# MORPHOMETRICAL STUDY OF SEMINIFEROUS TUBULES OF MICE AFTER USING ARSENIC AND *CHLOROPHYTUM BORIVILIANUM.*

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## Summary

The aim of the present study was to determine the morphometrical changes in seminiferous tubules induced by arsenic and its possible protection by *Chlorophytum borivilianum* root extract. Different groups were made: (i) Control group – vehicle DDW (double distilled water) (ii) *C. borivilianum* group – 800 mg/kg b.wt. orally (iii) Arsenic group – 4.0 mg/kg b.wt. orally (iv) Combination group – *C. borivilianum* and arsenic. Quantitative assessment of germ cells such as spermatogonia A and B, primary and secondary spermatocytes, spermatids and tubular diameter were done. Arsenic treatment showed a significant decrease in the germ cells population and tubular diameter. Combined treatment of *C. borivilianum* and arsenic showed significant increase in spermatogonia A and B, primary and secondary spermatocytes, spermatids and tubular diameter as compared to arsenic treated group. The result indicates that *C. borivilianum* root extract may be useful in reducing the toxic effects induced by arsenic.

Key Words:- *Chlorophytum borivilianum,* Arsenic, Seminiferous tubule, Morphometrical changes.

## Introduction

Arsenic is a widespread environmental contaminant with mutagenic, teratogenic, and carcinogenic effects (1). Arsenic is used as herbicide, fungicide, rodenticide and causes air, soil and water pollution. Drinking polluted water is a common cause of arsenic poisioning (2). Arsenic exposure has also been associated with severe metabolic disorders such as diabetes, gastrointestinal tract disorders, cardiovascular diseases (3,4),neurological, respiratory, hepatic, hematological (5) skin, bladder, liver, and lung cancers ( 6,7) and reproductive toxicity (8).

Male germ cells may be susceptible to oxidative stress because of high concentration of polyunsaturated fatty acids and low antioxidant capacity (9).Arsenic exerts its toxicity by generating reactive oxygen species (ROS) during redox cycling (10) and metabolic activation processes that causes tissue damages (1,11). Free radicals damage biomembrane, reflected by increased lipid peroxidation (12) oxidation of nucleic acid and protein, there by compromising cell integrity and function (13). In the testis enhanced production of ROS causes significant alteration in tissue physiology, spermatogenic process or induce oxidative damage to DNA , which is potential risk to offspring (14).

*Chlorophytum borivilianum* a medicinal plant belonging to family liliaceae has been traditionally used as adaptogenic drug. *C. borivilianum* root is aphrodisiac, adaptogen, antiageing, health restorative and health promoting (15). Root extract has been previously studied for antidiabetic, antistress, immunomodulatory, anti-inflammatory, antioxidant and antimicrobial activities and these activities are due to presence of saponin, glycosides and alkaloid (16). It contains saponin, sapogenin, fructans (17) simple sugars such as glucose, fructose (18) protein, phenolics, triterpenoids, gallo-tannins and mucilage (19). The aim of the present study was, therefore undertaken to investigate the protective role of *Chlorophytum borivilianum* against arsenic induced morphometrical changes.

## Materials and methods

### Test system

Adult male Swiss albino mice (6–8weeks old,weighing 25±2g) maintained in the animal house as inbred colony (Procured from IVRI, Izatnagar, India) under controlled conditions of temperature (25±2°C), relative humidity (50±15%) and normal photoperiod (12 h light and 12 h dark). Mice were given standard mice feed (Hindustan Lever Ltd., India) and tapwater *adlibitum*. 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.

### Test chemical

Arsenic in the form of $NaAsO_2$ of analytical grade was obtained from standard commercial suppliers [Himedia, Mumbai, India Ltd.].The dose 4.0 mg/kg b.wt was selected and it was dissolved in double distilled water (DDW) and administered orally.

