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Arsenic induced histological alterations in testis of Swiss albino mice and protection by *Chlorophytum borivilianum*

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ABSTRACT

Objective: To investigate the sodium arsenite induced damages on the reproductive system of male mice as well as to examine whether *Chlorophytum borivilianum* (*C. borivilianum*) is able to ameliorate the damages. **Methods:** A different group of adult Swiss albino mice was made such as control group, sodium arsenite group, post combination group, pre and post combination group. After the treatment, the testis was removed, weighed and processed for histopathological observation. Cauda epididymis was used to observe abnormal sperms. **Results:** A highly significant depletion was found in all the germ cell populations such as spermatogonia A and B, primary and secondary spermatocytes, spermatids in arsenic treated group with respect to control whereas recovery is found in combination groups. **Conclusions:** The results conclude that *C. borivilianum* significantly protects against arsenic-induced damages.

1. Introduction

Health hazards caused by heavy metals have become a great concern to the population due to their environmental pervasiveness. Metal intoxication particularly neurotoxicity, genotoxicity or carcinogenicity are widely known. The toxic manifestations of these metals are caused primarily due to imbalance between pro-oxidant and antioxidant homeostasis which is termed as oxidative stress and also due to a high affinity of these heavy metals for thiol groups on functional proteins[1].

Arsenic, a metalloid, occurs naturally, being the twentieth most abundant element in earth's crust and is a component of more than 245 minerals[2]. Exposure to lower levels of arsenic can cause nausea, vomiting, anemia, abnormal heart rhythm, damage to blood vessel and a sensation of "pins and needles" in hands and feet[3].

Exposure to arsenic contaminated drinking water causes several health problems such as hypertension, diabetes mellitus, cancers of skin, liver, kidney, lung and bladder in humans[4], blackfoot disease, disturbances in nervous system, skin lesions, cardiovascular effects[5] and diseases of the respiratory system[6].

Arsenic exposure was shown to depress the antioxidant defense system leading to oxidative damage to cellular macromolecules including DNA, proteins, lipids[7], wreak havoc in biological system by tissue damage, altering biochemical compounds and corroding cell membranes [8]. It interferes with metabolism of essential antioxidant molecules responsible for metabolism and excretion of xenobiotics[9].

Inorganic arsenic has a suppressive influence on spermatogenesis and androgenesis in male reproductive system. It causes testicular toxicity probably by affecting the pituitary testicular axis[10]. Environmental sodium arsenite is a toxin that is associated with male infertility due to decreased and abnormal sperm production[11].

Testis is one of the sensitive reproductive organs because of cell renewal system. The protection of these cells is therefore of prime importance since any harmful effect of heavy metal exposure will pass through generation and may affect progeny.

Remedies from plant sources have proved to be effective in primary healthcare in Indian tradition. *Chlorophytum borivilianum* (Liliaceae) (*C. borivilianum*) also known as 'Safed Musli' is a traditional rare Indian medicinal

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herb which has many therapeutic applications. Major phytochemical components reported from the roots of *C. borivilianum* include saponins, fructo-oligosaccharides (FOS), acetylated mannans, phenolic compounds and proteins[12]. Its root contains steroidal and triterpenoidal saponins, sapogenins and fructans which act as therapeutic agents and play vital role in many therapeutic applications. *C. borivilianum* root is aphrodisiac, adaptogen, antiageing, health restorative and health promoting[13]. Roots of the plant are used to treat oligospermia, arthritis, diabetes and dysuria. Its immunomodulatory, antiviral, anticancer, antistress, aphrodisiac[14], antimicrobial, anti-inflammatory and antioxidant[15] activities have been evaluated. The objective of the conducted study was to investigate the protective effect of *C. borivilianum* against arsenic induced testicular alterations.

2. Materials and methods

2.1. Test system

Adult male Swiss albino mice (6–8 weeks old, weighing 25 ± 2 g) maintained in the animal house as inbred colony (Procured from IVRI, Izatnagar, India) under controlled conditions of temperature (25 ± 2 ℃), relative humidity (50% ± 15%) and normal photoperiod (12 h light and 12 h dark). The animals were housed in standard polypropylene laboratory cages containing 5 cm deep layer of sawdust bedding. Mice were given standard mice feed (Hindustan Lever Ltd., India) and tapwater *ad libitum*. Once in a fortnight tetracycline water was given as a preventive measure against infection. The ethical committee of Department of Zoology, University of Rajasthan, Jaipur (India) has approved to carry out the experimental protocol.

