ORIGINAL ARTICLE

Histology of Rabbit's Testes as Influenced by Municipal Waste Water

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ABSTRACT

Relative effect of different concentrations of municipal wastewater i.e., (1) tape water (control), (2) 50% concentration of municipal wastewater and (3) 100% concentration of municipal wastewater on histology of rabbit's testes was studied at the Animal House of Gomal Medical College, Dera Ismail Khan during the year 2013-14. The study was laid out in randomized control trail (RCT). Sixty male rabbits classified into three groups A, B and C and each group further subdivided into two subgroups viz., A1, A2, B1, B2, C1 and C2 respectively. Group A1, B1, C1 was treated for 30 days whereas group A2, B2, C2 was treated for 60 days with tape water, 50% and 100& concentrations of municipal wastewater respectively. Data was recorded on various morphological and microscopic parameters viz; degenerative cells in the lumen, sertoli cells count, leydig cells/HPF, cell count/tubule, degenerative changes in epithelium and vacuolization. Results revealed highly significant (P≤ 0.01) differences among various concentrations of municipal wastewater for all the studied traits in comparison to control (tape water) whereas the duration of treatments were found to be statistically at par with each other. Maximum cell counts/tubule, leydig cells count and sertoli cells count was recorded in control and decreased steeply with an increase in municipal wastewater concentration. Similarly maximum degenerative changes in epithelium, degenerative changes in lumen and intraepithelial vacuolization were observed in 100% concentration of municipal wastewater. The instant results suggest that municipal wastewater adversely affects the testicular histology.

Keywords: Municipal wastewater, spermatogenesis, histology, rabbit's testes

INTRODUCTION

Water is the basic requirement for the entire living organism of the biosphere.[1] Human being are using the water systems for various purposes such as drinking, irrigation, fisheries, industrial processes, transportation and waste disposal. In the ecosystem, the environmental contamination of water has become a global threat to the existence of different plants and animals[2]. Sewage pollutants consists of organic and inorganic substances, pathogens, plant nutrients, radioactive materials, sediments, oxygen demanding wastes, heat and oil[3]. In polluted waters the main impurities are plant nutrients and organic materials, but disease causing microbes are very likely to be present in the domestic sewage also[4,5]. Various heavy metals like Cu, Cd, Cr, Pb, Hg & Se get their way into the water from different sources including industries, automobiles, exhaust, mines and even from natural soils[6,7]. Crop and plants that are grown in contaminated soils or irrigated with municipal wastewater when consumed, can result in health concerns such as diarrhea, mental retardation, liver and kidney damage and even might cause death[8,9,10]. These heavy metals after the administration, might react with different biomolecules which might harmfully affect the reproductive, nervous, gastrointestinal, immune, renal, cardiovascular, skeletal, muscular and hematopoietic systems as well as developmental processes[11].

These toxic heavy metals can induce impairment in different systems like blood and cardiovascular system, detoxification pathways of color, skin and liver, endocrine system, energy production pathways, enzymatic and urinary system[12]. Various plants accumulate various amount of these heavy metals and the accumulation mainly depends upon the plant biomass. These heavy metals are mostly accumulated by leafy vegetables[13]. These heavy metals when ingested, might retard the hydrolysis. It may also interfere with the enzyme action by changing the metal ions from metaloenzymes. Similarly it might affect the male reproductive system which is a global health concern.[14] Different studies have been carried out on male reproductive system using careless and excessive use of heavy metals showed a disturbing trend. Previous studies also showed an increase in abnormal sperm frequencies and decrease in total sperm count in subjects exposed to heavy metals[15,16,17,18].

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Keeping in view the heavy metals as the most alarming health concern and their effect on male reproductive system, the present study was conducted to study the effects of municipal wastewater on histological features of seminiferous epithelium of rabbit's testis.