### Plant material

The roots of *C. borivilianum* were collected from the market and were identified (RUBL No.19902) from the herbarium of Department of Botany, University of Rajasthan, Jaipur, India. The roots were air-dried in shade, powdered and extraction was carried out with DDW in soxhlet apparatus for 36 h at 40°C. The extract was filtered and then vacuum evaporated to get powdered form.

***C.borivilianum* drug tolerance study and optimum dose selection**

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. Among the doses, 800mg/kg. b.wt./day was selected for the study, the dose was decided on the basis of previously performed experiments in our lab (20) and it was dissolved in double distilled water (DDW) and administered orally.

**Experimental design**

Mice selected from inbred colony were divided into 4 groups.

| Groups | Number of animals | Treatment |
|---|---|---|
| **I Control** | 30 | Only vehicle DDW for 30 days |
| **II *C.borivilianum* treatment** | 30 | *C.borivilianum* root extract 800mg/kg b.wt/day orally in DDW for 30 days . |
| **III $NaAsO_2$ treatment** | 30 | Arsenic at 4 mg/kg b.wt./day for 30 days |
| **IV *C.borivilianum*+$NaAsO_2$+ *C.borivilianum*(Combination group)** | 30 | *C.borivilianum* root extract (orally800mg/ kg b.wt.) was administersd 10 days before $NaAsO_2$ (4 mg/kg b.wt.) and both were continued up to 30 days |

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

**Quantitative study of spermatogenesis**

The testis was fixed in Bouin's fixative and embedded in paraffin wax. Sections of 5 µm thickness were taken from testis and stained with hematoxylin and eosin and examined under a light microscope. Quantitative study of spermatogenesis was carried out by counting the spermatogonia A, spermatogonia B, primary spermatocytes, secondary spermatocytes, spermatid according to the method of Clermont and Leblond (1953)(21). Diameter of seminiferous tubules was also assessed.

**Statistical analysis**

The data were expressed as mean ± SE. The values at each autopsy interval for each experiment was compared with control, i.e. Control (Group I) vs C.borivilianum (Group II) /Arsenic (Group III) ; Arsenic (Group III) vs C.borivilianum +Arsenic + C.borivilianum (Group IV). Statistical significance between the groups was determined by student's t-test (22). Significance level was set at $P < 0.05$(a), $P < 0.01$(b) and $P < 0.001$ (c).

## Results

The control group showed all the stages of active spermatogenesis (Figure 1) whereas arsenic treated group showed depletion of germ cells (Figure 2). Combination group showed re-population of germ cells with maintained spermatogenesis (Figure 3). In control group the tubular diameter was between 151.10±0.81 µm to 154.10±0.96 µm upto 30 days (Figure 4) however no significant changes were observed in *C.borivilianum* treated group. Arsenic treated group showed highly significant ($P < 0.001$) reduction in tubular diameter from day 1 to day 30 (149.15±0.36 µm to 146.10±0.65 µm). Combined treatment of *C. borivilianum* and arsenic showed a highly significant increase in tubular diameter with respect to arsenic intoxicated mice.

A highly significant ($p<0.001$) depletion was observed in both A and B spermatogonia, primary and secondary spermatocytes and spermatid in arsenic intoxicated mice however germ cell population were fully maintained in *C.borivilianum* treated group. Combined treatment of *C. borivilianum* and arsenic showed a significant increase in both A and B spermatogonia, primary and secondary spermatocytes and spermatid as compared to arsenic intoxicated mice. (Figure 5 ,6,7,8 &9)

**Figure 1** Control group : Showing spermatogonia A (SgA), spermatogonia B (SgB), Primary Spermatocytes (Pri S), Secondary Spermatocytes (Sec S), spermatids (Sd).