2.2. Test chemical and Plant material

Arsenic in the form of $NaAsO_2$ (mol. wt. 129.9) was obtained from standard commercial suppliers [Himedia, Mumbai, India Ltd.]. The roots of *C. borivilianum* were collected from the market and it was identified (RUBL No.19902) from the herbarium of Department of Botany, University of Rajasthan, Jaipur, India. The animals were administered *C. borivilianum* root extract dissolved in DDW orally up to 30 days (100, 200, 400, 800 mg/kg b.wt.) and LPO and GSH contents were measured in the liver. The optimum dose selection of *C. borivilianum* root extract was decided on the basis of previously performed experiments in our own laboratory[16]. Among the doses 800 mg/kg b.wt./day was selected for the study.

2.3. Experimental design

Mice selected from inbred colony were divided into 4 groups such as Group I (Control group): – Animals received double distilled water (DDW) as vehicle orally for 30 days. Group II (Heavy metal treated group):– Arsenic dissolved in DDW, was administered orally for 30 days at 4 mg/kg b.wt/day. Group III (Post combination group): – In this group, animals were given C. borivilianum (800 mg/kg b.wt. orally) 30 minutes after the oral administration of sodium arsenite (4 mg/kg b.wt.) and both sodium arsenite and *C. borivilianum* were continued upto 30 consecutive days. Group IV (Pre and post combination Group):– In this group, only *C. borivilianum* (800 mg/kg b.wt.) was given orally to animals for 10 days. From day 11, animals were administered with Chlorophytum (800 mg/kg b.wt. orally) 30 minutes after oral treatment of sodium arsenite (4 mg/kg b.wt.). Thus the animal received $NaAsO_2$ and *C. borivilianum* root extract both upto 30 days. The total period of experiment was of 40 days.

The animals from all the groups were weighted and sacrificed on 1, 3,7, 15,30 days. Testis were removed and blotted.

2.4. Histopathological studies

2.4.1. Qualitative assessments of various cell types in the testis

The testes from the autopsied animals were fixed in Bouin's fluid for 24 hrs. The tissue was further processed by standard method. The sections were cut at 5 μ and stained with Harris haematoxyline and eosin to observe various cell types in the testis [Haematoxyline and eosin staining (400×)].

2.4.2. To observe the various stages of spermiogenesis

To observe spermiogenesis stages (golgi phase, cap phase, acrosome phase, maturation phase) the testes were fixed in Carnoy's fixative and the sections were stained by Standard Feulgen method using Schiff's stain [17].

2.4.3. Sperm dynamics

Abnormal sperms– Sperm smears were stained with nigrosin–eosin stain[18] and abnormal sperms were observed.

3. Results

In control group the tubules showed all the stages of spermatogenesis with mature sperms in lumen. Leydig cells and Sertoli cells appeared normal. In arsenic treated group on day 1 there was a reduction in all the germ cell populations such as spermatogonia A and B, primary and secondary spermatocytes, spermatid. On day 3 basement membrane showed wavy outline due to shrinkage. Exfoliation of germ cells in the lumen with prominent cytoplasmic vacuolization was observed. Interstitium was filled with oedematous fluid. On day 7 cytoplasmic vacuolization was evident due to badly affected germ cell population such as spermatogonia A and B, primary and secondary spermatocytes, spermatids and in the lumen broken sperms were observed. Shrinkage in Leydig cell nuclei were evident. On day 15 spermatogonial population along with primary spermatocytes were depleted.

Spermatids and sperms were badly affected. On day 30 Depletion of almost all the germ cell population along with cytoplasmic vacuolization was noticed. Shrinkage of Leydig cell nuclei and Sertoli cell were evident. From day 1 to day 30 there is significant recovery in all the germ cell population in both combination groups. In combination groups all the successive stages of germ cells i.e. from spermatogonia upto spermatids were present. Prominent increase in spermatogonial population along with all the germ cells (Figure 1–7).

In the present study abnormal sperm morphology such as sperms with broken head, broken tail, head anomalies such as anonymous shape, banana shape was observed in arsenic treated group (Figure 8).

