International Journal of Basic and Applied Chemical Sciences ISSN: 2277-2073 (Online)
An Online International Journal Available at http://www.cibtech.org/jcs.htm
2012 Vol. 2 (2) April-June, pp.25-31/Pateja.
Research Article

THE EFFECT OF CHRONIC ADMINISTRATION ON ETHIDIUM BROMIDE ON THE HISTOLOGY OF TESTIES OF ALBINO MICE

***R.K. Pateja**
Department of Chemistry G.N.Khalsa College, Mumbai-19
**Author for Correspondence*

ABSTRACT
Adult Mice (B.Wt. 30 gms and 40gms) were treated at a 5mg\kgB Wt. and 10 mg \kg BWt. of Ethidium Bromide for 10 days in drinking water.Control animals were given equal dose of deionised water. Quantitative and qualitative changes were studied in Testies. The overall cellularity of the testies, was noticed. The changes were charecterised by**,** the number of seminiferous epithelial cell layers (NSECL), the thickness of seminiferous tubules (TST) and the interior diameter of the seminiferous tubules (DST) in the testis section and injury to testicular tissue**,** were noticed. Also reduction in visceral epithelial cell number was noticed..The size of the testies slightly decreased and interstitium shrinked. Also a Slight change in testies weight was recorded.Body weight of the animal was slightly altered after challenge with Ethidium Bromide. Oxidative stress and apoptotic changes in the testes were detected. Typical morphological changes of apoptosis were observed using a variety of methods (HE Staining). These results led to the conclusion that Ethidium Bromide causes damage as well as apoptosis.

Key Words*: Ethidium Bromide, Toxicity, Testies, Histopathology, AlbinoMic*

INTRODUCTION

Testes are components of both the reproductive system and the endocrine system. The primary functions of the testes are to produce sperm (Spermatogenesis) and to produce androgens, primarily testosterone. Both functions of the testicle are influenced by gonadotropic hormones produced by the anterior pituitary. Luteinizing hormone (LH) results in testosterone release. The presence of both testosterone and follicle-stimulating hormone (FSH) is needed to support spermatogenesis. Germ cells develop into spermatogonia, spermatocytes, spermatids and spermatozoon through the process of spermatogenesis.
Sertoli cells are the true epithelium of the seminiferous epithelium, critical for the support of germ cell development into spermatozoa. Leydig cells are cells localized between seminiferous tubules that produce and secrete testosterone and other androgens important for sexual development and puberty, secondary sexual characteristics like facial hair, sexual behavior and libido, supporting spermatogenesis and erectile function. Testosterone also controls testicular volume. Blood supply and lymphatic drainage. Blood supply and lymphatic drainage of the testes and scrotum are distinct. The paired testicular arteries arise directly from the abdominal aorta and descend through the inguinal canal, while the scrotum and the rest of the external genitalia is supplied by the internal pudendal artery (itself a branch of the internal iliac artery) (Chapple *et al.*, 2011) . Many anatomical features of the adult testis reflect its developmental origin in the abdomen. The layers of tissue enclosing each testicle are derived from the layers of the anterior abdominal wall. Notably, the cremasteric muscle arises from the internal oblique muscle. (Richard 2009). The blood–testis barrier large molecules cannot pass from the blood into the lumen of a seminiferous tubule due to the presence of tight junctions between adjacent Sertoli cells. The spermatogonia are in the basal compartment (deep to the level of the tight junctions) and the more mature forms such as primary and secondary spermatocytes and spermatids are in the adluminal compartment. The function of the blood–testis barrier (red highlight in diagram above) may be to prevent an auto-immune reaction. Mature sperm (and their antigens) arise long after immune tolerance is established in infancy. Therefore, since sperm are antigenically different from self tissue, a male animal can react