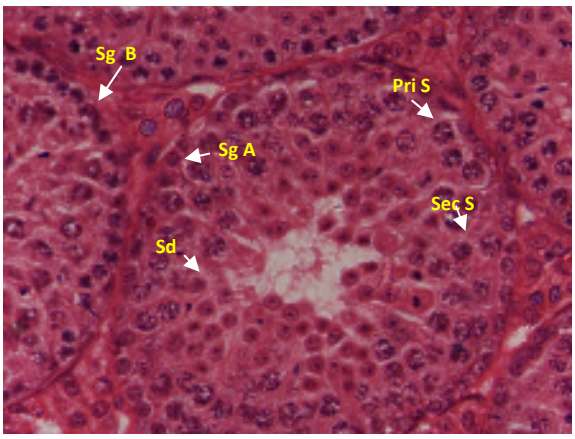



**Figure 2** Aresnic treated group : Shrinkage of tubular diameter and depletion of germ cell population.

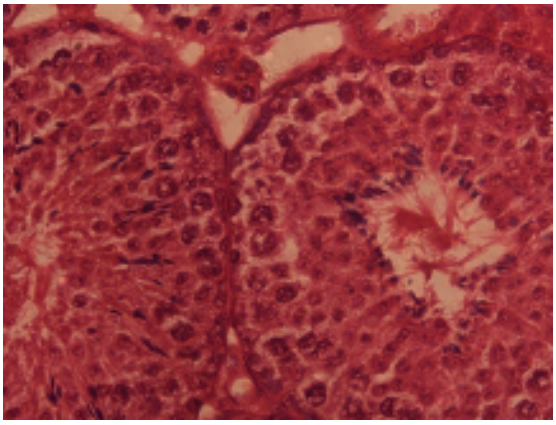


**Figure 3** Combination group : Re-population of germ cells with maintained spermatogenesis.

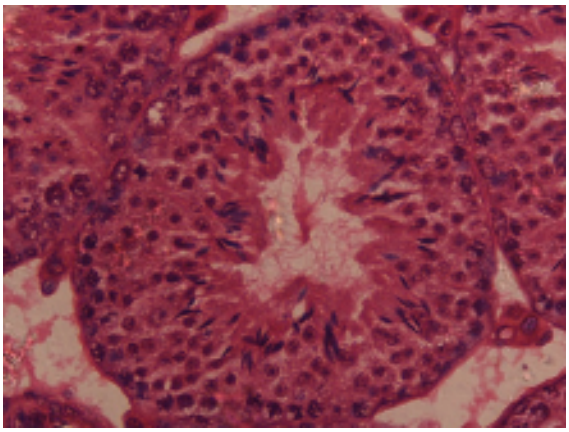


**Figure 4** Variation in tubular diameter (in μm) of male Swiss albino mice in different treated groups. Significance level was set at P < 0.05(a), P < 0.01(b) and P < 0.001 (c). Statistical comparison were done as: Control Vs Arsenic / C.borivilianum ; C.borivilianum +Arsenic + C.borivilianum Vs Arsenic .