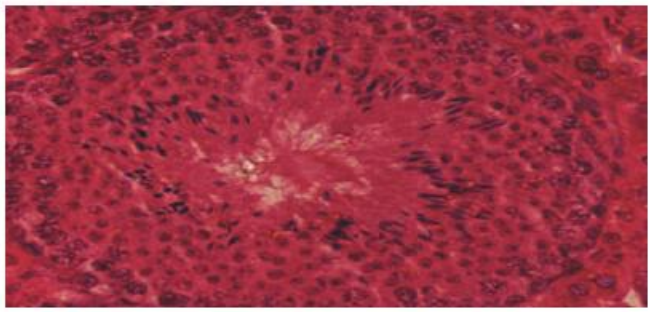

Figure 1. Control group showing active spermatogenesis with spermatogonia A (SgA), spermatogonia B (SgB), primary spermatocytes (PriS), secondary spermatocytes (SecS), spermatids (Sd).

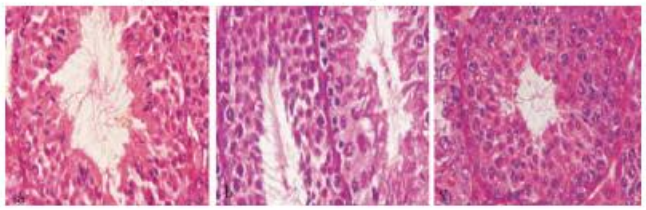

Figure 2. Day 1 (a) Arsenic treated (b) Post combination (c) Pre and post combination.

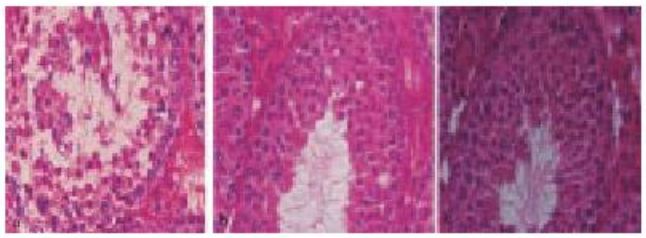

Figure 3. Day 3 (a) Arsenic treated (b) Post combination (c) Pre and post combination.

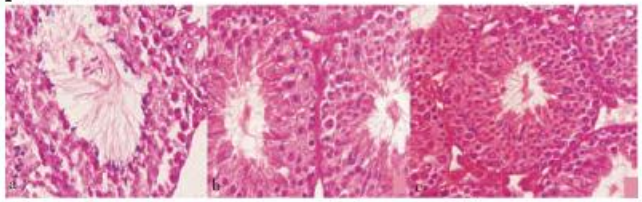

Figure 4. Day 7 (a) Arsenic treated (b) Post combination (c) Pre and post combination.

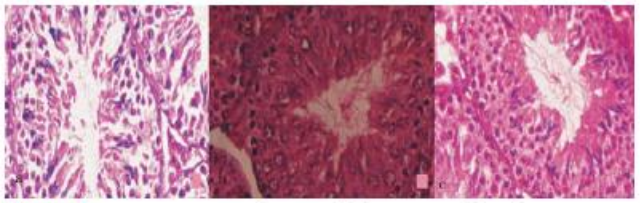

Figure 5. Day 15 (a) Arsenic treated (b) Post combination (c) Pre and post combination.

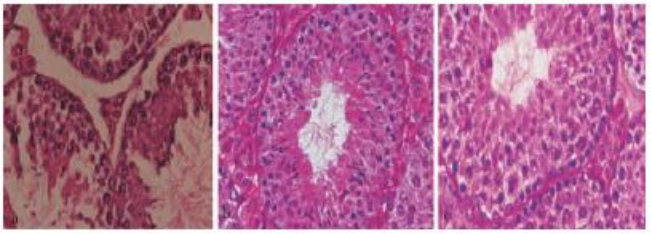

Figure 6. Day 30 (a) Arsenic treated (b) Post combination (c) Pre and post combination.

