Original Paper

(-)-Epigallocatechin-3-Gallate Inhibits Arsenic-Induced Inflammation and Apoptosis through Suppression of Oxidative Stress in Mice

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Key Words

arsenic • (-)-epigallocatechin-3-gallate (EGCG) • Immunotoxicity • Oxidative stress • Apoptosis

Abstract

Background/Aims: Exposure to arsenic in individuals has been found to be associated with various health-related problems including skin lesions, cancer, and cardiovascular and immunological disorders. (-)-Epigallocatechin-3-gallate (EGCG), the main and active polyphenolic catechin present in green tea, has shown potent antioxidant, anti-apoptotic and anti-inflammatory activity *in vivo* and *in vitro*. Thus, the present study was conducted to investigate the protective effects of EGCG against arsenic-induced inflammation and immunotoxicity in mice. **Methods:** Serum IL-1 β , IL-6 and TNF- α were determined by ELISA, tissue catalase (CAT), malonyldialdehyde (MDA), superoxide dismutase (SOD), glutathione (GSH), nitric oxide and caspase 3 by commercial kits, mitochondrial membrane potential with Rh 123, mitochondrial ROS with 2',7'-dichlorofluorescein diacetate (DCFH-DA), apoptotic and necrotic cells and T-cell phenotyping with Flow cytometry analysis. **Results:** The results showed that arsenic treatment significantly increased oxidative stress levels (as indicated by catalase, malonyldialdehyde, superoxide dismutase, glutathione and reactive oxygen species), increased levels of inflammatory cytokines and promoted apoptosis. Arsenic exposure increased the relative frequency of the CD8+(Tc) cell subpopulation (from 2.8 to 18.9%) and decreased the frequency of CD4+(Th) cells (from 5.2 to 2.7%). Arsenic exposure also significantly decreased the frequency of T(CD3) (from 32.5% to 19.2%) and B(CD19) cells (from 55.1 to 32.5%). All of these effects induced by NaAsO₂ were attenuated by EGCG. **Conclusions:** The present *in vitro* findings indicate that EGCG attenuates not only NaAsO₂-induced immunosuppression but also inflammation and apoptosis.

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Introduction

Arsenic is a toxicologically important compound that is widely distributed in the environment due to its natural existence and anthropogenic applications [1]. Arsenic exposure is associated with considerable human morbidity and mortality. There is accumulating evidence that chronic exposure to arsenic, even at fairly low levels, leads to increased risk of infectious diseases, such as polyneuritis, diarrhoea, and immunosuppression of the T-dependent antibody response [2, 3]. Moreover, arsenic exposure in individuals promotes the generation of reactive oxygen species and nitric oxide and is involved in the production of pro-inflammatory cytokines [4-6]. Elevated levels of interleukin-6 (IL-6) and tumour necrosis factor alpha (TNF- α) produced by the immune system play an important role in the immune response. These factors deteriorate in arsenic-related dysfunction of the immune system, particular immunosuppression [7]. Additionally, such alterations of these factors in intracellular and intercellular homeostasis probably lead to cell death and mitochondrial dysfunction, thereby strengthening the activation of stress-related genes [3, 8]. Meanwhile, arsenic exposure through contaminated food materials is quite common in areas with high levels of arsenic in the groundwater, which is becoming an urgent issue in the field of public health. Given the increasing risk of arsenic toxicity, recently accumulating evidence supports the notion that chronic arsenic exposure is associated the suppression of immune system. Furthermore, arsenic is known to induce oxidative stress by reactive oxygen stress (ROS) generation and DNA damage and increases levels of oxidative stress markers, which may affect the production of T cells and impair cell function. In addition, oxidative stress-responsive genes, such as ANGPTL7, NME5, and MBL2, were reduced by several folds in relation to elevated arsenic concentrations [9, 10]. Thus, treatment with immune modulators has been shown to regulate low immune activity induced by arsenic.

