample collection and processing

After completion of the experimental period, the animals were fasted overnight with free access to water. The animals were sacrificed by giving chloroform anesthesia. Immediately, blood was collected by puncturing the heart. After allowing blood to clot, serum was separated and used for testosterone hormone estimation. Both cauda epididymides were collected in phosphate buffered saline (PBS) of pH 7.2, sliced and incubated at 37 °C for 10 min to release spermatozoa from the tissue. Then, this sample was used for sperm analysis (kinematic and functional attributes). Testes were removed immediately, washed in PBS (ice-cold) and blotted with filter paper to remove excess buffer. The testes were randomly selected from each group and preserved in neutral buffered formalin (10%) for histopathological study. For biochemical studies (*i.e.,* LPO, GSH and antioxidative enzymes), testes were immediately snap frozen in liquid nitrogen (LN_2) and preserved at −80 °C until analysis.

2.3. Sperm analysis

2.3.1. Sperm concentration

Semen sample (50 μL) was diluted in PBS (500 μL) and 5 μL of 0.3% glutaraldehyde was added. Then, 10 μL of diluted sample was placed onto each compartment of Neubauer hemocytometer and sperm cells were counted under a microscope at 1000× magnification. Sperm count data were expressed as million spermatozoa per epididymides [27].

2.3.2. Sperm kinematic attributes

The kinematic attributes in mice epididymal semen samples were assessed using Computer Assisted Semen Analyzer-Sperm Class Analyzer (CASA, version 3.2.0; Microptic, Spain). The 10 μL of semen sample was directly placed onto a prewarmed (37 °C) microscopic slide and covered with a cover slip (18 × 18 mm). A minimum of 10 consecutive digitalized images of 25 frames/s was captured using 10× negative phase-contrast objective (Nikon Eclipse 50*i*, Nikon, Japan). The following parameters were set in CASA for mice sperm motility (including total motility) and velocity parameters: spermatozoa concentration (5–8 × 10^6/ml), particle size (5–70 μm^2), progressive forward motility (> 50% straightness, STR); circular (< 50% linearity, LIN), static (< 10 μm/s); slow motile (> 10– < 15 μm/s); medium motile (> 15– < 45 μm/s), rapid (> 45 μm/s) and type A sperm (> 80% STR with ALH > 2.5 μm). Based on the motility attributes, average path velocity (VAP), curvilinear velocity (VCL) and straight line velocity (VSL) were calculated [9].

2.3.3. Sperm structural membrane integrity (SMI)

The sperm SMI was assessed by using eosin (5%) and nigrosin (10%) stain (EN stain) [28]. Semen sample (10 μL) was mixed with EN stain (10 μL), and after 30 s at 37 °C the samples were smeared on to a warm glass slide. A minimum of 200 spermatozoa were observed under microscope at 1000× magnification (E200, Nikon, Japan). The unstained spermatozoa were considered as viable sperm, whereas partially and completely purple stained spermatozoa were considered as non-viable sperm. The percentage (%) of viable spermatozoa was calculated to the total number of cells counted.

2.3.4. Sperm functional membrane integrity (FMI)

Hypoosmotic solution (HOS) was used to assess sperm tail FMI [9,29]. Briefly, 30 μL of semen samples were added to 300 μL of 150 mOsm (hypoosmotic) and 300 mOsm (isoosmotic) solutions and the samples of semen were kept at 37 °C for 30 min, following which, a drop of 0.3% glutaraldehyde was added to fix the spermatozoa. Then, the samples were smeared on to the glass slides and observed under 400× magnification and a minimum of 200 sperms were counted. The spermatozoa with hair-pin bend in the principal piece of the tail were counted as HOS positive in both the solutions and the percentage of functional membrane integrity positive sperms were calculated as: HOS positive spermatozoa (%) = 150 mOsm HOS positive (%) – 300 mOsm HOS positive (%).

2.3.5. Sperm mitochondrial membrane potential (MMP)

The MMP in mice spermatozoa were assessed by using lipophilic, cationic fluorescent dye *i.e.,* JC-1 (Tetrachloro tetraethyl benzimidazolyl carbocyanine iodide, Sigma-Aldrich, USA) stain [9]. Briefly, 1 μL of JC-1 stain (1.53 mM in dimethyl sulfoxide) was added to a 50 μL of epididymal sperm sample and allowed to incubate for 30 min at 37 °C. Following incubation, the samples were smeared on to the glass slides