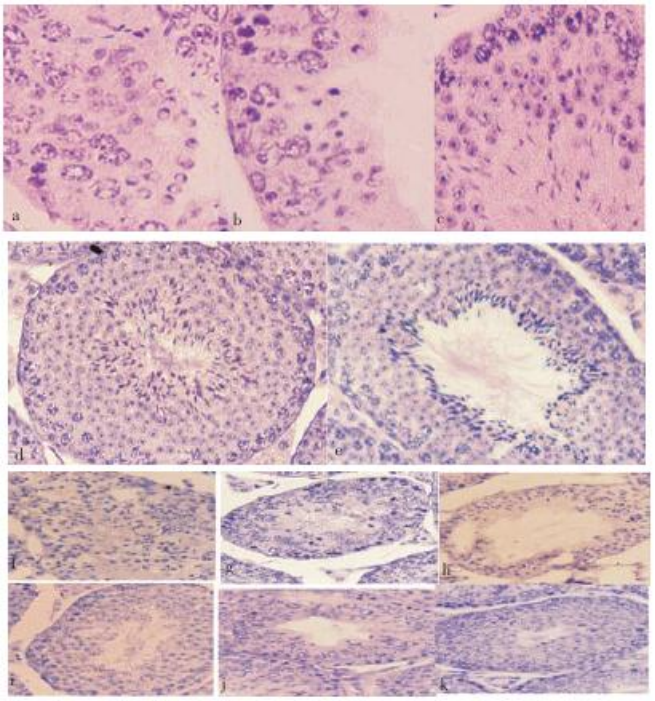

Figure 7. Various stages of spermiogenesis (Standard Feulgen method using Schiff's stain).

a–c: Photomicrograph of testis showing stages of spermiogenesis (The Golgi phase (G), Cap phase (C), Acrosome phase (A), Maturation phase (M). (1000×); (d): Control group showing all the stages of spermatogenesis; (e): C. borivilianum treatment showed active spermatogenesis in testis(400×); (f):Arsenic treatment showed disorganized germ cells; (g–h):– Arsenic treatment showed shrinkage of seminiferous tubules and depletion of germ cell population (400×); (i–k): Combination group showed recovery of germ cell populations along with all the spermiogenetic stages(400×).

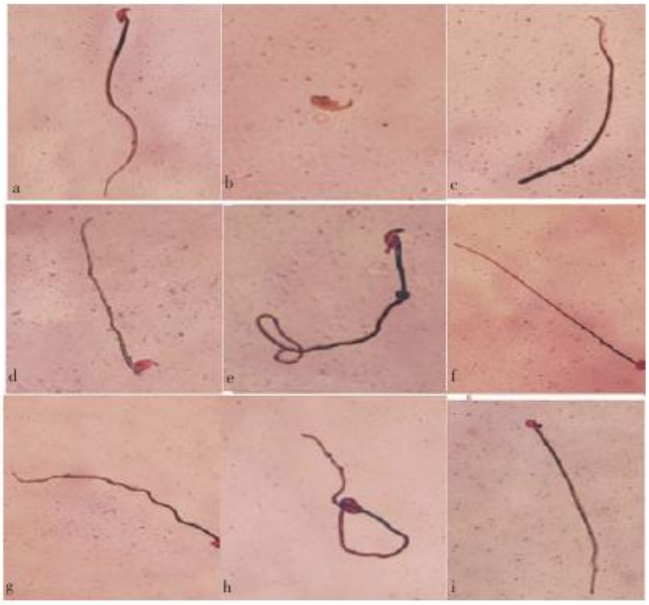

Figure 8. Photomicrograph of sperms from cauda epididymis (Eosine & Nigrosine Stain) (1000 ×).

(a): Normal sperm with head, an acrosome, neck, mid piece and tail; (b): Abnormal sperm with broken head; (c): Abnormal sperm with only tail; (d): Abnormal sperm with bent head; (e):Abnormal sperm with coiling tail; (f–i) Abnormal sperms with abnormal head.

4. Discussion

Spermatogenesis is a cellular process by which a subpopulation of type A spermatogonia, namely spermatogonial stem cells, divide and differentiate into sperm[19]. Spermatogonial stem cells are maintained in specialized microenvironment called the niche that is composed of the male germ cells, somatic cells, and extracellular matrix[20]. Sertoli cells or nurse cell are the major somatic cells and lie along the basement membrane and they are in close contact with male germ cells throughout spermatogenesis and envelope the developing sperm during spermatogenesis. Sertoli cells, through the formation of the blood–testis barrier, protect post–meiotic germ cells from exogenous toxicants brought by testicular blood and lymph. The Sertoli cell is the primary cellular target for toxicants[21].

Leydig cells are the endocrine cells in the testis that produce testosterone from cholesterol via a series of enzymatic pathways and steroidal intermediates under the control of luteinizing hormone (LH) from the pituitary[22,23].

In the present study, in sodium arsenite treated group Sertoli cell and Leydig cell nuclei showed shrinkage as compared to control. Heavy metals disturbed the integrity of the inter Sertoli cell tight junctions, and caused rapid and severe testicular oedema followed by haemorrhage and testicular necrosis[24].

In the present study, shrinkage of tubules was evident with almost significant reduction in tubular diameter. A highly significant reduction was found in all the germ cell populations such as spermatogonia A and B, primary and secondary spermatocytes, spermatids in arsenic treated group with respect to control.

