

Stereological Study of the Protective Role of Curcumin on Histological Changes of Seminal Vesicle in Mice Following Treatment with Sodium Arsenite

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Abstract

Background & Aims: Sodium arsenite causes oxidative stress and endocrine disorders in the reproductive system. The aim was to investigate the protective role of curcumin as an antioxidant on the seminal vesicle tissue in adult mice treated with sodium arsenite.

Materials & Methods: Adult male Naval Medical Research Institute (NMRI) mice were randomly divided into 4 groups (six mice per group) including control, Sodium arsenite (5mg/kg/day b.w.), Curcumin (15mg/kg/day b.w.) and Sodium arsenite + Curcumin. After 5 weeks of treatment, the mice were anesthetized and the seminal vesicles were removed and weighed. This followed by evaluating of the mean total volume of seminal vesicle and the mean volume of parenchyma, vesicular fluid, epithelial cells, vessels, connective and muscular tissues using stereological techniques. The results analyzed statistically by one-way ANOVA and Tukey's test and the means were considered significantly different at $p < 0.05$.

Results: A significant reduction in the mean total volume of seminal vesicle and mean volume of vesicular fluid ($p < 0.001$), and a significant increase in the mean volume of connective tissue ($p < 0.001$) were seen in the sodium arsenite group when compared to the control ones. The mentioned parameters were compensated to the control level in the sodium arsenite + curcumin group.

Conclusion: Our results showed that curcumin could prevent the adverse histological changes induced by sodium arsenite treatment on the seminal vesicle in mice.

Keywords: Sodium arsenite, Curcumin, Seminal vesicles, Stereology, Mice

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Introduction

Arsenic is an inorganic compound widely distributed in the environment (1) and is used in the production of pesticides, fungicides, wood preservatives, metallurgy, glass (2), coal-fired power plants and cosmetics (1). So, arsenic can be released into the atmosphere from natural sources and by human industrial activities and can pollute the water, air and soil (2, 3). Arsenite (trivalent form) and arsenate (pentavalent form) are among the most common forms of arsenic; in the meantime, trivalent arsenic has a greater potential for toxicity compared to the pentavalent (2). Due to the high reactivity of sodium arsenite to endogenous thiol groups, especially dithiols, it is being considered among the most harmful arsenic compounds (4) which damages DNA and ultimately changes cell performance through the production of Reactive Oxygen Species (ROS) and oxidative stress (6, 5). Arsenic is a potential occupational carcinogen and recently is known as one of the predominant causes of disorders such as keratosis, peripheral neuropathy, vascular disease and male infertility (7). Studies have shown that arsenic toxicity in laboratory animals increases the lumen of the seminiferous tubules, reduces sperm storage, reduces the number of mature sperm, reduces sperm viability and motility and thus reduces the ability of sperm and oocytes (7, 8, 9). According to the researches, arsenic can impair of male reproductive system through the inhibition of spermatogenesis, decreasing the activity of antioxidant defense system in the testes, reducing the amount of testosterone and gonadotropins and reducing testis weight and appendix sexual organs (1, 10, 11). Reports also showed decreased seminal vesicle weight of animals treated with sodium arsenite (1, 2, 3) In addition, high levels of radioactive arsenic have been found in the epididymis and the seminal tract of rodents (2, 7).

Curcumin or C₁₂H₂₀O₆ is a polyphenol and the most active component of curcuma longa (12) which acts as a free radical cleansing and is considered as an effective antioxidant against oxidative stress damage which can significantly inhibit the production of reactive oxygen species both in Vivo and in Vito (13, 14). Antioxidant properties of curcumin and male reproductive system protection against oxidative stress and environmental pollutants such as nitrates, aflatoxins, cadmium, chromium, acrylamide and lindane have been noted in many studies (14-19). However, few studies have reported the effects of curcumin on the seminal vesicle, indicating its effect in offsetting the adverse effects of metronidazole (20) and Chrome on seminal vesicle (14). According to the studies, no report has been provided about the effect of curcumin on rat's seminal vesicle treated with sodium arsenite yet. Since the seminal vesicle is a male sexual organ and its function is to secrete a part of semen and plays an important role in activating of sperm and sperm survival in the vagina acidic environment (21, 22), the aim of this study was to investigate the protective effect of curcumin as a powerful antioxidant on the seminal vesicle tissue of mice after treatment with sodium arsenite using stereological technique.