immunologically to his own sperm (Skau 2003). In fact, he is capable of making antibodies against them. Human testicles are smaller than chimpanzee testicles but larger than gorilla testicles (Zhang et al., 2004). Ethidium Bromide is one of the most toxic environmental and industrial pollutants, is known to exert gonadotoxic and spermiotoxic effects. In the present study, we examined the toxic effect of Ethidium Bromide on the testis of freshwater crab, Sinopotamon henanense. Crabs were exposed to different Cd concentrations (from 0 to 116.00 mg•L−1) for 7 d (Wang *et al.,* (2011). Ethidium bromide is commonly used in molecular biology laboratories to stain electrophoresis gels (Huang and Fu, 2005). The compound forms fluorescent complexes with nucleic acids (Waring 1965) and these can be viewed under UV light. Ethidium bromide (EB) is described to be mutagenic (Singer *et al.,* 1999) and moderately toxic after an acute exposure (National Toxicology Program, 2005). EB can be absorbed through skin and therefore it is important to avoid direct contact with the chemical.Ethidium bromide as it is a known mutagen in certain animal and microorganism test systems (Ohta *et al.,* 2001). Although the compound has not been thoroughly evaluated in humans, based on current toxicity data and its interaction with DNA it should be handled with considerable caution. Ethidium bromide is a large, flat basic molecule that resembles a DNA base pair. Because of its chemical structure, it can intercalate into a DNA strand. Not enough evidences are available in mammals, therefore this study was planned. From current study it has been observed to be very nephrotoxic. Therefore all individuals should regularly review their risk assessments and work practices for EtBr. Ethidium bromide may be a mutagen, carcinogen or teratogen although this depends on the organism and conditions. In the laboratory the intercalating properties have long been utilized to minimize chromosomal condensation when a culture is exposed to mitotic arresting agents during harvest. The resulting slide preparations permit a higher degree of resolution and thus more confidence in determining structural integrity of chromosomes upon microscopic analysis. Despite the performance advantage of using SYBR dyes instead of EtBr for staining purposes, many researchers still prefer EtBr since it is considerably less expensive. Ethidium bromide is thought to act as a mutagen because it intercalates double stranded DNA, thereby deforming the molecules. This can affect DNA biological processes, like DNA replication and transcription (Huang Q and Fu WL 2005). If the level is high enough, that exposure may interfere with replication of mitochondrial DNA in some human cell lines, although the implications of that are not clear. Testing in mice and humans and longer studies in any mammalian system is required. A low dose of ethidium bromide leads to an increase of total mitochondrial DNA while higher concentrations induce the mt-DNA 4997 deletion in a human neuronal cell line (Wurmb-Schwark *et al.,* 2006). It is used as a molecular probe for staining nucleic acids in fluorescent microscopy studies of multidrug resistance (Neyfakh 1988). It is also used as a DNA probe for various studies including characterizing and quantifying DNA (Green, 1990). It is also used as a derivatizing analytical reagent in clinical settings for continuous monitoring of levels of anticancer drugs in biological fluids, including blood, serum and urine by measurement of dose-critical levels of DNA-binding.

According to Lunn and Samsone (1987) safe handling of EB in laboratories to avoid human exposures to mutagenic solutions containing EB has been addressed by Lunn and Sansone (1987) and Quillardet and Hofnung (1988). They concluded that EB should be handled as a carcinogen in terms of identifying methods of safe waste disposal. EB is not known to occur naturally. No information was found in the available literature on detection of EB in environmental media. Several spill clean-up and disposal methods have been recommended in the available literature for EB. They are based on careful removal to achieve elimination of mutagenicity of solutions by decontamination and degradation. Published methods include treatment with potassium permanganate/hydrochloric acid or hypophorous acid/sodium nitrite, adsorption on activated charcoal and incineration at high temperatures (Quillardet and Hofnung 1988).

The American Conference of Governmental Industrial Hygienists (ACGIH) has not adopted a time-weighted average/threshold limit value (TLV/TWA) for this compound. EB is categorized as an acute hazard under SARA sections 311/312 (40 CFR 370.21) (Anonymous, 1994b).

MATERIALS AND METHODS

Tissue Preparation

Young Mice of B.Wt. 30 gms and 40gms were used as a model in the present study treated at a 5mg\kgB Wt. and 10 mg \kg BWt.(each) of Ethidium Bromide for 10 days in drinking water. Control animals were given equal dose of deionised water. Total five groups of mice were set in the experiment. Each group had 6 mice. They were acclamitised to laboratory conditions for 15days prior to the commencement of the treatment. Mice were kept in open air cages at room temperature. Mice were fed standard rodent palate diet (Hindustan Lever ltd). Experimental animals were given Ethidium bromide orally through drinking water.

