

## Exposure to arsenic induces histomorphological alterations in testis of rabbits

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### Abstract

With the expansion and development of industrial technology, frequent application of various synthetic chemicals like pesticides, insecticides and arsenic has led to contamination of both aquatic and terrestrial ecosystem across the globe. Among different synthetic chemicals arsenic poisoning has become a huge threat to ground water in many parts of the world. Therefore, this study was planned to examine the adverse effects of sodium arsenite on the morphology (gross and microscopic structure) of testis in rabbits. A total of 20 adult male rabbits were procured and randomly divided into two groups. Group (A) served as control and rabbits in group (B) were exposed to sodium arsenite orally (@ dose of 10mg/kg/day for a period 60 days. Results indicated significant ( $p < 0.05$ ) lower values of testicular weight, scrotal length and width, testicular volume, volume occupied by 10 seminiferous tubules and volume of the individual Leydig cells in treated rabbits. The exposure induced degenerative changes in testis including wavy contour of seminiferous tubules, vacuolization in the germinal epithelium and germ cell loss was recorded in the treated group. In conclusion, it was observed that arsenic may induce male reproductive effects by negatively affecting the testicular volume, Leydig cell population and volume of the Leydig cells.

**Keywords:** Arsenic toxicity, Rabbit testis, Histomorphology, Pakistan

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## Introduction

Various agricultural and industrial processes

persistently release different synthetic compounds or waste materials into natural water bodies leading to deleterious effects on both public health and aquatic



life (Sharaf et al., 2020; Ghaffar et al., 2020). Monitoring, identification and management of different chemicals / pollutants is of vital importance and crucial to reduce the toxic effects on target and non-target exposed organisms (Latif et al., 2020; Gul et al., 2020). Water is an imminent source of life on earth and water pollution has become a global threat to human and animal health. In Pakistan, there are numerous imminent origin to pollute ground water. Chemical and biological contagion of drinking water has been documented all over the country (Tahir and Bhatti, 1994; Kahlowan et al., 2004; Raza et al., 2017). Such contagion is accredited to effluence from sewage system, intermittent water supply, and shallow water tables, herbicides, floods, fungicides, untreated municipal waste are extremely hazardous for drinking water (Ghaffar et al., 2018; Hussain et al., 2020). Owing to swift industrialization and overwhelming civilization, the heavy metal toxicity is a leading health condition in the world (Ghaffar et al., 2016; Rehman, 2018). The evaluation of the heavy metals exposure has become central area of research in the medical related field since last decade of the nineteenth century. Metals have been reported to be negatively affect the process of spermatogenesis in experimental animals (Verstraeten et al., 2008; Naz et al., 2020). Due to continual nature and affinity to accumulate in organisms, heavy metals are considered as strong biological poisons (Kamble and Muley, 2000; Imran et al., 2020). Among different heavy metals, arsenic is metabolically multifarious and as a result perilous to health. The principal utilization of this heavy metal is as preservative, insecticide, pesticide, herbicide, algacide, growth promoter, in some alloys and electronic industry. Arsenic may be released into the environment through explosions, rocks and soil. It contaminate the air due to coal combustion, manufacturing waste and use of agriculture pesticides containing arsenic (DEFRA and EA, 2002).

Recent studies have reported arsenic poisoning as a massive epidemic in most of the globe and particularly in Pakistan due to arsenic ground water contamination and toxicity (Malik et al., 2010; Samrana et al., 2017; Shaikh et al., 2018; Sohail et al., 2019). Epidemiological investigations conducted in the globe including Bangladesh, Taiwan, China and Ukraine have exposed that arsenic contaminated food and water is the major cause of reproductive disorders (Yang et al., 2003). Most of the heavy metals are reported as classical testicular toxicants