Materials and Methods

Animals:

In this experimental study, adult male NMRI rats with an average weight of 31.05 ± 2.25 g were purchased from the Institute Pasteur Institute of Iran – Tehran and were kept at the Animal House of Arak University for 12 hours in darkness, 12 hours in light and at the temperature of 21 ± 2 °C with free access to food and water. The mice were randomly divided into 4 groups (n = 6 per group) of control, sodium arsenite (5 mg per kg of body weight per day, Merck of Germany), Curcumin-treated group (at a dose of 15 mg per kg of body weight per day, Sigma Corporation of

America) and sodium arsenite-curcumin treated group. The treatment was performed by intraperitoneal injection for 5 weeks. The doses used in this study were selected according to the studies (23, 24).

Histological and stereological studies:

After 5 weeks of treatment, mice were anesthetized by diethyl and seminal vesicles of both sides were taken out after opening the lateral anterior wall of the abdomen and their weights were measured. Then the primary volume (V_{primary}) of seminal vesicle was measured by dipping method and tissues were placed in Bowen fixative to prove for 24 hours (20). In this method, a beaker containing distilled water was weighed by scales (W1), seminal vesicle was then floated inside by a very thin thread and the beaker containing seminal vesicle was re-weighed (W2). Since the density of distilled water was one, the weight difference was considered as the initial volume in cm^3 of the seminal vesicle. Then, isotropic uniform random (IUR) parts of seminal vesicle tissue were prepared (20, 25) using Orientator method (25, 26). For this purpose, each lobe of the gland had been laid on phi (ϕ) with 10 equal distances. A random number was selected between zero and 10 and tubers were cut in two halves along the selected number. Then the cut surface of one of the halves was put along and the other was tangent to theta (θ) of axis 0-0 which was divided into 10 unequal parts and were cut along a randomly selected number. So that each half is divided into slabs and total 9-12 slabs were collected from seminal vesicles of each mouse. Round pieces of slabs of seminal vesicle were prepared by trocar in order to calculate the shrinkage. Two randomized diameter of round parts of gland were measured by caliper and their area (AB) were calculated. Then, tissue processing of the slabs and the round parts was performed by Tissue prosessor (Leica, Histokinette; Germany) and dipped and molded in paraffin. the samples' order in the templates containing paraffin was

in a format that each IUR section of a rat seminal vesicle were put within two or three blocks and pieces of the trocar were individually put inside a block of paraffin to calculate the shrinkage. After completing these steps, 5-micrometer thick sections prepared by microtome and histological staining was performed by hematoxylin-eosin method. The diameter and the area of circular pieces (AA) were measured again after coloring and shrinkage was calculated using the following formula (20, 25).

$$\text{Volume shrinkage} = 1 - (\text{AA}/\text{AB})^{1.5}$$

AA and AB are the volumes of round parts, respectively, before and after tissue processing, sectioning and staining. After estimating the shrinkage, the final volume of the seminal vesicle was estimated using the following formula (20, 25):

$$V_{\text{final seminal vesicle}} = V_{\text{primary}} \times (1 - \text{volume shrinkage})$$

Counting method was used to estimate the volume of tissue and vesicular fluid (20, 27), as the density of these components were estimated using optical microscope (Olympus, BX51) and magnification of 10 \times . For this purpose, the point probe was thrown randomly on microscopic images and then the points dealt with parenchyma surface or vesicle fluid and whole sections of the seminal bag were counted and their volume fraction (V_v) was estimated by the formula:

$$V_{V(\text{structure/gland})} = \frac{\sum_{i=1}^n P_{(\text{structure})}}{\sum_{i=1}^n P_{(\text{gland})}}$$

Where $\sum_{i=1}^n P_{(\text{structure})}$ is the total number of points dealt with profiles parenchyma, or vesicle fluid and $\sum_{i=1}^n P_{(\text{gland})}$ is

the number of points dealt with all sections of seminal vesicle. Then parenchymal volume and vesicle fluid then were determined by multiplying the bulk density in the final volume of seminal vesicle (20, 27).