Monsees *et al.*, [21] reported that reproductive toxicants may alter germ cell attachment, disturb apical cytoskeletal transport, or induce micro–tubule dependent transport defects. This in turn will lead to germ cells loss and disruption of the seminiferous epithelium.

Jana *et al.*, [10] reported that the spermatogenic disorder has been reflected by the diminution in the number of different generations of germ cells at stage VII of spermatogenic cycle after arsenic treatment.

A significant gradual dose dependent regression was observed in the number of spermatogonia, resting spermatocyte, pachytene and round spermatid in 30 and 40 mg/L of sodium arsenite over a period of 60 days. The spermatogonia and primary spermatocytes are the most sensitive cell stages of spermatogenesis to the toxic elements. These finding acts as an indicator that the maturation of spermatogonia through the process of meiosis has been severely distrupted following arsenic exposure[25].

Oxidative stress is a major causative factor of male reproductive failure because of high concentration of polyunsaturated fatty acids and low antioxidant capacity, male germ cells could be susceptible to oxidative stress[26]. Ahmad *et al.*,[27] reported that due to arsenic toxicity there were atrophic changes in testis and degenerative changes in spermatogenic and Leydig cells. There was decrease in diameter of seminiferous tubules, thickening of the basement membrane, early arrest of spermatogenesis, damaged Leydig cells, prominent Sertoli cells and collapsed blood vessels, showing generalized atrophy of the testes in arsenic treated group.

Histological examination of the experimental testes tissues revealed atrophy of seminiferous tubules with complete loss of spermatogenic cell layers in association with the absence of the centrally located spermatozoa in $NaAsO_2$ intoxicated animals[28,29].

Free radicals damage biomembranes, reflected by increased lipid peroxidation[30], oxidation of nucleic acid and protein, there by compromising cell integrity and function [31]. In the testis enhanced production of ROS causes significant alteration in tissue physiology, spermatogenic process or induces oxidative damage to DNA, which is potential risk to offspring[32].

Shi *et al.*,[33] provided evidence that arsenic generates free radicals that leading to cell damage and death through

the activation of oxidative sensitive signaling pathways. Generation of reactive oxygen species, alterations in the signal cascade and an imbalance in antioxidant levels, in turn triggers cellular apoptosis in cells[34] including spermatogenic cells[35] and deregulation of ROS alters expression of genes[36-38].

Chlorophytum borivilianum or safed musli is considered as the major herbal plant which has high commercial importance and is widely cultivated through India, thirteen species of Chlorophytum have been reported from India [39], among which *C. borivilianum* has highest therapeutic applications in ayurvedic system of medicine[40]. Safed Musli is celebrated as a Divya Aushad with unparalleled medicinal properties. It is a chief ingredient in the preparation of over a hundred Ayurvedic formulations. Major phytochemical components reported from the roots of *C. borivilianum* include mainly steroidal saponins, fructans and fructoligosaccharides (FOS), acetylated mannans, phenolic compounds and proteins, fibers, vitamins and Zinc. It has various therapeutic values as total rejuvenator, antioxidant and immunomodulator[41].

Arsenic initiates cytotoxicity by introducing oxidative damage. Oxidative stress arises when reactive oxygen species such as free radicals, lipid hydroperoxides, aldehydes, hydrogen peroxides are generated, which can react with cellular constituents such as thiols and lipids and alter the antioxidant defense systems[36].

In the pre and post treatment group, a highly significant increase was found in spermatogonia, spermatocytes and spermatid population along with normal Sertoli cells and Leydig cells. Mitotic figures were also observed in spermatogonial population.

Luo *et al.*,[42] and Thakur and Dixit, [43] have shown that C. borivilianum root polysaccharides (fructans and fructooligosaccharides) were effective in protecting against testicular damage and promote rejuvenation of testicular architecture as well as on sexual behaviour.

C. borivilianum enhances the sexual arousal, vigor and libido in diabetic Wistar rats and has a very promising future as a potent and safe herbal supplement [44] and are aphrodisiac, adaptogenic, antiaging, health restorative and health promoting[13] and beneficial in treating male infertility due to oligospermia[45]. In the combination groups present study showed normal testicular size and maintained spermatogenesis.

Results of the present study suggest that *C. borivilianum* root extract have modulatory influence on arsenic induced testicular histopathological alteration which may be due to its antioxidant, immunomodulatory and pharmacological properties.

Conflict of interest statement

We declare that we have no conflict of interest.

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