(Sharma and Garu, 2011). Without any doubt, testes are the major organs for male sexual development and fertility. Male reproductive health upshot of arsenic was first studied in mice, then in fishes (Shukla and Pandey, 1984). Arsenic exposure in experimental rats has revealed impairment of spermatogenesis, oxidative hassle and genotoxic effects on the testis (Biswas et al., 2006; Chang et al., 2007). Many workers have reported the arsenic reprotoxicity in experimental rat shown by the reduction in testicular and other sex organ mass, necrotic changes in the testicular tissue, degenerative changes in the germ cells and oxidative stress (Chinoy et al., 2004; Ahmad et al., 2008; Sanghamitra et al., 2008; Mukherjee and Mukhopadhyay, 2009). Keeping in view the persistent exposure of general population to arsenic through ground water in Pakistan (Shahid et al., 2017; Podgorski et al., 2017; Samrana et al., 2017; Shaikh et al., 2018; Sohail et al., 2019) and lack of significant male reproductive toxicity data on morphology of testis in the literature, current research plan was designed to investigate the histomorphometric and anatomical testicular tissue damage after induced arsenic poisoning in rabbits in the natural ecology of Pothohar area of Pakistan.

## Material and Methods

In order to circumvent experimental animal distress, the methodology was designed conforming to the guiding principle approved by the animal welfare ethical committee of the PMAS-Arid Agriculture University.

### Experimental animals

Twenty adult healthy male rabbits were kept in cages in the Animal House of Department of Biomedical Sciences, Faculty of Veterinary and Animal Sciences, PMAS-Arid Agriculture University Rawalpindi. The rabbits were allowed to acclimatize for duration of three weeks before the commencement of the experiment.

### Treatment regime

After acclimatization period, the rabbits were divided at random into two groups (n=10 for each group); group A (control) was kept as control, group B (Sod. Arsenite treated) was kept as sodium arsenite (@ dose of 10mg/kg/day), the drug administration was given orally for 60 days (Zubair et al., 2014).



### Body and testes weight (gms)

Body weight and testis weight was measured before and after the experimental period. Before the start of experiment and at the end of investigational period, the animals were euthanized and testes were collected and weighed individually with the electrical balance.

### Scrotal biometry of testis

Scrotal length (cm) and width (cm) of the testis of experimental animals before the start and at the end of the experimental period of all the groups was measured and recorded.

### Histomorphology

Small pieces of the testicular tissue were taken from the mid of the testis and fixed in Bouin's solution till processed for paraffin embedding technique (Bancroft and Gamble, 2008). Tissue sectioning was performed by rotary microtome, 5 µm thick sections were stained with hematoxylin and eosin for histomorphometric studies under light microscope: Testicular volume was determined by using the formula described by (Johnson and Neaves, 1981):

$$\text{Testicular volume (cm}^3\text{)} = \text{testis weight (g)} / \text{Testis density (1.052)}$$

The volume occupied by 10 seminiferous tubules cross section was calculated by the formula described by Moura and Erickson (1997):

$V_{st} = \pi \times h \times (d^2/4)$ , where h was the section thickness (5 µm), and d was tubular diameter (µm). The volume of an individual Leydig cell was determined by the formula:  $Lc = (4/3) \times \pi \times D^3$  where D is the

average diameter (µm) of Leydig cells

### Statistical analysis

The data were subjected to statistical analysis by using Two-way ANOVA followed by student t-test to find out the significance difference between different treatments maintained under controlled laboratory conditions.

## Results and Discussion

The main objective of this experiment was to study the macro and microscopic changes in the testis of rabbits which may leads to reproductive health hazards due to the arsenic poisoning, and may affect the reproductive efficiency and hence the animal production. The experimental rabbits were randomly divided into two groups; group-A (control) was kept as control, group-B (Sod. Arsenite treated) was kept as sodium arsenite (@ dose of 10mg/kg/day) treated. The data of mean total body weight and testicular weight was recorded before and after the experimental period.