In order to estimate the volume fraction of the parenchyma components, 10-14 microscopic fields per box was studied with a magnification of 40×. Systematic random selection method was used to select the microscopic field. For example, the position of the first field was randomly selected out of the incisions and then subsequent microscopic fields were selected by moving the microscope field at equal distance along the X and Y axis until the study of all slices on a slide. Point counting method and the following formula were used for the calculations (20, 27):

$$V_{V(\text{structure/parenchyma})} = \frac{\sum_{i=1}^n P_{(\text{structure})}}{\sum_{i=1}^n P_{(\text{parenchyma})}}$$

Where $\sum_{i=1}^n P_{(\text{structure})}$ is the total number of points collide with epithelial cells profiles, vessels, connective tissue and smooth muscle tissue and $\sum_{i=1}^n P_{(\text{parenchyma})}$ is the total number of points collide with parenchyma profiles. Volume fraction of each parameter was multiplied by the total volume parenchyma to calculate the volume of the above-mentioned components.

Statistical analysis:

Statistical analysis of the data, using software (version 16) SPSS and one-way analysis of variance (One-way ANOVA) and Tukey test have been conducted. The difference was considered as significant at the level of $P < 0.05$.

Results

Histological study of the seminal vesicle:

Histological studies of seminal vesicle showed that epithelial cells were columnar in the control group and vesicle fluid volume was normal with uniformity and

the structure and the thickness of connective and muscle tissue was normal (Figure A -1), but seminal vesicle atrophy, vesicle fluid volume reduction and epithelial cell clutter had been observed in sodium arsenite. In addition, the epithelial cells had been deformed to cylindrical cubic forms and accumulation of abnormal masses of epithelial cells of seminal vesicle gland was observed in some areas of connective tissue, probably due to the loss of cells (Figure B-1). The tissue structure of the group which received sodium arsenite and curcumin simultaneously, was similar to the control group and the vesicle fluid volume had increased to a large extent as compared to sodium arsenite group (Figure C-1). Histological observation in curcumin group showed that normal seminal vesicle structure tissue similar to the control group (Figure D-1).

The mean total volume of seminal vesicle, parenchymal volume and vesicle fluid volume:

The mean total volume of seminal vesicle and vesicle fluid volume showed a significant reduction in the sodium arsenite group compared to the control group ($P < 0.001$) while no significant difference was observed in the comparison of the mean tissue volume in different groups ($P > 0.05$). A significant increase in mean total volume of seminal vesicle and vesicle fluid volume was observed in sodium arsenite + curcumin group compared to sodium arsenite ($P < 0.001$) and curcumin was able to reach the value of this parameter the same as in the control group (Table 1).

The average volume of epithelial cells, vascular volume and the volume of connective tissue and muscle:

No significant difference had been observed in the comparison of the average volume of epithelial cells and vascular volume in different groups ($P > 0.05$). The mean volume of seminal vesicle connective tissue of

mice treated with sodium arsenite was significantly higher compared with the control group ($P < 0.001$). The volume of muscle tissue may also be increased partly in sodium arsenite group compared with the control, but the increase was not significant ($P > 0.05$).

Body weight and seminal vesicle weight:

No significant difference has been observed through comparison of the mean body weights of mice

in different groups ($P > 0.05$). Seminal vesicle weight in sodium arsenite group was significantly lower than control ($p < 0.01$), while curcumin increases seminal vesicle weight in sodium arsenite + curcumin group compared to sodium arsenite ($p < 0.05$). A significant difference was observed through comparison of weight of seminal vesicle treated with sodium arsenite + curcumin and control ($P > 0.05$) (Table 3).