MATERIALS AND METHODS

The study was conducted in the Animal House of Gomal Medical College, Dera Ismail Khan during the year 2013-14 (From 20th December, 2013 to 19th Feb, 2014). Sexually mature male rabbits weighing 3-4 kg were utilized in the study. The study was conducted in Randomized control trail for 60 days. They were provided with daily food and water ad libitum for two weeks to acclimatize. They were kept under optimum temperature (24 ±2°C) and hygienic conditions with observation of dark and light cycles of 12 hours in iron cages.

Municipal wastewater was collected from main drain near Shobrah Hotel, Dera Ismail Khan, Pakistan. Different concentrations of municipal wastewater were prepared by mixing distilled water. The samples were prepared in large quantity and used. The animals were divided by using random table number into three groups, group A, B and C. Each group consists of 20 animals. Each group is further divided into two comprised of 10 animals each treated for 30 and 60 days. Each group is kept in separate iron cages, labeled with cards to indicate each group. The procedure was started after two weeks of acclimatization.

Group A serves as a control, group B treated with 50% municipal wastewater and group C treated with 100% MWW in a quantity of 100ml/kg body weight. The animal dose was adjusted on the basis of body weight. Municipal wastewater and tape water was administered via Nasogastric (NG) tube.

Data was recorded on cell count/tubule/section, degenerative cells in the lumen, intraepithelial vacuolization, leydig cell count/HPF and sertoli cell count/tubule.

Data analysis procedure: Data was collected and entered into computer and analyzed via Statistical Package for Social Sciences (SPSS) version 20. Analysis of Variances (ANOVA) was used for all variables to compare more than two groups. Least Significant Difference (LSD) test was applied to compare two groups as suggested by Steel and Torrie, 1980[31].

RESULTS

Cell count/tubule/section: The data pertaining to cell count/tubule/section is presented in table 1. Statistical analysis revealed that among various groups highly significant (P≤0.01) differences were observed. Cell count/ tubule/section were maximum in control (273.50) as compared to 50% MWW and 100% MWW. 100% MWW concentration revealed minimum cells might be due to adverse effects of municipal wastewater. Different durations of municipal wastewater have no influence on the cell/count/tubule/section presented in table 1, as non-significant differences were observed between 30 and 60 days.

Fig. 1: A photomicrograph of the histological section of the rabbit of control group showing normal, seminiferous epithelium (A), tubular lumen (B), blood vessels (C) and interstitial cells of leydig (D). H&E stain 100x.

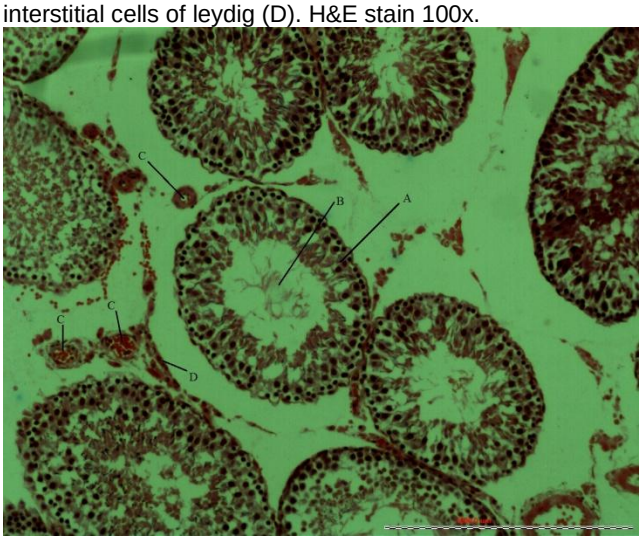
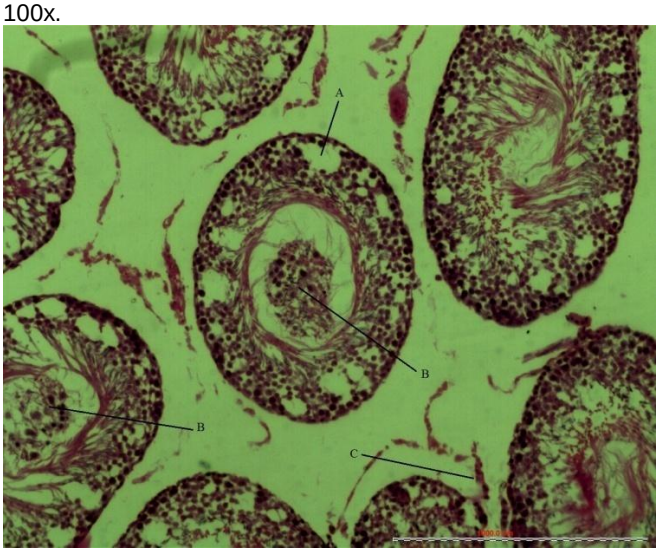