Recently, a number of plant products, their active constituents and herbal agents have received great attention as antioxidants against arsenic-induced toxicity. The most abundant polyphenol in green tea, (-)-epigallocatechin-3-gallate (EGCG) (Fig. 1), is a potent antioxidant and exhibits extensive pharmacological activity including antioxidative, anti-inflammatory, anti-cancer and immunomodulatory properties [11-14]. The antioxidative activity of EGCG plays a crucial role against the above mentioned disease. It has been reported in the literature that EGCG regulates the function of T cells in autoimmunity and transplantation [15]. Further studies have confirmed that EGCG modulates the functions of dendritic cells after ultraviolet A and B irradiation and prevents the immune suppression induced by ultraviolet radiation [16]. Our recent study showed that EGCG can protect cardiac tissue against the cardiotoxicity induced by arsenic [17]. However, the effects of EGCG on the immune dysfunction induced by arsenic have not been investigated. The present study has therefore been carried out to understand the protective role of EGCG against arsenic-induced inflammation, immunotoxicity and apoptosis in mice.

Materials and Methods

Ethics statement

All animal experiments were performed according to institutional ethical committee guidelines and approved by the Medical Ethics Committee of Hunan Normal University (Grade II, Certificate No. 2015A054, Hunan Normal University) and were in accordance with the National Institutes of Health Regulations for the Care and Use of Animals. All efforts were made to minimize suffering.

Drug preparation

EGCG and NaAsO₂, with a purity of 98%, were purchased from Sigma Aldrich Corporation (Shanghai, China). A stock suspension was prepared with distilled water and stored at 4°C for two days (concentration: 50 mg/mL). Throughout all the experiments, the characteristics of all the drugs were unchanged.

Animals and pharmacological treatment in vivo

Male BALB/c mice (15 ± 2 g) at 8 weeks of age were maintained in our animal house under standard conditions. They were fed with standard rodent pellet chow and water ad libitum. Our animal house and breeding facility are registered with the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA); Government of China and CPCSEA guidelines were followed. The mice were randomly divided into four groups with 10 animals/group. Arsenic was dissolved in distilled water and administered by gavage. EGCG was also suspended in distilled water and given by gavage. Both of the treatments (arsenic and EGCG) were given by interval. There was a gap of 6 h between the first arsenic treatment and second EGCG treatment. Treatment was given for 30 days. All animals were randomly assigned to the following groups:

Group 1-control (received vehicle only).

Group 2-toxin control (received NaAsO_2 orally at a dose of 10 mg/kg body weight, *per os* daily for 30 days).

Group 3- NaAsO_2 (10 mg/kg body weight, *per os* daily for 30 days) followed by EGCG (10 mg/kg body weight, *per os* daily for 30 days).

Group 4- EGCG (10 mg/kg body weight, *per os* daily for 30 days).

Blood/tissue collection

Blood/tissues were collected as previously described [18]. At the end of the experimental period (30 days), a set of animals were sacrificed by cervical dislocation. In another set, blood was quickly collected by heart puncture for the separation of serum. For the assessment of different inflammatory markers, the thymus and spleen were isolated from mice as previously described [7].

The thymuses and spleens of five mice per group were cleaned and placed in phosphate-buffered saline (PBS, pH 7.4) and subsequently processed for measures of immunological parameters. The remaining thymus and spleen in each set were placed in ice-cold saline solution (0.15 M), blot-dried, weighed, and then immediately processed for use in the assessment of arsenic level in both tissues.

Preparation of thymocyte and splenocytes suspension

The dissected thymuses and spleens from mice were processed into single-cell suspensions under aseptic conditions. The suspensions were passed through stainless steel mesh and centrifuged at $200 \times g$ at 4°C for 10 min. The thymocytes were resuspended in PBS. The splenocytes were suspended in 5.0 ml hypotonic erythrocyte lysing solution (2.42 g Tris and 7.56 g NH_4Cl in 1000 ml deionised water, pH adjusted to 7.2). After 5 min incubation followed by centrifugation, cells were resuspended in PBS, counted on a haemocytometer and then diluted with PBS to appropriate concentrations for use in immunological assays.

The spleens were dissected from male BALB/c mice (4–6 weeks old), and a single-cell suspension was prepared under aseptic conditions. The suspension was passed through $100\ \mu\text{m}$ stainless steel mesh, treated with 5.0 ml hypotonic NH_4Cl solution (2.42 g Tris and 7.56 g NH_4Cl in 1000 ml deionised water, pH adjusted to 7.2) for 5 min at room temperature to lyse red blood cells, centrifuged and washed with FBS-free medium. The cells were suspended in complete cell culture medium (RPMI 1640 containing HEPES and 2 mM glutamine, supplemented with 10% FBS and 1% antibiotic-antimycotic solution). The cell density was adjusted to 1.5×10^6 cells/ml, and the viability of the freshly isolated cells was always over 95% (trypan blue exclusion test). After cell preparation, the monitoring of various parameters was performed in the present investigation.

