



HISTOPATHOLOGICAL CHANGES DUE TO ARSENIC KUSHTA IN TESTES OF WISTAR RATS.

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INTRODUCTION

Arsenic (As) is a well-known semi-metal and 20th most common element in nature (PCRWR, 2002). It is also considered as a heavy metal which can gain entry into the human body via food, air, water and skin.

Arsenic is known as a human carcinogen.¹ It is a unique metal as it can cause cancers both by ingestion as well as inhalation.² The Agency of Toxic Substances and Disease Registry (ATSDR) in 2007 published a report that ranked arsenic as a no.1 substance in Comprehensive, Environmental, Response, Compensation and Liability Act (CERCLA) Priority List of Hazardous Substances. According to this act the hazardous substances are listed on basis of toxicity, potential for human exposure and frequency of their occurrence in nature.³

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ABSTRACT... Objectives: To see the detrimental effects of arsenic kushta (kushta-e-Sammulfur) induced toxicity in wistar rats. **Study Design:** Experimental Study. **Setting:** Department of Morbid Anatomy and Histopathology, University of Health Sciences, Lahore. **Period:** 02nd June 2016 to 30th September 2016. **Material & Methods:** This experimental study was conducted on a total of 48 wistar rats, each weighing approximately, 200-300grams. These rats were then randomly divided into four groups each of them comprising of 12 rats. Group I was taken as a control group which was given flour pellets. Group II was given a single dose of 180 mg/kg of arsenic kushta for 2 weeks, whereas Groups III was given 150 mg/kg of arsenic kushta only for 12 weeks and Group IV was also given dose of 150 mg/kg of arsenic kushta along with 75mg of Bovine Serum Albumin for 12 weeks. Histopathological changes were then seen in the germinal epithelium, stages of spermatogenesis and Interstitium of testes of rats. **Results:** Changes in germinal epithelium, stages of spermatogenesis and interstitium were seen in all the above groups except group 1 which was the control group. Maximum changes were seen in groups C and D which were given high doses of arsenic kushta along with injection of bovine serum albumin. **Conclusion:** Arsenic kushta preparation of kushta-e-Sammulfur causes testicular toxicity in wistar rats and have similar toxic effects in human beings.

Key words: Arsenic, Germinal Epithelium, Histopathological Changes, Interstitium, Kushta, Spermatogenesis.

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It has traditionally been used widely in South East Asia in various popular herbomineral preparations known as kushta by the Hakeems (traditional healers). If it is not properly prepared in right amount it may cause poisoning in the human body. Kushta is derived from Persian word, kushtan which literally means to kill.⁴ It is usually prepared by constantly heating metal (arsenic) and then wet grinding with different herbal extracts, various plants and fruit juices. The finished preparation of kushta then contains oxidized metals. As now very toxic concentration of metal is present, it has a great risk of causing heavy metal poisoning.⁵

This preparation is commonly known as Kushta-e-Sankhaya (kushta Sam-ul-Fur) containing approximately 18.89% by weight in the compound in its organic form.⁶ It has been used for various diseases especially for gastric problems and

arthritic complaints.⁷

No authentic research has been done before in the past in our country except for in India to see the adverse effects of kushtas trending among the locals. Owing to its extensive use as medication for various diseases by the traditional healers and its growing importance in our society, the present study was conducted to assess the toxic effects Kushta-e-Sankhaya on the testes of wistar rats.

MATERIAL & METHODS

This experimental study was carried out in the Department of Morbid Anatomy and Histopathology, University of Health Sciences, Lahore from 02nd June 2016 to 30th September 2016. Total 48 rats were taken of approximately 6-10 weeks age and weighing 200-300 grams from the University of Veterinary and Animal Sciences Lahore. They were then further divided into four groups, as Control group and other three experimental groups. Each group consisted of 12 rats. The dose calculation was done using LD₅₀ of wistar rats as mentioned in the given literature.