Fig. 4: A photomicrograph of a section of seminiferous tubules from C1 group rabbit testis treated for 30 days. Intraepithelial vacuolization (A), degenerated cells in the lumen (B) and interstitial cells of leydig (C). H&E stain 100x.



Degenerative cells in the lumen: The mean number of degenerative cells in the lumen of seminiferous tubule in various groups are presented in table 1. As evident from table, the number of degenerated cells in the lumen are significantly affected. The animals treated with 100% concentration of MWW were found with maximum degenerative cells as compared to animals treated with 50% concentration of MWW. Control group treated with normal tape water was found with minimum number of degenerative cells in the lumen of tubule. The data regarding degenerative cells in the lumen for different durations and concentrations are given in table 1. Visual differences were found in groups of different durations treated with MWW, however statistically insignificant differences were observed regarding duration.

Intraepithelial vacuolization: Intraepithelial vacuolization was studied in the rabbits treated with different concentrations of municipal wastewater. Animals treated with 100% MWW were observed with maximum number of intraepithelial vacuolization (52.2) as compared to 50% MWW (28.5). The vacuolization was very low in control group where no MWW was used. The animals treated with various concentrations of MWW for different durations were also evaluated, statistically no significant differences was observed in groups treated for different durations. Intraepithelial vacuolization was statistically at par with each other treated for 30 and 60 days.

Leydig cell count/HPF: The number of leydig cells in control group was 7.82. The number of leydig cells was significantly decreased in animals treated with 50%MWW and 100%MWW when analyzed statistically (table 1). The lowest number of leydig cells was observed in animals treated with 100% MWW, followed by 50%MWW treatment and maximum in control group (7.82) treated with tape water. Statistically non-significant difference was observed in animals treated with MWW for 30 and 60 days.

Sertoli cell count/tubule: The sertoli cell count/tubule/section as found in various groups of rabbits are presented in table 1. It was found that highly significant (P≤0.01) differences were found among various treatments of municipal wastewater. Maximum sertoli cell count/tubule of 35 were found in control group which was treated with normal tape water followed by the group treated with 50% concentrations of municipal wastewater. Whereas minimum sertoli cells/tubule/section was recorded in the rabbits treated with 100% concentration of municipal wastewater. This minimum sertoli cells/tubule might be attributed to the adverse effects of municipal wastewater. The mean values regarding 30 and 60 days duration are also given in table 1. Non significant differences were observed in the rabbits treated for 30 and 60 days duration, however number of sertoli cells/tubule was more in the groups treated for 30 days and somewhat reduced when exposed upto 60 days.

	Cell count/ tubule/ section	**Degenerative cells in lumen**	**Intraepithelial vacoulizaiton**	**Leydig cells**	**Sertoli cells**
Control (tape water)	273.50 a	2.5 c	2.85 c	7.82 a	35 a
50% mww	223.50 b	38 b	28.5 b	6.06 b	28.5 b
100% mww	196.50 c	54 a	52.5 a	5 b	17.5 c
$LSD_{0.05}$	14.02	8.78	17.30	1.22	4.64
30 days	235.33	29.33	24.56	6.32	28.66
60 days	227	33.66	31.33	6.26	25.33
$LSD_{0.05}$	NS	NS	NS	NS	NS
Control x 30 days	275	2	2.7	7.64	36
Control x 60 days	229	35	33	6.12	30
50% mww x 30 days	202	51	48	5.22	20
50% mww x 60 days	272	3	3	8	34
100% mww x 30 days	218	41	34	6	27
100% mww x 60 days	191	57	57	4.78	15
$LSD_{0.05}$	NS	NS	NS	NS	NS

