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The Histopathological Effect of Alum on the Testis of Male Wistar Rats

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ABSTRACT

Alum is a nontoxic substance commonly used in water treatment to purify drinking water. This method of water purification is still practised in most parts of Nigeria. Marked reduction in sperm count in otherwise healthy men has been reported. The aim of the present study was to investigate the relationship that might exist between the use of alum in the purification of drinking water and impaired testicular function. A total 15 adult male albino wistar rats weighing between 130-220g were assigned to three groups (A, B and C) consisting of five rats each. Groups A and B were fed with animal feed and alum-water solution while group C was fed with animal feed and water only for 4 weeks. The alum-water solution was a mixture of 1g of granulated potash alum and 1 litre of water for group A and 40mg of granulated potash alum and 1 litre of water for group B. At the end of the 4th week, the rats were sacrificed, the testis extracted were weighed and histologically evaluated. Histological analysis of the testis showed normal histological features for group C and B while group A showed reduced spermatogenic layer. This study suggests that high dose of potash alum causes testicular atrophy resulting in hypospermatogenesis in adult male albino wistar rats.
Key words: Aluminium Potash, testicular toxicity, water purification.

The mean sperm count and sperm volume have been reported to have declined by 50% worldwide in the past 50 years (Carlessen et al, 1996). This decrease can be correlated with the increase in the pollutants of daily digestibles such as drinking water.

Alum is any combination of a trivalent metal (especially aluminum) and a univalent metal or radical (Chambers Dictionary, 1983). Generally, alum is a salt which is a combination of akali metal (such as sodium, potassium or ammonium) and a trivalent metal (such as aluminum, iron or chromium). Alum is both a specific chemical compound and a class of chemical compounds. The specific chemical compound is the hydrated potassium aluminum sulfate, $KAl(SO_4)_2 12H_2O$. The wider class of compounds known as alum has the related stoichiometry $AB(SO_4)_2 12H_2O$. There are many types of alum such as potash alum, soda alum, ammonium alum and chrome alum.

Potash alum is the type relevant to this study and it is the common alum of commerce. It is used as an astringent and antiseptic in various food processes and as a coagulant for water purification. A common method of producing potash alum is leading of alumina from bauxite which then reacts with potassium sulfate. Potash alum is found in naturally occurring minerals, alunite and kalinite.

The various medical uses of alum are as follows:

- as an ingredient used in some brands of toothpaste or toothpowder
- alum in crystal or powder form is sometimes applied to cuts to prevent or treat infection (Line 2002)
- powdered alum is commonly cited as a remedy for canker sores
- alum is used in many subunit vaccines as an adjuvant to enhance the body response to immunogens (Vibhu et al, 2007)
- preparations containing alum are used by pet owners to stem bleeding associated with animal injuries caused by improper nail clipping.
- Alum is used to purify water, by neutralizing the electrically double layer surrounding the fine suspended particles, allowing them to coagulate. After coagulation, the particle becomes large enough to settle and can be removed.

In many areas of Nigeria potable water is a problem hence people resort to the use of alum in purification of water for drinking. The aim of the study, therefore, is to evaluate through an animal study the relationship that might exist between impaired testicular function in male albino wistar rats and the use of alum (potash alum) in water treatment in our

environment.

MATERIALS AND METHODS

Animals: A total of 15 male albino wistar rats of about 3 months of age with weight ranging from 130-220g were used. The rats were randomly assigned into three groups. A, B and C with 5 in each group. Groups A and B (n=10) served as treatment groups while C (n=5) was the control. The rats were obtained from the Saint Francis Lab. Coal Camp Enugu and maintained in iron cages in the animal house of the Faculty of Basic Medical Sciences, Anambra State University, Uli. The animals were fed with guinea growers animal feed obtained from Bendel Feed and Flour Mill Ltd, Km 85 Benin Auchi Road, Ewu, Edo State, Nigeria.

Preparation of Alum Solution: 500g of granulated potash alum manufactured, by the May and Baker Ltd, Dagenham England, was obtained from Bridge Head Market, Onitsha, Nigeria. The alum was without adulteration (standard condition) 1g and 40mg of alum were measured using Satorius Chemical Weighing Balance and each dissolved in 1L of water after which the solution was shaken for effective dissolution. 40mg of alum is the standard dosage used for treatment of 1L of water (Martinsville, 2007).

Experimental Design: After the grouping, the alum-water solution was transferred into containers and then connected to the iron cages housing groups A and B. A high dose of Ig/L of alum solution was administered to group A and to group B, a normal dose of 40mg/L of alum solution was administered orally respectively. Group C, which serves as the control received only water. They were all fed with guinea growers animal feed produced by Bendel Feed and Flour Mill Ltd, Km 85 Benin-Auchi Road, Ewu Edo State, Nigeria.

The feed has the following nutritional contents:

- Protein 5.0g/100g
- Fat and oil 7.0g/100g
- Phosphorus 00.7g/100g
- Methionine 0.36g/100g
- Salt 0.30g/100g
- Lysine 0.75g/100g
- Energy 2700kcal

Tissue Processing: After the period of administration of the alum solution, the animals were sacrificed by cervical dislocation. A midline incision was made along the abdominopelvic region and another incision was made horizontally along the upper part of the pelvis. After the contents of the abdomen and pelvis were exposed, the testes were reflected from the scrotum and the spermatic fascia covering were removed. The cord was also removed. The ductile system and surrounding fats were weighed immediately and fixed in Bouin's fluid. The tissue was processed using the Automatic Tissue processor (Histokinett bench model). It was stained in Ehrlich's Haematoxylin and Eosin stains.