The arsenic containing kushta was provided by the Hamdard Laboratories and then chemical analysis was done by PCSIR (Pakistan Council of Scientific and Industrial Research Laboratories) for exact quantification of arsenic in kushta. The dose was calculated according to the body weight of wistar rats and pellets were formed after mixing and homogenizing with wheat flour. Group 1 was the control group given four pellets. Group II was given a single dose of 180mg/kg of arsenic kushta for 2 weeks; Group III received 150mg/kg of arsenic kushta. Group IV was also given 150mg/kg of arsenic kushta along with 75mg of Bovine Serum Albumin according to 250 mg/kg of body weight. BSA is used to increase the capillary permeability and causes serum sickness. After the experiment was completed the rats were sacrificed and the specimens were fixed in formaline. After that they were brought to the Department of Morbid Anatomy and Histopathology, UHS.

They were allocated specific laboratory numbers. After detailed gross examination of all the

specimens, appropriate sections were taken from the tissues and paraffin embedded blocks were made. Sections were cut and stained with Hemotoxyline and Eosin by conventional method. Microscopy was done and the respective forms were filled by the relevant data.

The data was entered and analysed by the SPSS software version 20.0. Frequencies and percentages were given for the qualitative variables like histopathological changes in testes which were then analyzed using Fisher's exact tests. A p value of ≤ 0.05 or equivalent was considered as statistically significant.

RESULTS

The testicular histopathology in terms of germinal epithelial changes showed no characteristic of degeneration, necrosis and hyperplasia in the control Group A. Degeneration was seen focally in only Group C while diffusely in only Group D (Figure-1) whereas it was totally absent in Group B. (Figure-2) Necrosis and hyperplasia were seen totally absent in Groups C and D also (Table-I).

Normal shape of seminiferous tubules was seen in Groups A, B, C and D. While according to the Johnson scoring for spermatogenesis normal scoring was present in the control group A and other experimental group such as B (Figure-2) whereas groups C and D showed the lower Johnson score and spermatogenesis (Table-II) (Figures-3,4).

None of the characteristics of basement membrane changes such as thickness, fraying and necrosis. The testicular histopathology also depicted no oedema, inflammation, fibrosis, sclerosis and hyalinization in the control group A. Oedema was seen in Group D whereas Groups C and D showed hyalinization (Table-III).

Rest of the characteristics were absent in the experimental groups as well.

		Groups				P-Value
		Group-A	Group-B	Group-C	Group-D	
Degeneration	focal	0	0	3	4	0.024
	diffuse	0	0	0	1	
	absent	18	12	9	7	
Necrosis	focal	0	0	0	0	-
	diffuse	0	0	0	0	
	absent	18	12	12	12	
Hyperplasia	focal	0	1	0	0	0.312
	diffuse	0	0	0	0	
	absent	18	11	12	12	

Table-I. Germinal epithelial changes in testes. Note: Significance Level*: p-value<0.05

	Groups	N	Mean	SE
	Johnson scoring for spermatogenesis	Group-A	18	9.98
Group-B		12	9.70	0.09
Group-C		12	8.96	0.32
Group-D		12	8.50	0.25

Table-II. Johnson scoring for spermatogenesis.

		Groups				P-Value
		Group-A	Group-B	Group-C	Group-D	
Edema	Present	0	0	0	1	0.312
	Absent	18	12	12	11	
Inflammation	Present	0	0	0	0	0.000
	Absent	18	12	12	12	
Fibrosis	Present	0	0	0	0	0.000
	Absent	18	12	12	12	
Sclerosis	Present	0	0	0	0	-
	Absent	18	12	12	12	
Hyalinization	Present	0	0	2	3	0.061
	Absent	18	12	10	9	

Table-III. Interstitial changes in testes. Note: Significance Level*: p-value<0.05