Animals of experimental and control group were sacrificed on tenth day of treatment by cervical dislocation. The testies of experimental and control group of mice were fixed in formalin for 4 hrs. They were dehydrated, in graded EtOH series, cleared in xylene, infilterated with and embedded in pure filtered paraffin wax (M.P.58 degree centigrade). Deparaffinised sections (5-7 microns) were stained by haematoxylin and eosin to monitor the extent of changes in the spleen histoarchitechture. Every alternate section of the testies was microscopically examined and appropriate areas were microphotographed and enlarged. Disintegration was also microphotographed to record the vulnarebility to Ethidium Bromide toxication. The behaviourable changes in mice were also observed. The changes were charecterised by, the number of seminiferous epithelial cell layers (NSECL), the thickness of seminiferous tubules (TST) and the interior diameter of the seminiferous tubules (DST) in the testis section and injury to testicular tissue, were noticed. Also reduction in visceral epithelial cell number was noticed. The degenerating cells were identified on the basis of desquamation of cells, nuclear pycnosis, chromatolysis and loss of shape. Necrotic and hyperplasia patches were seen and microphotographed. Alternate Serial sections were examined to assess testies structure.

RESULTS AND DISCUSSIONS

The testies of control mice weighed 0.536 mgs (mean value). The weight of both control and experimental mice were recorded before and after the experiment. The weight of Testies in all the groups of mice before and after the experiment were observed according to TABLE-I. The overall shape of the testies was not altered nor there was any significant change in organ weight as compared with control. But still slight changes in the weight were noticed.

Behavioural Observations

Control group:

All animals showed normal bevahiour and there was no mortality or lingering of animals.

Treated Group (30gms mice):

Administration of Ethidium Bromide to rats resulted in marked alterations in bevahiour revealing nervous manifestations(abnormal neurobevahiour)in the treated groups as increased landing of the limbs,weakness of the muscles,general emasciation .The severity of clinical science was dose and time dependent as these manifestations appeared on the 3rd day of EtBr treatment in 30gms mice given with a dose of 10mgs\kg BWt.Two out of six died. But just lingering was observed in mice with 30gms weight with a dose of 5mgs\kg BWt.in 3 out of 6 mice.

Treated Group (40gms mice):

Animals weighing 40 gms administered 5 milligram per kg body weight showed normal bevahiour and their appetite was normal but the animals weighing 40 gms administered with 10 milligram/kg body weight of the dose showed drousiness and their apetite was reduced.No mortality and lingering was observed in the mice.

Table 1: Weight of Testies before and after experiment

Groups	Testies Weight Before Experiment(av)	Testies Weight After Experiment(av)
Control	0.20gms	0.20gms
30grms(5mgs\)	0.20gms	0.19gms
30gms(10mgs\)	0.20gms	0.18gms
40gms(5mgs\)	0.22gms	0.21gms
40gms(10mgs\)	0.20gms	0.18gms

Table 2: Percent Degenerative changes in Testicular cells

Groups	%Degenerative Changes in Testicular Cells
Control	0%
30grms(5mgs\)	35%
30gms(10mgs\)	60%
40gms(5mgs\)	30%
40gms(10mgs\)	25%

Histological Observations of Testis

Histopathological changes of Ethidium Bromide treated rats of 30gms BWt with a dose of 5mgs/kg body wt showed little degenerative changes characterized by injury, Lymphoid Necrosis/Apoptosis.Ethidium Bromide treated mice with a body weight 30 gms, and a dose of 10mgs\kgBWt showed the maximum degenerative changes. When evaluating a testicular specimen by light microscopy on an HandE stained section. In Figure 1 normal structure of testies was seen. The control mice testis showed normal seminiferous tubules with various stages of spermatogenesis, sperm bundles in the lumen and interstitial Leydig cells (Figures 1). In our studies we also found that Ethidium Bromide caused disorganization, denudation and reduction in germinal epithelial cells of the seminiferous tubules and an accompanying absence of sperm in the lumina in histological sections. These histopathological changes are in accord with the results of Cui *et al.*,14 and of Chinoy *et al.*, 22. Thus the number of seminiferous epithelium cell layers (NSECL), the thickness seminiferous tubules (TST) and the diameter of seminiferous tubule (DST) were significantly reduced in the high Ethidium Bromide group with a dose of 10mgs/kg Bwt(in mice with BWt 30gms) (Figures 2 and 3). In mice with 40gmsBWt, treated with 5 mgs/kgBwt, the NSECL, TST and DST did not show very significant changes in the testis tissue,although loosening of the seminiferous epithelium cell walls and the lack of spermatozoa in the lumen can be distinctly observed. Both the reduction of the NSECL, TST and DST and the histopathological changes are evident in 40 gms mice treated with10mgs/kgBWt. Our results thus show that damage by Ethidium Bromide to testicular tissues changes depends on the .toxic exposure of dose /kg BWt**.** (Figures 4 and 5). Slight change in Testies weight was recorded. Body weight of the animal was slightly altered after challenge with Ethidium Bromide. No study has been done on the histopathological changes in Testis of Albino mice due to Ethidium Bromide toxicity. Hence the present study has been done.