NS= Non significant, mww= municipal wastewater

DISCUSSION

As municipal wastewater were frequently used for irrigation purposes to cultivate vegetables, having different affinity for various micropollutants and heavy metals present in it. Which have different physiological influences depending on the nature of micropollutant, and type of metal ingested by organism or human beings leading to poisoning or death[5]. These metals may form different complexes with biomolecules and react adversely affecting the reproductive, gastrointestinal, immune, renal, nervous, hematopoietic, muscular and cardiovascular systems along with developmental processes[20].

Prolonged use of vegetables cultivated on municipal wastewater may lead to toxic levels of these heavy metals in living organism[21]. In various parts of the country municipal wastewater has been used for agriculture purposes, having higher concentrations of heavy metals with little differences in their composition[22,23]. As these heavy metals adversely affects different systems of the body[24,25].

In the present study histological features of rabbit's testes were evaluated against various concentrations of municipal wastewater and significant to highly significant differences were observed in any of the variables.

In the present study cell count/tubule/per section in rabbit's testes was adversely affected by increased concentration of municipal wastewater. Statistically significant decrease in cell count is observed in animals exposed to various concentrations of municipal wastewater, containing various amounts of different heavy metals. Heavy metals exceeding the permissible limits may cause such changes. Cd adversely affect the male reproductive system, these changes are dose dependent, which are in agreement with the present findings as Cd in the municipal wastewater was present in manifold increased concentration[26]. As a result metallothionein (MT) concentration become insufficient to bind with the Cd present resulting in oxidative stress and disruption of spermatogenesis[27].

Similarly Pb concentration also exceeds the permissible limits of WHO in the MWW, causing injury to spermatogenic and leydig cells[25]. Degenerative cells in the lumen of seminiferous tubule were also adversely influenced by various concentrations of MWW. The reason behind this adverse effect might be the presence of various heavy metals above permissible limits. Similar degenerative changes were also observed in testicular tissue of rats treated with textile waste water having heavy metals above permissible limits which confirms the present findings[28]. Animals treated with sewage sludge consisted of various heavy metals revealed similar changes[29].

Intraepithelial vacuolization in animals treated with different concentrations of MWW was observed. Seminiferous epithelium show intraepithelial vacuolization along with degenerated germ cells in the lumen of the tubule and multinucleated spermatid aggregates with apoptotic nuclei in animals with compromised defense system of the testicular tissue[26].

Leydig cell count was decreased in the animals exposed to different concentrations of MWW when compared to control, leading to decreased level of testosterone[39]. Another study conducted on animals exposed to Cd alone resulted in decreased levels of testosterone. This decreased count of leydig cell and testosterone level results in decreased body size and delayed gonadal maturation in fish collected from five different sites from the Furnas Reservoir, Grande River[30,31].

Sertoli cell count in animals exposed to MWW was significantly decreased irrespective of the duration of exposure, in agreement with the results of previous research work reported in the literature, where significant decrease in the sertoli cell count and sperm count in animals exposed to textile wastewater.[28] Another research contradicting the present results as sertoli cell count was not affected in animals exposed to lead acetate only[25]. The reason for this contradiction is that in present research cumulative effect of heavy metals was observed.

CONCLUSIONS

The present findings showed the progressive histological alterations caused by increased doses of municipal wastewater because of their direct effect on testes histology. The instant results highlighted the direct relationship of municipal wastewater concentration to the histological alterations of rabbit's testes. Moreover the increased concentrations of municipal waste water causing different degrees of damage in testes histology, overcoming the natural defences of these tissues.

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