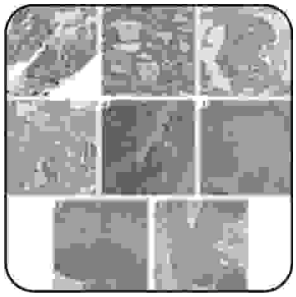
ORIGINAL PROF-1092

ARSENIC INDUCED MICROSCOPIC CHANGES IN RAT TESTIS



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ABSTRACT... The present study was designed to observe the changes in the testis of rats due to arsenic in higher doses. Distilled water and sodium arsenite were administrated intra-peritonealy to control and experimental groups respectively. Animals were sacrificed, their testis were weighed and cut into small pieces. After observing the plucking and stringing phenomenon of the seminiferous tubules the pieces of tests were embedded in paraffin and then 5µm thick section were made. These sections were stained with PAS-sulfurous acid haematoxylin and examined microscopically for qualitative assessment of germinal epithelium. **Results:** In the rats of experimental group mean weight and average tissue ratio of the paired testes was 1.140gm and 0.0037 respectively, which was significantly less than the control. 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 experimental group. **Conclusion:** In arsenic toxicity there are atrophic changes in testis due to degenerative changes in spermatogenic and leydig cells.

Key words: PAS-Sulfurous, Haematoxylin Stain, Arsenic Toxicity, Seminiferous Tubules, Spermatogenic Cells, Leydig cells and atrophic changes in testis.

INTRODUCTION

Arsenic is used as herbicide, fungicide and rodenticides. It causes air, soil and water pollution. Drinking polluted water is a common cause of arsenic poisioning[1,2,3]. Exposure to arsenic is associated with various metabolic disorders, hypertrophy of adrenal gland[4] and anemia[5]. A number of proteins and enzyme systems containing sulfhydral group have been found to be altered by arsenic[6]. Arsenic effects mitochondrial enzymes and impairs tissue respiration, which seems to be related to the cellular toxicity[7]. Gonadal effects of arsenic were fist evaluated in mice, then in fishes[8,9,10]. Most of the

available data on arsenic toxicity indicates that the main concern is with the developmental toxicity on the fetus[12]. Till to date there is little study available on the effect of arsenic on the microscopic anatomy of testis[11,13].

PURPOSE OF STUDY

The present study was carried out to observe the toxic effect of arsenic in higher doses on the morphology and histology of testis in the albino rats.

MATERIAL AND METHODS

In this experimental study twenty male albino rats were used which were divided into two equal groups A and B. The animals in group A were administered distilled water intra-peritonealy and served as control while animals in group B were administered sodium arsenite in a dose of 6gm/kg for 30 days. These animals were fed on normal protein diet (21% protein). The animals were housed in standard stainless steel cages and exposed to 14:10 hour light and dark photo-period at 20 degree centigrade and 40% humidity.

Body weight of each animal was recorded weekly and then at the end of experiment all the animals were ensanguined under ether anaesthesia. There testes were removed and weighed. Average tissue ratio was determined according to the formula.

$$\text{Average tissue ratio of paired testis} = \frac{\text{Weight of paired testis in grams}}{\text{Weight of body in grams}}$$

Testes were cut into small pieces (less than 05cm)in size and the tubules were plucked out from one of the pieces with the help of forceps to see the plucking phenomenon. The pieces of testis were then fixed in Zenker-formalin solution (Helly's solution) for 18 hours and the tubule on the cut surface were observed for stringing phenomenon. The pieces of testis were than processed and paraffin blocks prepared. Lastly 5µm thick sections were cut and stained by PAS sulfurous acid haematoxylin. Stained sections of the testis were examined microscopically for qualitative assessment of germinal epithelium. Diameter of the seminiferous tubules was measured with the help of ocular micrometer. One observation in each section was noted. Ten sections from each animal were studied and in this was 100 observations were made in each group. Total data regarding body weight, weight of paired testis and the diameter of the seminiferous tubules was analyzed statistically by applying student's 't' test.

OBSERVATIONS AND RESULTS

General Physical Appearances

All the rats in control group A remained active and healthy with normal feeding behaviour. Their mean body weight (MBW) at the end of experiment was 357gm as shown in (Table I). In group B animals were less active, more irritable and have lost considerable degree of weight. Their MBW was (318gm that is significantly less than that of control group).

Table-I. Comparison of mean body weight of animals in gm experimental versus control group on weekly basis.

Group	1st week	2nd week	3rd week	4th week
A (n = 10)	360	358	357	357
Control	±8.45	±8.56	±10.2	±10.1
B (n = 10)	357	348	324*	318**
Arsenic treated	±5.38	±10	±7.15	±8.83

n = Total number of rats used
*Mean±SD: Student 't' test: * = P<0.05. ** = P<0.01.*
All significance has been calculated with respect to control group A.

SCROTUM

In group A scrotal sacs were normal in size, shape and color having freely hanging testes. There was no sign of inflammation. While in group B scrotal sac size was reduced and skin had lost its normal luster.