Assay of nitric oxide levels

Levels of nitric oxide in the serum were estimated using a nitric oxide assay kit procured commercially (Calbiochem, USA). The assay principle involves the conversion of nitrate to nitrite by the enzymatic action of nitrate reductase. The addition of 2,3-diaminonaphthalene (DAN) and sodium hydroxide converts nitrite to a fluorescent compound, 1(H)-naphthotriazole, which is measured spectrofluorometrically (excitation 430 nm/emission 450 nm) using a multi-well plate reader.

Fig. 1. Structure of (-)-epigallocatechin-3-gallate (EGCG).

Determination of oxidative stress-related enzymes

Supernatant samples from cells were assayed for the determination of oxidative stress-related enzymes (CAT: catalase, MDA: malonyldialdehyde, SOD: superoxide dismutase, GSH: glutathione) by kits using an automatic chemical analyser (7060, Hitachi, Tokyo, Japan), following the standard procedure according to previous methods [7].

Assay of inflammatory factors (IL-1 β , IL-6, TNF- α)

The levels of IL-1 β , IL-6 and TNF- α in the serum of mice exposed to arsenic and simultaneous treatment of arsenic and EGCG were quantified by an ELISA method using the Quantikine mouse IL-1 β , IL-6 and TNF- α kit. The intensity of the colour measured was in proportion to the amount of mouse IL-1 β , IL-6 and TNF- α in the sample. The levels of IL-1 β , IL-6 and TNF- α were determined using a standard curve.

Assessment of T and B lymphocyte population in thymocytes and splenocytes

T-cell phenotyping was conducted on the basis of CD4 and CD8 surface molecules. Quadrants were positioned on FITC/PE dot plots, and CD4⁺CD8⁺ (double positive; DP) and CD4⁺CD8⁻ (double negative; DN) subpopulations were distinguished. The lymphocyte population among splenocytes was assessed based on CD3 (for T-cells) and CD19 (for B-cells) surface molecules. Assessment of T and B lymphocyte population was performed as previously described [19, 20]. The data were analysed with Cell Quest software, and the mean fluorescence intensity was obtained by histogram statistics.

Estimation of arsenic levels in thymus and spleen

Arsenic levels in the thymuses and spleens of mice exposed to arsenic and co-treated with EGCG were estimated with an automatic fluorescence spectrometry system, the hydride system 60 atomic absorption spectrophotometer (HSAAS, ZEEnit 700), as previously described [19]. Briefly, 1 ml of concentrated nitric acid was added to 100 mg of tissue, followed by 1 ml of perchloric acid. The sample was then digested over a sand bath until the solution became yellow in colour. If the colour of the digest was brown, more nitric acid and perchloric acid were added, and the oxidation was repeated. The digest was diluted to a predetermined volume with deionized water. Aliquots of this were used to estimate the quantity of arsenic using an atomic absorption spectrophotometer. A calibration curve was constructed by adding known standard amounts of arsenic to calculate arsenic levels in the thymus and spleen.

Measurement of ROS production

Measurement of mitochondrial ROS was performed as previously described [21, 22]. Thymus cells from each group were suspended in PBS and incubated with 50 μ M 2',7'-dichlorofluorescein diacetate (DCFH-DA) in modified Krebs solution (135 mM NaCl, 5.9 mM KCl, 1.5 mM CaCl₂, 1.2 mM MgCl₂, 11.5 mM glucose, 11.6 mM HEPES, pH 7.4) supplemented with 0.1% bovine serum albumin and 0.02% Pluronic F-127 at 37 °C for 1 h in the dark at room temperature. ROS generation was recorded at excitation wavelength 488 nm and emission wavelength 525 nm using fluorescence microscopy.