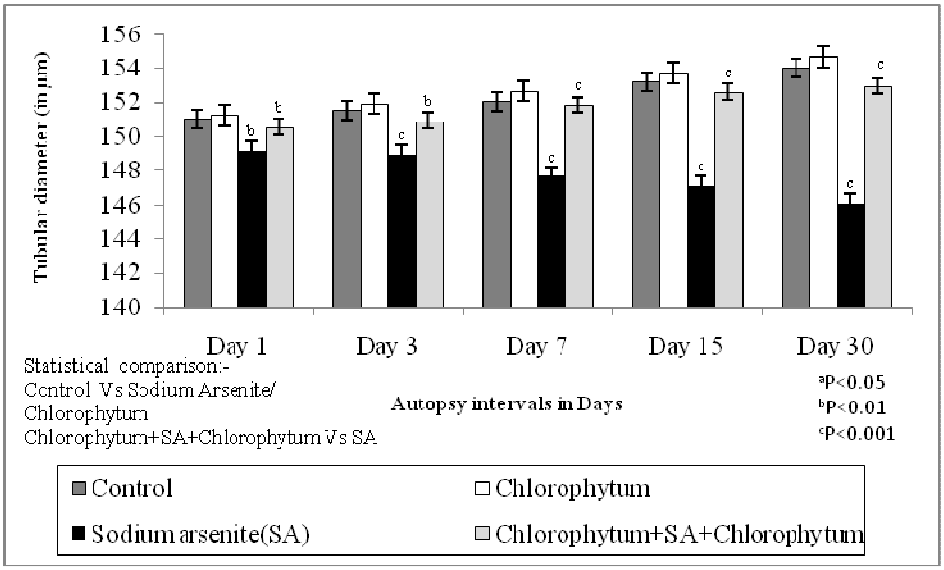



**Figure 5** Variation in number of Spermatogonia A of male Swiss albino mice in different treated groups. Significance level was set at P < 0.05(a), P < 0.01(b) and P < 0.001 (c). Statistical comparison were done as: Control Vs Arsenic / C.borivilianum ; C.borivilianum +Arsenic + C.borivilianum Vs Arsenic .

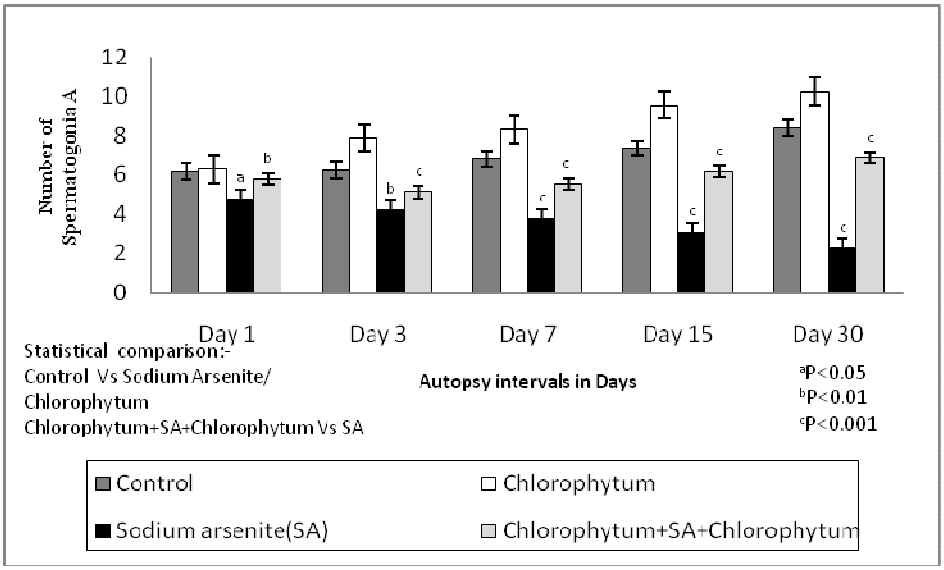


**Figure 6** Variation in number of Spermatogonia B of male Swiss albino mice in different treated groups. Significance level was set at P < 0.05(a), P < 0.01(b) and P < 0.001 (c). Statistical comparison were done as: Control Vs Arsenic / C.borivilianum ; C.borivilianum +Arsenic + C.borivilianum Vs Arsenic .