### Body weight

Initial mean body weight of the rabbits of control and treated groups was recorded as 905.45±48.51 gms and 915.00±20.15 gms and at the end of experiment the final mean body weight was recorded as 910.50±50.14 gms and 900.14±48.12 gms respectively (Table 1). There was non-significant difference in body weights of all the rabbits among two groups.

**Table-1: Comparison of body weight (gms), testicular weight (gms), scrotal length (cm) and scrotal width (cm) of the experimental rabbits**

Parameters	Treatment			
	Before		After	Mean ± SD
Body Weight (gms)	Control	905.45±48.51	910.50±50.14	900.40±31.80
	Treated (@ dose of 10mg/kg/day)	915.00±20.15	900.14±48.12	914.50±14.61
Paired testicular Weight (gms)	Control	2.48.25±35.51	2.52±43.12	2.51±30.10
	Treated (@ dose of 10mg/kg/day)	2.20±25.16	1.02±33.10	1.89±12.21
Scrotal Length (cm)	Control	2.30.20±21.21	2.25±35.10	2.28±50.20
	Treated (@ dose of 10mg/kg/day)	2.23±12.15	1.35±30.16	1.76±44.11
Scrotal Width (cm)	Control	1.15.10±31.11	1.55±12.18	1.35±32.10
	Treated (@ dose of 10mg/kg/day)	1.18±32.12	0.88±10.19	0.99±37.21



**Testicular weight**

The sodium arsenite treated group of the rabbits was recorded as having significantly ( $p < 0.05$ ) low testicular weight ( $1.89 \pm 12.21$  gms) as compared to the testicular weight of control rabbits ( $2.51 \pm 30.10$  gms) (Table 1).

**Scrotal length and width (cm)**

The scrotal length (cm) was recorded as  $2.30.20 \pm 21.21$  cm and  $2.25 \pm 35.10$  cm before and after the experiment in control group of experimental animal while the same parameter (length) was documented as  $2.23 \pm 12.15$  cm and  $1.35 \pm 30.16$  cm before and after the experiment in treated group of the rabbits (Table 1). Statistical analysis revealed a significant ( $p < 0.05$ ) decrease in the scrotal length in the arsenic treated rabbits.

The scrotal width (cm) was recorded as  $1.15.10 \pm 31.11$  cm and  $1.55 \pm 12.18$  cm before and after the experiment in control group while the same parameter was measured as  $1.18 \pm 32.12$  cm and  $0.88 \pm 10.19$  cm before and after the experiment in treated group of the rabbits (Table 1). Statistical analysis revealed a significant ( $p < 0.05$ ) decrease in this parameter in the arsenic treated rabbits.

**Testicular volume and volume occupied by 10 seminiferous tubules (cm<sup>3</sup>)**

The testicular volume (cm<sup>3</sup>) was recorded as  $1.23 \pm 12.09$  cm<sup>3</sup> and  $0.70 \pm 22.10$  cm<sup>3</sup> in control and arsenic treated rabbits respectively while the volume occupied by 10 seminiferous tubules was documented as  $0.55 \pm 11.15$  cm<sup>3</sup> and  $0.23 \pm 10.19$  cm<sup>3</sup> in control and treated groups of the experimental animals respectively (Table 2). Statistical analysis revealed a significant ( $p < 0.05$ ) decrease in both parameter in the arsenic treated rabbits.