Measurement of mitochondrial membrane potential

The thymus and spleen cells were washed twice with PBS, scraped from the dishes, and suspended in 1 ml of cold isolation buffer. The cells were homogenized using a glass homogenizer. The homogenate was centrifuged at 1330 $\times$ g for 5 min at 4 °C. The supernatants were centrifuged at 21,200 $\times$ g for 10 min at 4 °C. The pellet was suspended in a 15% Percoll solution, and aliquots were layered onto discontinuous Percoll gradients (23% over 40%) and then centrifuged for 15 min at 30,700 $\times$ g at 4°C. The dense band of material at the interface between the 23% and 40% Percoll layers was collected, diluted 1:4 in fresh isolation buffer, and then centrifuged for 10 min at 16,700 $\times$ g at 4°C. The pellet was resuspended in 2 ml of isolation buffer and centrifuged again for 10 min at 6,900 $\times$ g. The mitochondrial pellet was suspended in 100 ml of PBS and used to measure mitochondrial membrane potential ($\Delta\psi$ m). For the detection of mitochondrial $\Delta\psi$ m, cells were incubated with Rh 123 (5 μ g/ml final concentration) for 60 min in the dark at 37°C, harvested and suspended in PBS. Mitochondrial $\Delta\psi$ m was measured by the fluorescence intensity.

Assay of caspase-3 activity

The activity of caspase-3 in thymocytes was measured using the CaspACE™ Assay System (fluorometric kit) according to the manufacturer's instructions. For the assay, the fluorogenic substrates for caspase-3 were labelled with the fluorochrome AMC. AMC was released from these substrates on cleavage

by caspase-3. The activity of caspase-3 was determined by monitoring the fluorescence produced by free AMC using a GloMax 20/20n Luminometer (Promega, Madison, WI, USA) at 360/460 nm.

Detection of apoptotic and necrotic cells

The distribution of apoptotic and necrotic cells was analysed by annexin V binding and PI uptake following a previously described procedure. Briefly, thymocytes were suspended in 1 ml binding buffer (1×), an aliquot of 100 µl was incubated with 5 µl annexin V-FITC and 10 µl PI for 15 min in the dark at room temperature, and 400 µl binding buffer (1×) was added to each sample. The FITC and PI fluorescence were measured through FL-1 (530 nm) and FL-2 filters (585 nm), respectively.

Statistical analysis

All assays were repeated at least three times independently. All data are expressed as the mean ± SEM. Data were analysed using one-way analysis of variance (ANOVA) followed by a Newman-Keuls test for multiple pairwise comparisons among the groups. Statistical significance was defined as $P < 0.05$.

Results

Effect of EGCG on nitric oxide levels

It has been reported that the NO level indicates an impaired antioxidative defence mechanism in the case of an arsenic-related oxidative injury. To investigate the changes of oxidative stress, we measured the levels of nitric oxide in the serum. As shown in Figure 2, exposure to arsenic was associated with an increase in the level of nitric oxide level compared to the control group. Administration of EGCG significantly decreased the level of nitric oxide in the serum compared with animals treated with arsenic alone. No significant effect was observed in treatment with EGCG alone compared with the control group.

Effect of EGCG on NaAsO₂-induced oxidative stress and antioxidant markers in mice

To assess the level of oxidative damage to the biological membrane, the activity levels of SOD, CAT and GSH were measured. As shown in Figure 3, the activity levels of SOD, CAT and GSH in NaAsO₂-treated mice decreased significantly, and the content of MDA increased. After treatment with EGCG, all of the abovementioned results were reversed. These results collectively suggested that EGCG could attenuate NaAsO₂-induced oxidative stress.

EGCG decreases the release of IL-1β, IL-6 and TNF-α in the serum of mice

IL-1β, IL-6 and TNF-α are of great importance in organ physiology and pathophysiology. Excessive release of inflammatory factors affects cellular immune responses, resulting in immunosuppression. To investigate whether IL-1β, IL-6 and TNF-α were involved in the effects of EGCG on NaAsO₂-induced inflammation, these inflammatory factors were tested. As

Fig. 2. Effect of EGCG on nitric oxide levels. Compared with the control group, ^a $P < 0.05$; compared with the arsenic-treated group, ^b $P < 0.05$. Values are expressed as the mean ± SEM of five animals in each group.

Fig. 3. Effect of EGCG on biochemical changes in mice. Compared with the control group, ^a $P < 0.05$; compared with the arsenic-treated group, ^b $P < 0.05$. Values are expressed as the mean $\pm$ SEM of five animals in each group. A: SOD; B: GSH; C: CAT; D: MDA.