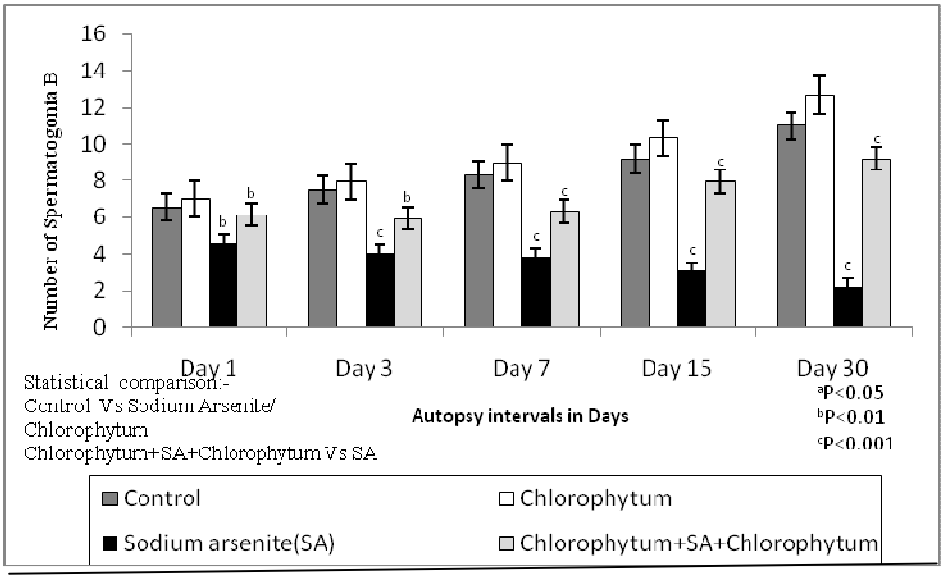



**Figure 7** Variation in number of Primary Spermatocytes of male Swiss albino mice in different treated groups. Significance level was set at P < 0.05(a), P < 0.01(b) and P < 0.001 (c). Statistical comparison were done as: Control Vs Arsenic / C.borivilianum ; C.borivilianum +Arsenic + C.borivilianum Vs Arsenic .

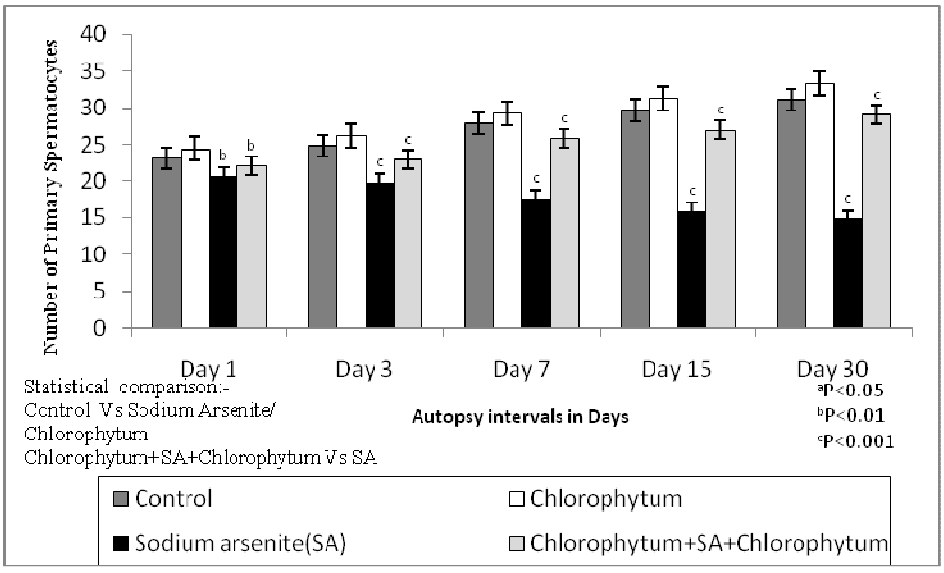


**Figure 8** Variation in number of Secondary Spermatocytes of male Swiss albino mice in different treated groups. Significance level was set at P < 0.05(a), P < 0.01(b) and P < 0.001 (c). Statistical comparison were done as: Control Vs Arsenic / C.borivilianum ; C.borivilianum +Arsenic + C.borivilianum Vs Arsenic .