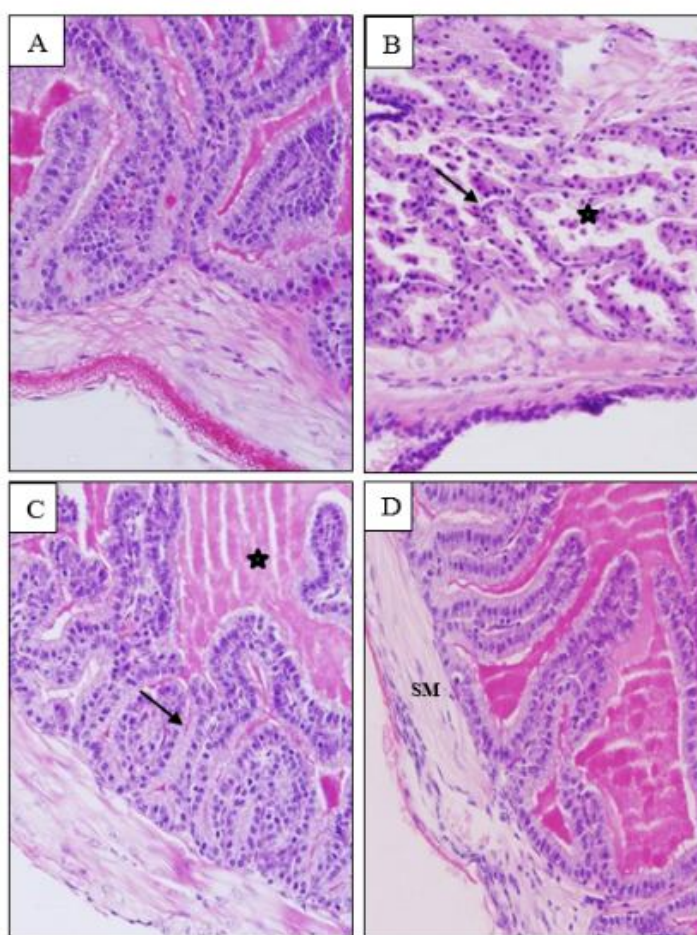


Figure 1. The histological images of the seminal vesicle in different groups of mice 5 weeks after treatment with sodium arsenite (5mg/kg b.w.), Curcumin (15mg/kg b.w.) and sodium arsenite + curcumin. **A)** Control group which represents normal seminal vesicle tissue. **B)** Sodium Arsenite Group, reduction of vesicle fluid (star) and the epithelial secretory destruction (arrows) can be seen. **C)** Sodium Arsenite + Curcumin Group, Indicative of a similar texture to the control group where an increase in vesicle fluid (star) and the normal secretory epithelium (arrows) can be seen. **D)** Curcumin Group, the normal structure of the seminal vesicle tissue can be seen. Smooth Muscle (SM) (5-micron slice, H & E staining, magnification of 40 \times).

Table 1. Comparison of the total seminal vesicle weight, Parenchymal volume and Vesicle fluid volume (mm³) in different groups of mice 5 weeks after treatment with sodium arsenite (5mg/kg b.w.), curcumin (15mg/kg b.w.) and sodium arsenite + curcumin. Values are as Means \pm SD. Means with different letter codes have significant difference (One-way ANOVA, Tukey's test, $P \leq 0.05$).

Groups	Total seminal vesicle weight	Parenchymal volume	Vesicle fluid volume
Control	198.67 ^a \pm 16.48	46.30 ^a \pm 5.10	152.37 ^a \pm 68.11
Sodium Arsenite	157.61 ^b \pm 16.75	52.89 ^a \pm 7.47	106.38 ^b \pm 08.13
Sodium Arsenite + Curcumin	191.81 ^a \pm 12.20	47.36 ^a \pm 3.80	144.45 ^a \pm 09.34
Curcumin	200.90 ^a \pm 16.76	47.98 ^a \pm 3.39	152.91 ^a \pm 14.47

Table 2. Comparison of the average volume of epithelial cells, vascular volume and the volume of connective tissues and muscles (mm³) in different groups of mice 5 weeks after treatment with sodium arsenite (5mg/kg b.w.), curcumin (15mg/kg b.w.) and sodium arsenite + curcumin. Values are as Means \pm SD. Means with different letter codes have significant difference (One-way ANOVA, Tukey's test, $P \leq 0.05$).