Fig. 4. EGCG decreases the release of IL-1 β , IL-6 and TNF- α in the serum of mice. Compared with the control group, ^a $P < 0.05$; compared with the arsenic-treated group, ^b $P < 0.05$. Values are expressed as the mean $\pm$ SEM of five animals in each group.

depicted in Figure 4, the present study showed that exposure to NaAsO₂ causes a significant increase in the levels of IL-1 β , IL-6 and TNF- α . In contrast, the release of IL-1 β , IL-6 and TNF- α by NaAsO₂ was suppressed by pretreatment with EGCG in mice. These results collectively suggested that EGCG could attenuate NaAsO₂-induced inflammation (Fig. 4).

Fig. 5. Effect of EGCG on T and B lymphocyte populations in thymocytes and splenocytes of mice. A: The ratio of CD4(Th) (%); B: The ratio of CD4(Tc) (%); C: The ratio of CD4(Th)/CD8(Tc) (%); D: The ratio of CD3(T) (%); E: The ratio of CD19(B) (%). Compared with the control group, ^a $P < 0.05$; compared with the arsenic-treated group, ^b $P < 0.05$. Values are expressed as the mean $\pm$ SEM of five animals in each group.

Fig. 6. Effect of EGCG on arsenic levels in thymus and spleen of mice. A: Arsenic level in the thymus; B: Arsenic level in the spleen. Compared with the control group, ^a $P < 0.05$; compared with the arsenic-treated group, ^b $P < 0.05$. Values are expressed as the mean $\pm$ SEM of five animals in each group.

Effect of EGCG on T and B lymphocyte population in thymocytes and splenocytes of mice

It has been reported that arsenic exposure in mammals impairs the activity of lymphocytes, monocytes, and macrophages and affects cellular immune responses, resulting in immunosuppression. To investigate the effects of arsenic on thymocytes and splenocytes in mice, the T and B lymphocyte populations were assessed. As indicated in Figure 5, arsenic exposure increased the relative frequency of the CD8 (Tc)-positive cell subpopulation from 2.8 to 18.9% and decreased the ratio of CD4 (Th)-positive cells from 5.2 to 2.7%. These changes were reversed following simultaneous treatment with EGCG. Both the CD8 and CD4 cells types came close to the control group at 2.5% and 3.4%, respectively, compared with the NaAsO₂-treated group. No significant effects on CD8 and CD4 cell ratios were observed in EGCG treatment alone compared with the control group.

Compared with controls, the arsenic-treated group showed a significant decrease in T (CD3)-cells from 32.5% to 19.2% and in B (CD19)-cells from 55.1 to 32.5%, whereas simultaneously treatment with arsenic and EGCG restored the proportions to 29.3% and 49.5%, respectively. No significant effects on T (CD3)- and B (CD19)-cells were observed in mice treated with EGCG alone compared with the control group.

Fig. 7. Effect of EGCG on NaAsO₂-induced ROS generation in the thymus of mice. Compared with the control group, ^a*P*<0.05; compared with the arsenic-treated group, ^b*P*<0.05. Values are expressed as the mean ± SEM of five animals in each group.

Effect of EGCG on arsenic levels in thymus and spleen of mice

To further confirm the effect of EGCG on NaAsO₂ in the thymus and spleen of mice, the levels of arsenic were determined by spectrophotometry. As shown in Figure 6, there existed a significant increase in the tissue levels of arsenic in animals exposed to arsenic for 30 days compared with the control group. Treatment with EGCG decreased the levels of arsenic in the thymus and spleen.

Effect of EGCG on NaAsO₂-induced ROS generation

Arsenic has been found to be associated with increased generation of ROS. NaAsO₂-induced ROS generation is supposed to be one of the major contributors to apoptosis and injury related to different organs. To determine whether pretreatment with EGCG mitigated NaAsO₂-induced oxidative stress, total ROS were measured by incubating the thymus with 10 μM DCF-AM fluorophore. Our data demonstrated that EGCG attenuated the NaAsO₂-induced increase in DCF fluorescence in the thymus. As shown in Figure 7, treatment with EGCG significantly inhibited the elevated intracellular concentration of ROS compared with the NaAsO₂-treated thymus.