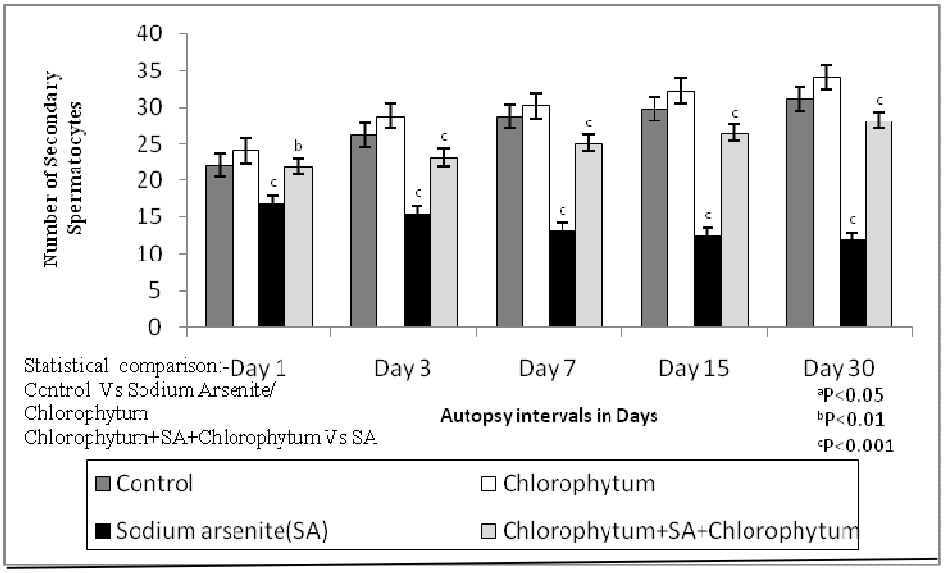
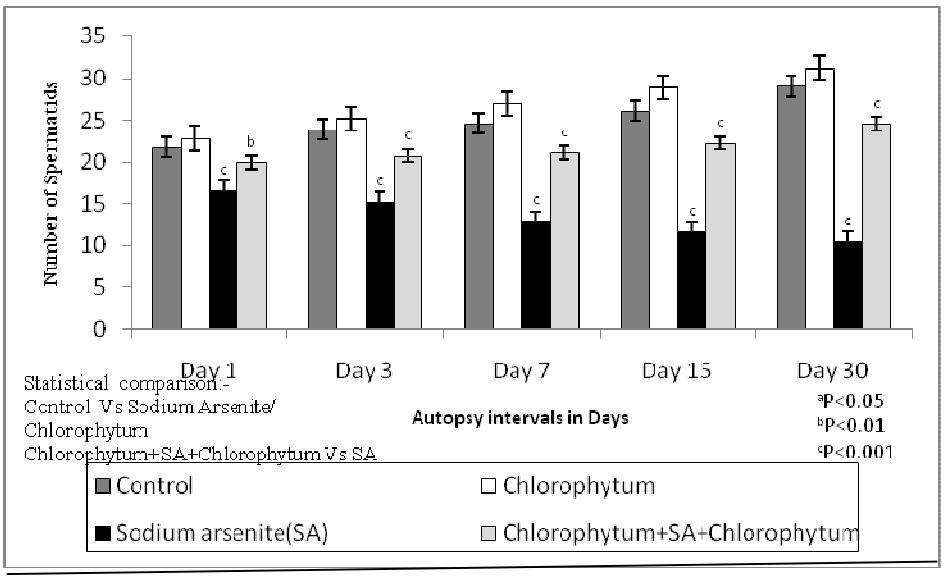



**Figure 9** Variation in number of Spermatids of male Swiss albino mice in different treated groups. Significance level was set at P < 0.05(a), P < 0.01(b) and P < 0.001 (c). Statistical comparison were done as: Control Vs Arsenic / C.borivilianum ; C.borivilianum +Arsenic + C.borivilianum Vs Arsenic .