**Volume of individual leydig cell ( $\times 10^{-12}$ ml)**

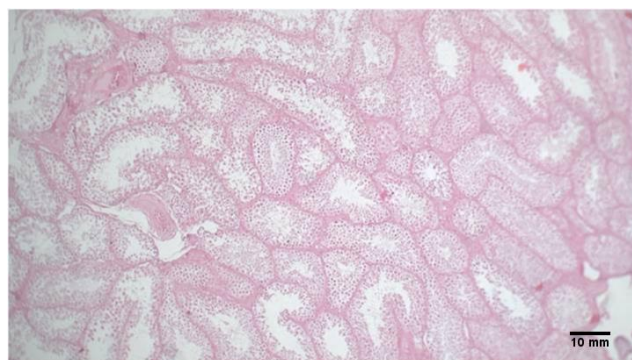
The volume of individual Leydig cell ( $\times 10^{-12}$ ml) was recorded as  $1,889.10 \pm 250.49$  ( $\times 10^{-12}$ ml) and  $1,001.11 \pm 249.10$  ( $\times 10^{-12}$ ml) in control and arsenic treated rabbits respectively (Table 2). Statistical analysis revealed a significant ( $p < 0.05$ ) decrease of individual Leydig cell volume in the arsenic treated rabbits as compared to the control group of the experimental animals.

**Table-2: Comparison of the testicular volume (cm<sup>3</sup>) and volume occupied by 10 seminiferous tubules (cm<sup>3</sup>) and Volume of the Individual Leydig cell ( $\times 10^{-12}$ ml) of the experimental rabbits**

Parameters	Control	Treated	Mean $\pm$ SD
Testicular Volume (cm <sup>3</sup> )	1.23 $\pm$ 12.09	0.70 $\pm$ 22.10	1.05 $\pm$ 12.40
Volume occupied by (10) seminiferous tubules	0.55 $\pm$ 11.15	0.23 $\pm$ 10.19	0.38 $\pm$ 21.14
Volume of the individual Leydig cell ( $\times 10^{-12}$ ml)	1,889.10 $\pm$ 250.49	1,001.11 $\pm$ 249.10	1,545.20 $\pm$ 227.30

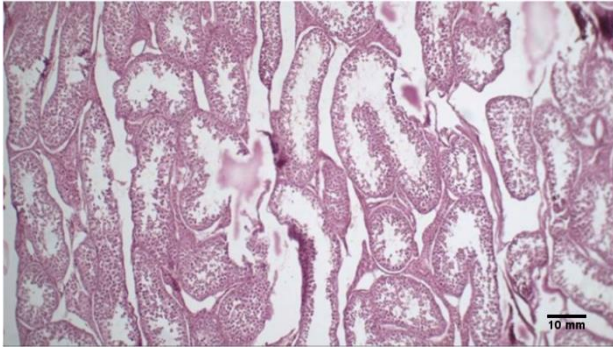
**Histopathological alterations in the testis**

The testes of control group showed normal appearance of the seminiferous tubules having normal arrangement of the germinal epithelial cells including sertoli cells, spermatogonia and spermatozoa (Fig. 1 and 3). Whoever histological observations of experimental group revealed various abnormal changes like intraepithelial vacuolization, signs of necrosis, reduced number of spermatogenic cells, rupturing of germinal epithelium and degenerative cells were observed in the testis of treated group of rabbits (Fig 2 and 4). The volume of seminiferous tubules and Leydig cells was also decreased significantly.

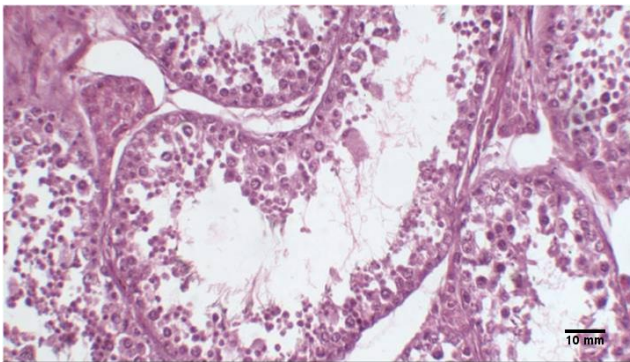


**Figure-1: Photomicrograph of testis showing the cross section of seminiferous tubules from Group -A (Control), showing normal seminiferous tubules with intact germinal epithelium (black arrow), tubular lumen (A), and interstitium (C). H&E stain 10 X**