Method of Analysis: The micrographs were analyzed using histopathological method. The data were processed using 2007 Microsoft Excel Data Analysis Programme. The student t-test was used to determine the difference between means. $P<0.05$ was considered significant.

Ethical Clearance: Ethical clearanve was obtained from the Research and ethic committee of the Department of Anatomy, Anambra State University, Uli. Ihis is in line with treatment and handling of experimental animals.

RESULTS

Morphological Assessment of the testes

The testes harvested were ovoid in shape without signs of wrinkle. The tunica albuginea and fascia covering each testis were consistent and the left testes were larger in most of the rats.

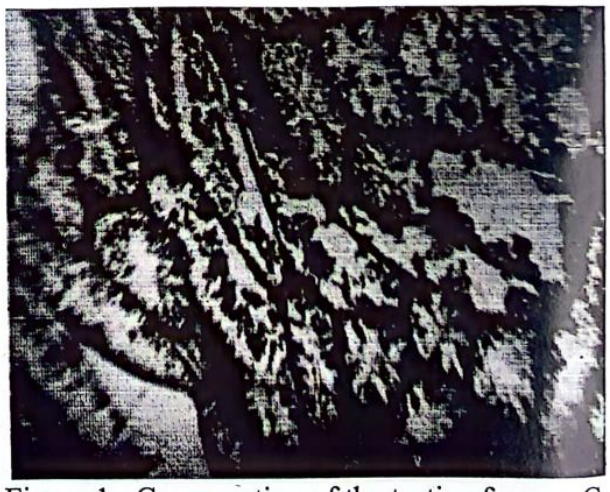

Figure 1: Cross section of the testis of group C (control) magnification x 100

Plate 2: Cross section of the testis of group B (40mg/kgbwt) magnification x 100

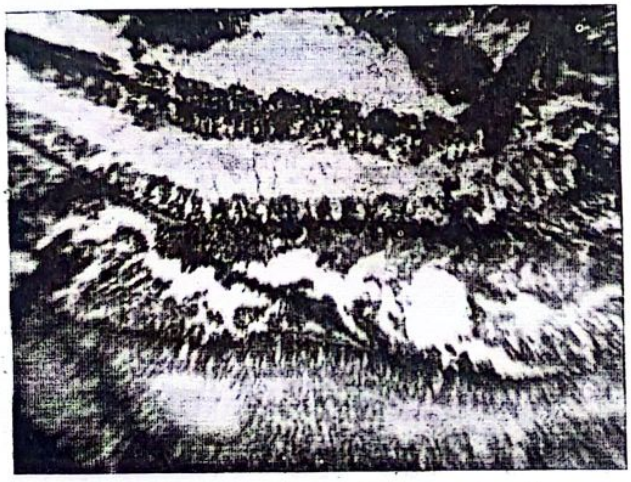

Plate 3: Cross section of the testis of group A (1g/kgbwt) magnification x 100

Histopathological studies

Plate 1 shows light micrograph for control group (Group C). Testes of control group (group C) showed normal histological features such as intact seminiferous tubules lined with complex stratified epithelium called germinal or seminiferous epithelium. Their outer wall is surrounded by a well-defined basal lamina and a fibrous connective tissue consisting of several layers of fibroblast. The innermost layer, adhering to the basal lamina, consists of flattened myoid cells, which have characteristics of smooth muscle. Interstitial (Leydig) cells occupy much of the space between seminiferous tubules. The tubules are lined in most cases by cells in various stages of spermatogenesis namely sustentacula cells (Sertoli cells), spermatogonia type A and B, primary and secondary spermatocytes, spermatids and spermatozoa are seen.

Group B (see plate 2) showed normal histological features like in those of control group, though there is mild atrophy with thickening of the basement membrane in some tubules.

Group A (see plate 3) Testes from group A showed reduced spermatogenic layers (only spermatogonia type A and B, spermatocytes and very few spermatids) due to atrophy resulting in moderate to severe hypospermatogenesis. Only about 40% of the tubules show evidence of spermatogenesis.

DISCUSSSION

Several studies have been carried out on the effect of potash alum on the histology of testis in experimental animals. The present study reveals a hypospermatogenic effect on the testes of Group A (high dose alum treated). This finding is in agreement with the works done by other researchers. Shahraki et al (2004) reported that aluminum significantly decreases sex hormone and spermatozoid concentration per gram of tissues in vas deference, epididymis and testes in male rats. Llobet et al (1994) also reported that high dose of aluminum significantly decrease testicular and epididymal weights in male mice. In a study by Yousef et al (1996), it was revealed that aluminum significantly decrease sperm motility and viability and response which was both concentration and time dependent. The result of this study also shows a reduction in spermatogenic layer in Group A (18/kgbw) testes when compared to group B (40mg/kgbw) form of atrophy (that can lead to hypospermatogenesis) may be caused by high dose of alum. This agrees with report of Boulikowski et al (1991) that potassium produces necrosis of spermatic and germinal epithelial cells. Aluminum causes significant decrease in testicular spermatid count and epididymal sperm count. Kutlubay et al (2007) also reported that aluminum causes testicular damage.

In conclusion, high concentration of alum causes hypospermatogenesis due to testicular atrophy thus affecting fertility in male albino wistar rats and by extension may induce testicular toxicity in man.

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