Groups	Epithelial cell volume	Vascular volume	Connective tissue volume	Muscle tissue volume
Control	29.84 ^a \pm 3.18	0.66 ^a \pm 0.09	2.03 ^a \pm 0.20	14.27 ^a \pm 2.15
Sodium Arsenite	32.78 ^a \pm 4.42	0.56 ^a \pm 0.10	2.98 ^b \pm 0.52	16.54 ^a \pm 3.15
Sodium Arsenite + Curcumin	28.66 ^a \pm 3.21	0.60 ^a \pm 0.11	2.38 ^{ab} \pm 0.27	15.67 ^a \pm 1.71
Curcumin	29.13 ^a \pm 3.17	0.65 ^a \pm 0.09	2.34 ^a \pm 0.28	15.93 ^a \pm 1.73

Table 3. Comparison of body weight and seminal vesicle weight (g) in different groups of mice 5 weeks after treatment with sodium arsenite (5mg/kg b.w.), curcumin (15mg/kg b.w.) and sodium arsenite + curcumin. Values are as Means \pm SD. Means with different letter codes have significant difference (One-way ANOVA, Tukey's test, $P \leq 0.05$).

Groups	Body weight	Seminal vesicle weight
Control	35.71 ^a \pm 1.97	0.24 ^a \pm 0.02
Sodium Arsenite	33.79 ^a \pm 1.87	0.19 ^b \pm 0.03
Sodium Arsenite + Curcumin	34.62 ^a \pm 2.30	0.23 ^a \pm 0.01
Curcumin	34.23 ^a \pm 2.43	0.25 ^a \pm 0.03

Discussion

The present study showed that the weight, the volume of seminal vesicle and vesicle fluid volume in mice of sodium arsenite had significant decrease compared to the control group and the amount of connective tissue showed significant increase and treatment with curcumin was able to compensate the factor in the control group. Other researchers reported

seminal vesicle atrophy and weight loss in laboratory animals treated with sodium arsenite (1-3). Seminal Vesicles Atrophy is mainly in connection with reduced androgens that manifests as wrinkling and reduced size of the seminal vesicles and histologically is along with a reduction in the release of seminal vesicle, the destruction of the epithelium and increment of muscle-fiber stroma (28).

Weight loss of Seminal vesicle as a tumor responsible for nutrients discharge of seminal fluid shows the impression of epithelial cell secretions by treatment with sodium arsenite. The decline in testosterone levels in animals treated with arsenic is the most important cause of seminal vesicle secretion (6, 10, 29). On the other hand, Reactive Oxygen Species (ROS) such as superoxide anion radicals and hydrogen peroxide H_2O_2 plays an important role in the development of endocrine disorders due to environmental chemicals and inhibits steroidogenesis in the testis (30). Sodium arsenite causes hormonal imbalance (31). Arsenic levels inhibit steroidogenesis and reduce the level of testosterone in the testicles by increasing mitochondrial ROS and reduction of the enzymes 3-beta-hydroxy steroid dehydrogenase (3β -Hydroxysteroid dehydrogenase: 3β -HSD), 17-beta-hydroxy steroid dehydrogenase (17β -Hydroxysteroid dehydrogenase: 17β -HSD) as an enzyme involved in the biosynthesis of steroid hormones.

Also, arsenic may reduce the levels of testosterone in the Leydig cells by reducing mRNA expression of StAR protein (steroidogenic acute regulatory protein) and cytochrome P450_{scc} (Cholesterol side-chain cleavage enzyme) (7, 10). StAR protein is essential for the synthesis of testosterone and is responsible for transporting cholesterol to the inner mitochondrial Leydig cells. P450_{scc} cytochrome enzyme in P450 cytochrome group is involved in the conversion of cholesterol to pregnenolone in the inner mitochondrial membrane of Leydig cells and is the first enzymatic step in the biosynthesis of testosterone (32). The decrease in both expressions by arsenic leads to a decrease in testosterone synthesis. According to the above researches, it can be said that sodium arsenite reduces testosterone levels by several mechanisms, resulting in dysfunction in the seminal vesicle as an androgen-dependent organ. Androgenic steroids can lead to structure adjustment and function of the seminal