Effect of EGCG on mitochondrial membrane potential

Loss of mitochondrial membrane potential ($\Delta\psi_m$) is a common index of mitochondrial damage that results from the surplus generation of ROS. To investigate the direct effect of EGCG on $\Delta\psi_m$, the $\Delta\psi_m$ was measured after incubation with Rh 123. As shown in Figure 8A, NaAsO₂ exposure caused a decrease in $\Delta\psi_m$. EGCG increased the $\Delta\psi_m$ in thymus compared with mice treated with arsenic. No effect on $\Delta\psi_m$ was observed in mice treated with EGCG alone compared with the control group.

Effect of EGCG on NaAsO₂-induced caspase-3 activation

Activation of the caspase cascade is crucial in the initiation of apoptosis in diverse biological processes. To investigate whether caspase-3 was involved in the effects of EGCG on NaAsO₂-induced cytotoxicity, the activity of caspase-3 was assessed. The present study showed that caspase-3 activity significantly increased after treatment with NaAsO₂. In contrast, activation of caspase-3 by NaAsO₂ was suppressed in the thymus of animals pretreated with EGCG (Fig. 8B).

Fig. 8. Effect of EGCG on mitochondrial membrane potential, cell apoptosis and oxidative stress in thymus of mice. A: Determination of mitochondrial membrane potential; B: Activity of caspase-3; C: Flow cytometry analysis of annexinV-FITC/PI staining. D: Quantification histograms indicate the percentages of cells in the early (annexin V-FITC positive and PI negative) and late stages of cell death. Compared with the control group, ^a $P < 0.05$, ^{aa} $P < 0.05$; compared with the arsenic-treated group, ^b $P < 0.05$. Values are expressed as the mean $\pm$ SEM of five animals in each group.

Effect of EGCG on apoptotic and necrotic cells of the thymus in NaAsO₂-treated mice

It is reported that there exists a progressive increase in apoptotic and necrotic cell death in thymocytes and splenocytes. Arsenic exposure caused an increase in apoptotic and necrotic cells compared with the control group. Treatment with EGCG decreased the number of apoptotic and necrotic cells in the thymus compared with mice treated with arsenic alone (Fig. 8C, D).

Discussion

The study presented herein clearly demonstrated the anti-inflammatory and anti-apoptosis properties of EGCG against NaAsO₂-induced immunotoxicity *in vivo*. Our data showed that treatment with EGCG significantly attenuates the enhancement of oxidative stress and NaAsO₂-related immunotoxicity (including dysfunction of CD8+(Tc) cells, CD4+(Th) cells, T(CD3) and B(CD19) cells), suppresses apoptosis of thymocytes and splenocytes, and inhibits the release of inflammatory factors in mice. Importantly, this study has verified that reduction of ROS production and decreased activation of caspase-3 were associated with the protective effects of EGCG. These results provide insight into the mechanisms by which EGCG acts a potential therapeutic prescription to ameliorate the immunotoxicity induced by NaAsO₂.

Chronic arsenic exposure through contaminated drinking water causes immunosuppression and affects the secretion of a variety of cytokines in the population [2, 23, 24]. Mitochondria are regarded as the main targets of arsenic toxicity [25]. In addition, it has been reported that these cytokines, including TNF- α , IL-1 β and IL-6, are involved in the generation of free radicals through the mitochondrial respiratory chain reaction in response to arsenic toxicity [26, 27]. These pro-inflammatory mediators are involved in various biological