DISCUSSION

Arsenic (As) is a semi-metal occurring in nature. It is found in the earth's crust in greater proportions in some areas of Pakistan due to which drinking water gets contaminated. It causes mild effects on our health which include nausea, vomiting, and diarrhea. Other well-known effects include thickening and discolouration of the skin, gastritis, and numbness in hands and feet, blindness and partial paralysis.³ Arsenic is highly toxic, naturally occurring heavy metal that causes wide range of deleterious effects on human health. Its poisoning occurs through inhalation, ingestion and injection or through dermal exposure. Arsenic in any form is toxic and cause nephrotoxicity, hepatotoxicity and testicular toxicity.⁸ Different treatment

modalities are being practiced in South East Asia, they include modern allopathic medicine, traditional and homeopathic medicines, which consist of a variety of herbs, salts and metals.⁹ One of the dosage forms of traditional medicine is herbo-mineral preparations popularly known as 'KUSHTAS' containing oxidized metals.¹⁰ This study is concerned about the effects of arsenic containing compound and kushta on testes in rats. Only few studies have been done in the past which suggested that toxicity from arsenic has occurred over its prolonged use. There is very limited data available to compare our findings, however there has been some similarity found between the findings of our study and previous studies.

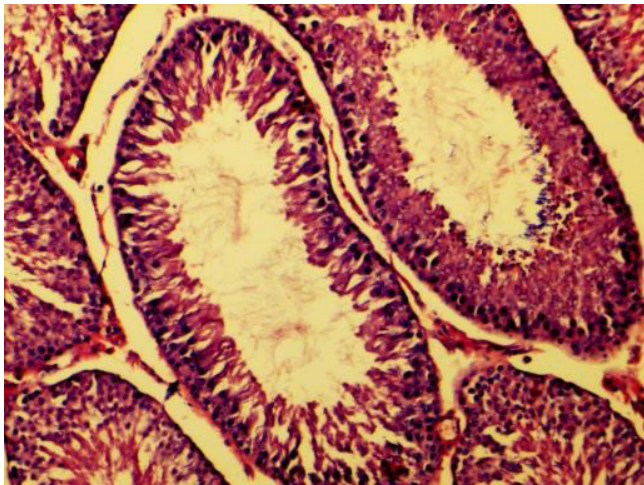


Figure-1. Photomicrograph of testis from experimental animal in Group D showing degeneration of tubules. (H&E, 40x)

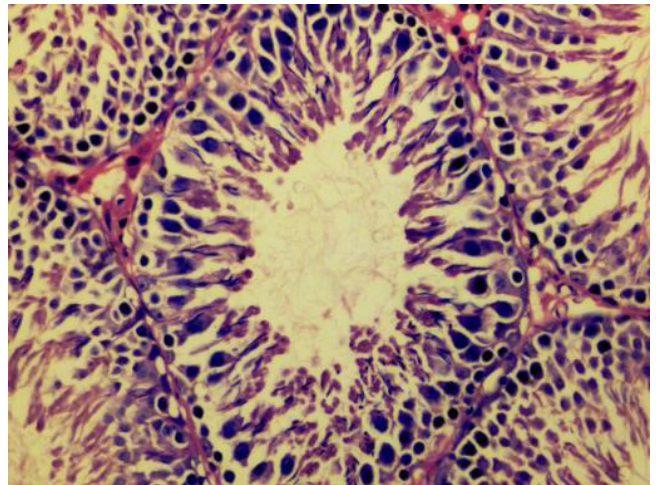


Figure-2. Photomicrograph of testes from experimental animal of Group B showing complete spermatogenesis. (H&E, 40x).

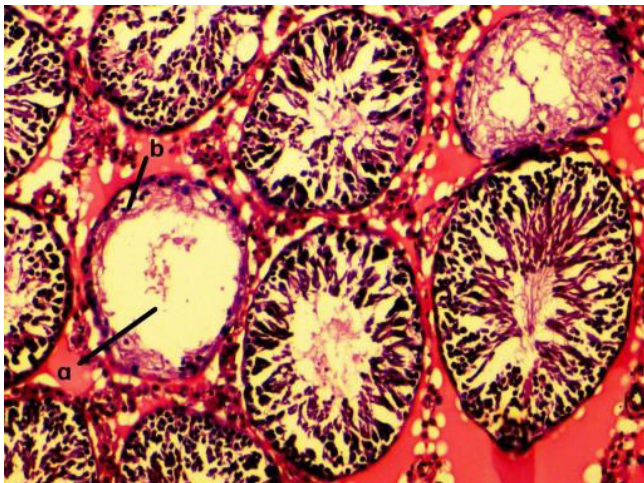


Figure-3. Photomicrograph of testes showing a) focal degeneration of tubules and b) incomplete spermatogenesis from experimental animal of Group C. (H&E, 40x).