vesicles, inhibition of apoptosis of epithelial cells and stimulation of cell proliferation in these organs (33). In addition, testosterone induces the secretion of fluids and electrolytes in the seminal vesicles. Seminal vesicle contains high levels of 5-alpha-reductase enzyme that indicates the ability of the organs in the conversion of testosterone to dihydrotestosterone (DHT) (21). As reported, the concentration of DHT is 8 times higher than testosterone in the rat seminal vesicle (33). DHT can affect the volume and viscosity of semen and leads to up-regulation of mRNA of seminal vesicles secreted proteins (21). The decrease in testosterone and subsequently DHT by sodium arsenite can reduce the secretion of the seminal vesicles. Fructose is a monosaccharide, an important material synthesized and secreted by the seminal vesicles and involves in the development of energy metabolic. It was found that sodium arsenite can reduce the formation ability of fructose by seminal vesicle (6). Fructose synthesis is regulated by androgens and is directly associated with testosterone levels (34). As in a study, Wistar rats' treatment with arsenic trioxide to the amount of 3mg / kg / day for 28 days showed a significant decrease in the fructose content of the seminal vesicle, and dysfunction of the organ in addition to the seminal vesicle weight loss (6). Thus, sodium arsenite leads to lower seminal vesicle secretions by reducing the level of testosterone and reduces the vesicle fluid volume and causes atrophy of seminal vesicle.

The results of the body weight showed that there was not significant difference between the treatment and control groups at the end of the treatment period. These results indicate that 5mg / kg / day sodium arsenite treatment of adult mice for 35 days has a significant impact on the metabolic activity of mice and thus a change in weight. This result is in line with the results of some previous studies (8, 9, 11). However, some studies have reported body weight loss

in the animals treated with sodium arsenite (1,2,3,25). The differences in the results might be due to treatment duration or the dose of arsenic (8).

As noted in the study, curcumin in the mice receiving sodium arsenite and curcumin simultaneously, increases the weight and volume of the seminal vesicle, increases the size of the vesicle fluid and reduces the volume of connective tissue normally. In certifying the results of the present study, in 2013, Khan and his colleagues showed that curcumin treatment leads to a significant increase in seminal vesicle weight as in the control group. In addition, curcumin improves parameters associated with oxidative stress, increases enzyme activity in the testes, increases mRNA expression of 17β -HSD and CYP450scc enzymes and level of testosterone in the testes of rats (10). Other studies indicate a protective effect of curcumin on the seminal vesicle tissue in laboratory animals treated with various pesticides which is in agreement with our results. So that intraperitoneal of 20mg / kg / day of curcumin for 26 days can increase the seminal vesicle weight and serum testosterone levels in rats treated with chromium (14). It was shown in another study that simultaneous administration of curcumin and metronidazole could prevent reduction in the size of the seminal vesicle, and vesicular fluid due to metronidazole in mice (20). In addition, curcumin improves the activity of 17β -HSD and 3β -HSD enzymes in the rats treated with aflatoxin (35). The results of various studies have shown that curcumin is a strong antioxidant, and one of the most powerful free radical cleansings and increases intracellular glutathione, which is capable of producing different kinds of ROS in vivo and in vitro (14, 15). In

fact, the presence of beta-Dy-ketone phenolic groups and methoxy groups in curcumin plays a role in free radical scavenging activity (36). By maintaining the activity of antioxidant enzymes such as superoxide dismutase, catalase and glutathione peroxidase in high levels, curcumin can reduce the lipid peroxidation (37). In addition, curcumin inhibits the oxidation of the thiol groups (38). The protection of the sexual glands antioxidant not only is due to their antioxidant capacity improvement, but also due to restructuring and adjustment of osteogenesis against the toxicity of arsenic (6).

The effect of curcumin against the toxicity of sodium arsenite on seminal vesicle had been studied for the first time in the present study. Results showed that curcumin could improve the histopathological lesions induced by sodium arsenite on the seminal vesicle tissue and possibly curcumin applies the effect by increasing the antioxidant defense and improving androgen levels. However, it is suggested to measure the effects of curcumin on serum testosterone level of rats treated with sodium arsenite in addition to measuring the levels of antioxidant enzymes to determine the exact mechanism of the effect of curcumin and sodium arsenite on seminal vesicle.

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