and cellular changes including growth factors, transcription factors and activation of pro-apoptotic proteins. Arsenic exposure weakens the activity of immune cells (lymphocytes, monocytes, and macrophages) and further influences cellular immune responses, resulting in immunosuppression [28]. Meanwhile, arsenic can decrease CD4⁺ cell numbers from the spleen, suppress contact hypersensitivity responses and increase CD8⁺ cell numbers. Accumulating evidence has shown that arsenic exposure interferes with the activation of T cells and inhibits immune cell migration in animal models [29]. The results of the present study suggested that arsenic exposure significantly increased the levels of TNF- α , IL-1 β and IL-6 compared with controls. Arsenic exposure increased the relative frequency of the CD8⁺ cell subpopulation and decreased the frequency of CD4⁺ cells. In addition, arsenic significantly decreased T (CD3) and B (CD19) cells compared with controls. These changes may cause cellular dysfunction due to the development of inflammatory reactions and the secretion of a variety of inflammatory cytokines. Several lines of study have also shown that supplementation of dietary antioxidants may reverse arsenic-induced oxidative damage and immunotoxicity [30]. Accumulating evidence has revealed that EGCG, as a potent antioxidative agent, can inhibit inflammation and enhance the survival of disc cells in oxidative stress [31]. The anti-inflammatory effects of EGCG on radiculopathic pain have also been investigated *in vitro* and may benefit from its inhibitory effect on the overexpression of pro-inflammatory mediators [13, 32]. Given the inhibition of immune cell-derived cellular functions by arsenic exposure and the antioxidative and anti-inflammatory properties of EGCG in antagonizing increased secretion of inflammatory mediators, simultaneous exposure to EGCG has been found to be beneficial in increasing arsenic mobilization and assisting the recovery of altered biochemical levels during chemical therapy. In the present study, simultaneous treatment with EGCG significantly restored the alterations in CD8⁺ and CD4⁺ cells and ameliorated the damage in the B and T cell subpopulations in the thymus and spleen of mice. These findings suggested that EGCG could modulate NaAsO₂-induced immunotoxicity in mice.

Numerous studies have shown that EGCG possesses extensive pharmacological actions in various diseases, including immunosuppression in mice [18]. It has been reported that there is a positive correlation between the application of EGCG and reduction of heavy metal poisoning-related risk in both epidemiological and experimental studies. These actions of EGCG originate partially from its antioxidant properties. Moreover, EGCG can also inhibit oxidative stress by increasing the activity of endogenous antioxidant enzymes and protein expression, decreasing apoptosis rate and attenuating ROS production. In the present study, arsenic treatment decreased the level of NO and the activity of SOD, CAT, GSH and increased the content of MDA in NaAsO₂-treated mice. These indices are well proven as special markers in the diagnosis of arsenic-related organ toxicity. Administration of EGCG apparently decreases the levels of these enzymes in the thymus of mice. These findings suggested that EGCG could attenuate oxidative stress in NaAsO₂-treated mice.

Arsenic has been shown to be a strong inducer of apoptosis in various models through excessive production of ROS, decreased mitochondrial membrane potential, activation of caspase, increased fragmentation of DNA and impairment of T-cell activity via up-regulation of immune and stress response genes associated with increased production of ROS, which might be involved in the apoptosis and necrosis that it induces in immune cells [10, 33-35]. Earlier reports demonstrated a strong link between immunotoxicity and formation of reactive oxygen species (ROS), which is related to arsenic exposure [36]. Generation of ROS induced by arsenic is involved in the regulation of the initiation of apoptosis in the lymphocytes and is also involved in the immunotoxic response. Given the important role of oxidative stress in arsenic-related immunosuppression and apoptosis, ROS scavengers or antioxidants may be a promising approach for modulating the immunotoxic response. It has been reported that EGCG acts as an antioxidant and immune booster both *in vivo* and *in vitro* [37]. Thus, we speculated that EGCG might play an important role in arsenic-related disorders. In the present study, we found that arsenic caused mitochondrial damage along with an excess of ROS production and finally promoted apoptosis in thymocytes and splenocytes, which is consistent with previous studies. Supplementation with EGCG protected against the harmful

action of free radicals and exhibited ameliorating effects on NaAsO₂-induced apoptosis. EGCG, as a potent antioxidant, can enhance antioxidative activity, which increases resistance to oxidative injury *in vivo* and *in vitro* [38]. In the present study, one of the most interesting observations is the ability of EGCG to reduce the concentration of arsenic in the thymus and spleen tissue, decreasing the arsenic burden in target tissues by its metal chelating properties. This action may play a crucial role in its immunoprotective effect. These findings suggest that the protective effect of EGCG against NaAsO₂-induced immunotoxicity may involve its potent antioxidant activity.

Conclusion

In conclusion, the present study revealed that EGCG could attenuate NaAsO₂-induced immunotoxicity and that the mechanism may involve its potent antioxidant capacity. These findings suggest that EGCG has clinical potential for the amelioration of NaAsO₂-induced inflammation and apoptosis.

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Disclosure Statement

There is no conflict of interest.

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