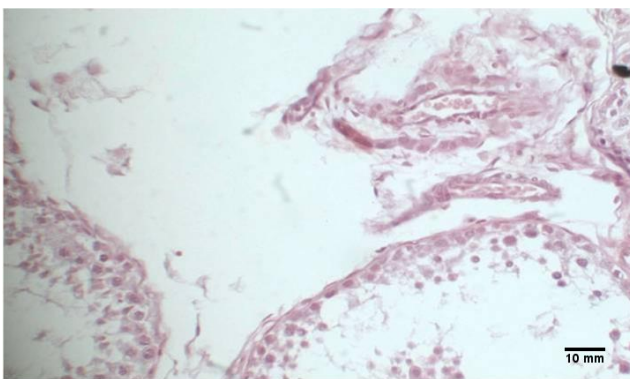




**Figure-2: Photomicrograph of testis showing the cross section of seminiferous tubules from Group –B (Treated), exposing degenerated cells in the lumen of tubules (A), and vacuolization in the germinal epithelium (black arrow). H&E stain 10 X**



**Figure-3: Photomicrograph of testis showing the cross section of seminiferous tubules from Group –A (Control), showing normal seminiferous tubules with intact germinal epithelium (A), tubular lumen (B), and Leydig cells (black arrow). H&E stain 40 X**



**Figure-4: Photomicrograph of testis showing the cross section of seminiferous tubules from Group –B (Treated), revealing degenerated cells in the lumen of tubules (A), interstitium (B) and vacuolization in the germinal epithelium (black arrow). H&E stain 40 X**

The present study was designed to explore the anatomic toxic effects of arsenic on the testicular tissues after induced arsenic poisoning in rabbits. This study revealed no significant difference among the body weights of the control and the arsenic treated rabbits which confirm the results of previous studies (Zubair et al., 2014; Sanghamitra et al., 2008). However, another study reported significantly decreased body weight in the arsenic treated rabbits (Sarkar et al., 2003). The divergence noted above from the values reported by Chang may be accredited to the duration or dosage of the treatment.

The sodium arsenite treated group of the rabbits was recorded as having significantly ( $p < 0.05$ ) low testicular weight ( $1.89 \pm 12.21$  gms) as compared to the testicular weight of control rabbits ( $2.51 \pm 30.10$  gms), these findings are in accord with the previous findings (Zubair et al., 2014; Sanghamitra et al., 2008).

This experiment showed the significant ( $p < 0.05$ ) decrease in the scrotal length and scrotal width in the arsenic treated rabbits as compared to the control group of the rabbits. Testicular mass and scrotal size is an important index of the testicular toxicity and damage to the germ cells (Chapin and Lamb, 1984). The Mean  $\pm$  SD of the testicular volume and volume occupied by seminiferous tubules was recorded as  $0.98 \pm 15.10$  and  $0.45 \pm 25.18$   $\text{cm}^3$  for control and treated animals respectively. These results revealed a significant damage to the testicular tissue induced by the arsenic toxicity which is in line with the results of arsenic toxicity in male rats (Morakinyo et al., 2010). This study exposed a significant ( $p < 0.05$ ) decrease of individual Leydig cell volume in the arsenic treated rabbits as compared to the control group of the experimental animals. These results confirm the findings of previous studies in mice and rats (Chinoy et al., 2004; Pant et al., 2001).

## Conclusion

Present study exposed the histomorphometric effects of arsenic on the testicular morphology subsequently it is concluded that arsenic induce male infertility by negatively affecting the testicular volume, Leydig cell population, volume of the individual Leydig cells and hence the functions of the reproductive system in rabbits.

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**Disclaimer:** None.

**Conflict of Interest:** None.

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### Contribution of Authors

Pasha RH & Khan MA: Investigation, sampling, research methodology and writing of original draft

Qureshi AS: Conceptualization and data collection

Ali A & Rehman SU: Literature review and editing of manuscript