**Discussion**

The structural and functional unit of testis are seminiferous tubules. Spermatogonia are adjacent to the basement membrane of seminiferous tubules. These cells are of two types one of them is the pale type A spermatogonia have a light-staining cytoplasm and round or ovoid nucleus with pale, finely granular chromatin. Other is the dark type B spermatogonia have a spherical nucleus with chromatin granules. B type spermatogonia produces primary spermatocytes. Primary spermatocytes, the largest germ cells in the seminiferous tubules occupy the middle region of the germinal epithelium. Their cytoplasm contains large nuclei with coarse clumps or thin threads of chromatin. The primary spermatocytes produces smaller secondary spermatocytes with less dense nuclear chromatin. Secondary spermatocytes give rise to spermatids. The spermatids grouped in the adluminal compartment of the seminiferous tubule and are closely associated with Sertoli cells where they are released as spermatozoa. Aresnic treated group shows shrinkage of tubular diameter and depletion of germ cell population while combination group shows re-population of germ cells with maintained spermatogenesis. Arsenic treated group showed highly significant reduction in tubular diameter as compare to control group. This observation is in corroboration with the earlier finding of Ahmad *et al*., (2008) (23) who reported that administration of arsenic disrupt structural integrity and degenerate the seminiferous tubules of rat testis. Manna *et al.,* (2008) (24) observed that arsenic exposure causes significant degeneration of the seminiferous tubules with necrosis and defoliation of spermatocytes. Combined treatment of *C. borivilianum* and arsenic showed a highly significant increase in tubular diameter.

The germ cell population showed a drastic decline with total arrest of spermatogenesis as observed by spermatogenic cell count. The spermatogonia and primary spermatocytes are the most sensitive cell stages of spermatogenesis to the toxic elements. A highly significant reduction was observed in both spermatogonia, primary and secondary spermatocytes and spermatid in arsenic intoxicated mice. Combined treatment of *C. borivilianum* and arsenic showed a significant increase in all the germ cells as compared to arsenic intoxicated mice. Our results are in agreement with Sarkar *et al*., (2008) (25) who reported that arsenic effects the processes of meiosis and post-meiotic stage of spermatogenesis and causes disruption of spermatogenesis in mice. The maturation of spermatogonia through the process of meiosis has severely distrupted following arsenic exposure. In arsenic exposed mice, a significant gradual dose dependent regression was observed in the number of resting spermatocyte, pachytene and round spermatid also confirm our results.

Sodium arsenite induces oxidative stress in animal cells which damages intracellular components such as lipids and proteins and DNA this in turn can impair cellular structure (26). Monsees *et al.* (2000) (27) reported that reproductive toxicants may alter germ cell attachment, disturb apical cytoskeletal transport, or induce micro-tubule dependent transport defects. This is turn will lead to germ cells loss and disruption of the seminiferous epithelium. A major function of Sertoli cells is their supportive role in maintaining spermatocytes and spermatids in adluminal compartment of the seminiferous epithelium. Several toxicants target the different sites of attachment between Sertoli cells and germ cells (28).

*C. borivilianum* roots are rich in alkaloid, vitamin, minerals, protein, carbohydrate, saponin, polysaccharide and steroid (29). *C. borivilianum* has been reported for its antioxidant activity (13) which reduces oxidative stress, decreases lipid peroxidase activity that indicates stability of cell membrane and arrest of cellular damages and maintain spermatogenic activity. Reduction in oxidative stress associated with free radical and ROS, could be its probable mechanism against arsenic induced toxicity. Thakur and Dixit (2008) (30) have shown that fructans and fructooligosaccharide (FOS) were effective in protecting against testicular damages and promote rejuvenation of testicular histoarchitecture. The overall constitution of aqueous root extract rich in steroidal saponin and FOS provides a prototype combination for combating the degenerative influence on sexual function caused by ROS (31). Saponin has spermatogenic property and is found useful in curing impotency (32).

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