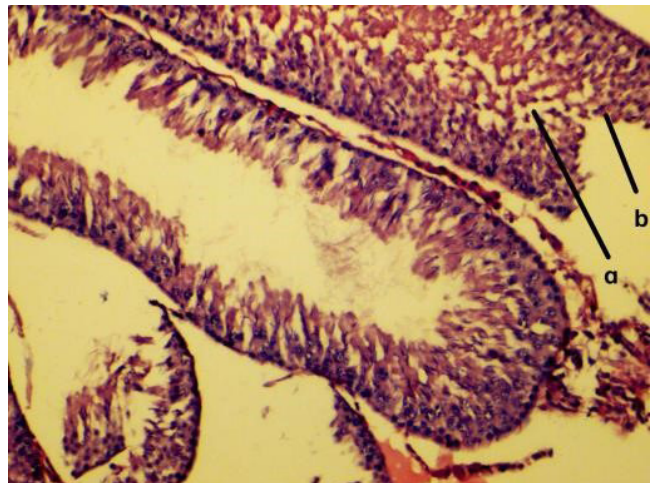


Figure-4. Photomicrograph of testis from experimental animal in Group D showing a) degeneration of tubules and b) incomplete spermatogenesis. (H&E, 40x).

Microscopy of testes depicted degeneration which was observed in the chronic toxic dose exposed groups. Arsenic may bind to sulfhydryl groups of enzymes and tissue protein such as glutathione and consequently inhibit cell division.^{11,12} All these effects in the experimental animals indicated degenerative changes that had taken place in the reproductive organs.¹³ Histological damages in testes due to arsenic exposure may also be seen due to the biochemical changes. Some of the key enzymes such as superoxide dismutase and catalase were inhibited and cause damage to spermatozoa integrity.¹⁴ Testosterone had a

definite role in attachment of different generations of germ cells to the seminiferous tubules. Therefore its low intracellular levels resulted in detachment of germ cells from seminiferous tubules and thus finally causing their apoptosis.¹⁵ Furthermore in arsenic treated experimental rats, the elevated plasma levels of testicular acid phosphatases and alkaline phosphatases were responsible for degeneration in testes, causing in turn depressed concentration of testosterone hormone.¹⁶ According to another study, the disintegration of germ cells by arsenic treatment was probably due to low intra testicular concentrations of

testosterone.¹⁷ It happened as high level of testosterone in testes was essential for normal process of spermatogenesis as well as for the preservation of structural morphology, adequate vascularity and typical physiology of seminiferous tubules¹⁸ In this study, Johnson scoring was used to determine the stages of spermatogenesis. In 1980, Johnson & et'al devised a 10 points scoring system for quantitatively assessing the germ cell elements and the relationship of spermatogenesis to seminal fluid sperm density for each tubule cross section examined. The scoring system overcomes the variation in microscopic variations in seminiferous tubules of males.¹⁹ Johnson scoring was done for every subject in the experiment and then means were calculated for each group and were compared to control and as well as other exposed groups. Exposure to arsenic kushta lowered the Johnson score in the exposed groups. Lowest Johnson score was seen in the group which was exposed to arsenic kushta in chronic toxic dose along with bovine serum albumin. However the microscopic examination of testicular basement membrane & interstitium did not show any significant changes in any of the groups. Ruptured follicles, reduction in number of spermatozoa & degenerated interfollicular septae were demonstrated by Hemalatha et'al in 2013 after exposure of rats to toxic doses of arsenic kushta.²⁰

CONCLUSION

Thus, arsenic kushta has detrimental effects over testes of wistar rats. These effects are thus increased by concomitant exposure to bovine serum albumin.






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2	Raheel Khan	Manuscript writing.	
3	A.H. Nagi	Editing and review of manuscript.	
4	Asma Fayyaz	Statistical analysis.	
5	Afra Samad	Drafting and critical reviewing.	
6	Nudrat Fayyaz	Analysis of data.	