GROSS APPEARANCES OF TESTES

The testes of the rats of the control group were easily pushed out of the scrotal sac were soft in consistency and well vascularized. They were pinkish in color and gave little resistance on cutting. The seminiferous tubules had normal plucking and stringing out phenomenon of the tubules. The mean weight and average tissue ratio of the paired testis were 1.40gm (Table II) and 0.0039 (Table III) respectively, while in rats of arsenic treated group B testes had gone reduction in size, pale looking, tough in consistency and showed resistance on cutting. It was difficult to pluck out any tubules from the testes and stringing out phenomenon was absent. The mean body weight and average tissue ratio of paired testis were 1.18gm (Table II) and 0.0037 (Table III) respectively.

Table-II. Comparison of mean weight paired testes in gms experimental versus control group at the end of therapy.

Group	1st week	2nd week	3rd week	4th week
A (n = 10) control	-	-	-	1.40±1.04
B (n = 10) Treated	-	-	-	1.18**±4.44

n = Total number of rats used
*Mean±SD: Student 't' test: * = P<0.05. ** = P<0.01.*
All significance has been calculated with respect to control group A.

Table-III. Comparison of average tissue ratio

$$\text{Average tissue ratio of paired testis} = \frac{\text{Weight of paired testis in grams}}{\text{Weight of body in grams}}$$

	Mean weight of paired testes (gm)	Mean body weight at end of experiment (gm)	Average tissue ratio
A Control	1.40	357	0.0039
B Arsenic treated	1.18	318	0.0037

MICROSCOPIC STRUCTURE OF THE TESTES

In group A, tunica albugenia and blood vessels were normal, seminiferous tubules were richly populated and gave healthy appearances. There was thin basement membrane. The mean tubular diameter (MTD) was 240.56µm (Table IV). All the cells of the spermatogenetic series such as spermatogonia, spermatocytes, spermatids and spermatozoa, even sertoli cells could be identified in the tubules. Lumen could easily be delineated in almost all the tubules and mature spermatozoa occupied most of it. Myoid and interstitial cells of leydig were present in between the tubules.

In group B animals, which were treated with arsenic, tunica albugenia was thickened and blood vessels were sparse and collapsed, majority of seminiferous tubules were shrunken and had a wavy outline. The basement membrane was thickened and hyalinized. The mean tubular diameter (MTD) were 118.30µm as shown in (Table IV). As the MTD was reduce, the myo-epithelial cells surrounding the seminiferous tubules moved closer to each other and were projected more prominently. Debris of shredded cells occupied most of the lumen of the seminiferous tubules. The spermatogenic cells index was 2-3. Most of the tubules contained spermatogonia and spermatocytes, which were large in size and contained darkly, stained nuclei. In some cells the nuclear membrane had been ruptured and was

accompanied by fragmentation of nucleus (karyorrhexis). Some of the tubules contained only spermatogonia, which were scantly in number and prominent due to disappearances of other cells in their neighbourhood. The blood vessels were sparse and collapsed. The interstitial cells of leydig were also reduced in number and their characteristic tendency of clumping together to form groups was also reduce. Nuclei of these cells were decreased in size and leaked the characteristic nucleoli. All these features were suggestive of atrophy of the testes.

Table-IV. Comparison of mean tubular diameter (MTD) of seminiferous tubules in (um) experimental versus control group at the end of therapy.

Group	1st week	2nd week	3rd week	4th week
A (n=10) Control	-	-	-	240.56 ±14.5
B (n =10) Arsenic treated	-	-	-	118.30 ** ± 7.6

DISCUSSION

The group B animals (treated with arsenic) for four weeks lost 16% of body weight . This significant drop in weight was due to loss of appetite and gastrointestinal disturbance .Majority of animals also developed ulcreative lesion at the sight of injections due to necrotic changes induced in skin by this metal which failed to respond to any conservative treatment. The testes were reduced in size and weight and thus the mean weight of paired testes of these animals was 5.6% less than that of control .The average tissue ratio ,which is a better way to assess the damage to the testes in relation to the body, was significantly reduced .All these effects were indicative of atrophic changes that had taken place in the testes .Plucking and stringing phenomenon were absent, suggesting degeneration of seminiferous tubules, thickening of basement membrane and condensation of the stroma. The pale colour of the testicular tissue was suggestive of reduced vascularity. The number of blood vessels in the interstitial tissues was reduced and most of them were collapsed as was evident by their reduced diameter. The population of leydig cells was decreased and they were rarely seen in-group or clumps. The nucleoli in majority of these cells were absent. The sertoli cells were spared because of their resistence to obnoxious agents as is documented by lesson and lesson[14]. In the end it is concluded that arsenic is injurious to spermatogenetic in higher doses and leyding cells, while the sertoli cells are resistant to such bad effects.

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THE RACE FOR PERFECTION HAS NO FINISH LINE

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