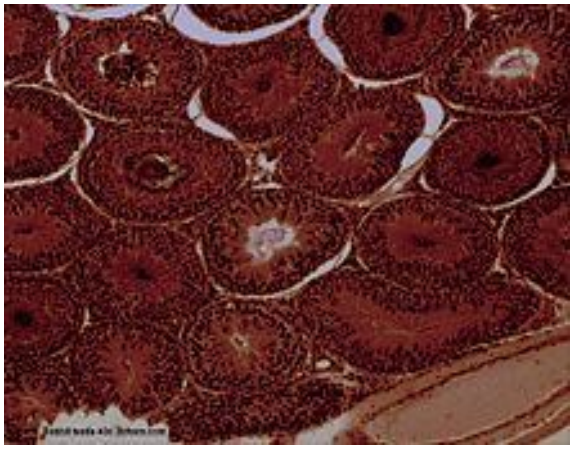

Figure 1: Normal Testies seen in control mice Figure

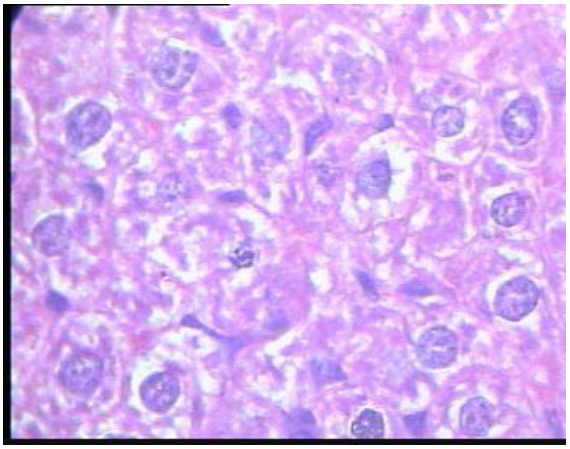

Figure 2: Abnormal Testies in 30gms mice treated with 5mgs/kg Bwt

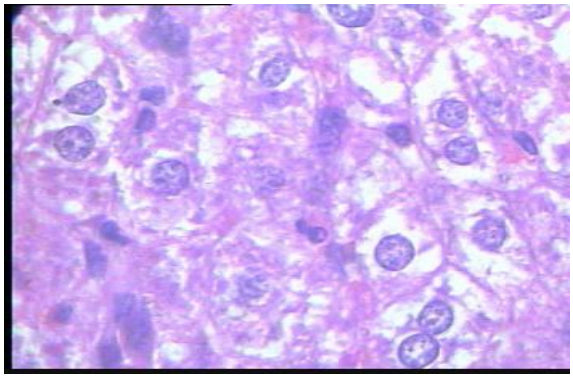

Figure 3: Abnormal Testies seen in30gms mice treated with 10mgs/kg Bwt

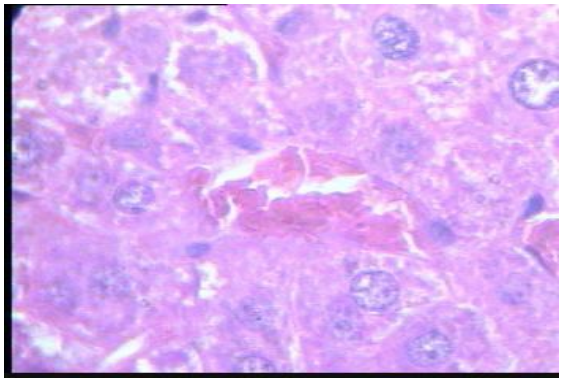

Figure 4: Abnormal Testies sin een 40gms mice treated with 5mgs/kgBwt

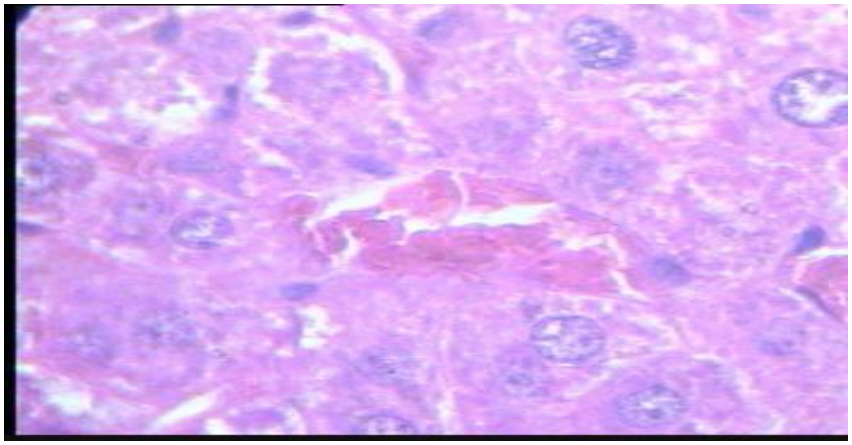

Figure 5: Testies of 40gms treated with 10mgs\kgBwt

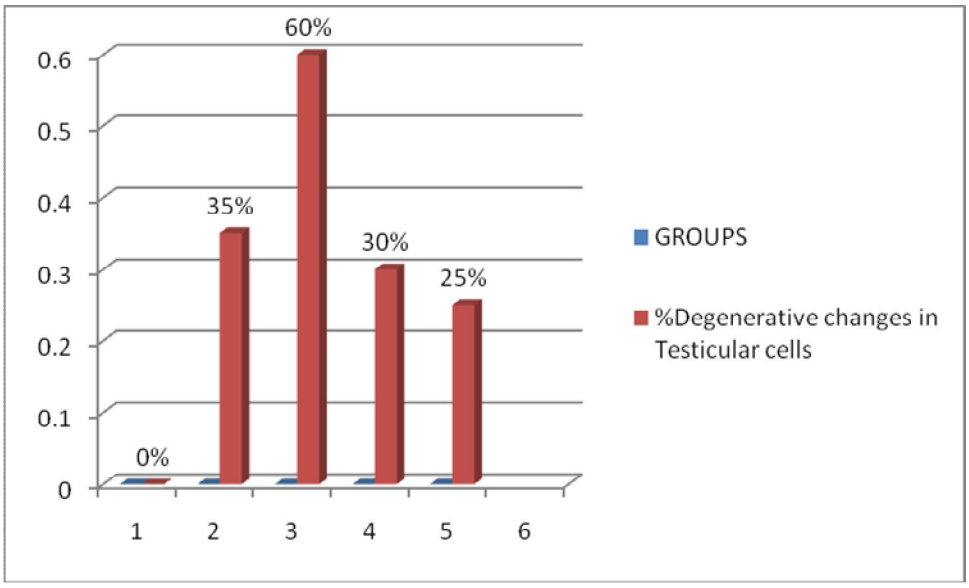

Figure 6: Graph showing percent degenerative changes in Testicular cells

ACKNOWLEDGEMENT
I dedicate my work to my family. I acknowledge my heartiest feelings for their sacrifice and inspiration rendered during the period of study.

REFERENCES

Anonymous (1994). Material Safety Data Sheet (MSDS): Ethidium bromide. *MDL Information Systems Inc.,*San Leandro, CA (on-line retrieval through STN database: MSDS-OHS) Clinical neuroanatomy books.google.co.in Richard S. Snell - 2009–542.

Green FJ (1990). The Sigma-Aldrich Handbook of Stains, Dyes andIndicators . Aldrich Chemical Co., Inc. *Milwaukee WI.*

Huang Q and Fu WL (2005). Comparative analysis of the DNA staining efficiencies of different fluorescent dyes in preparative agarose gel electrophoresis. *Clin. Chem. Lab. Med.* **43**(8) 841–2.

Jianzhi Zhang1, Xiaoxia Wang and Ondrej Podlaha (2004). Testing the Chromosomal Speciation Hypothesis for Humans and Chimpanzees. *Author Affiliations, Genome Research* 2004 **14** 845-851.

Lunn G and Sansone EB (1987). Ethidium bromide: destruction and decontamination of solutions. *Analytical Biochemistry* **162** 453-458.

MJ Waring (1965). Complex formation between ethidium bromide and nucleic acids. *Journal of Molecular Biology* **13**(1) 269–282.

National Toxicology Program (2005). Ethidium Bromide: Genetic Toxicity. Available at: *http://ntp.niehs.nih.gov/index.cfm?objectid=BDAF3AE4-123F-7908-7BE09D1BEA25B435.* [Accessed on: September 30, 2009].

Nayfakh AA (1988). Use of fluorescent dyes as molecular probes for the study of multidrug resistance. *Exp. Cell. Res.* **174**(l) 168-176.

Ohta T Tokishita S Yamagata H (2001). Ethidium bromide and SYBR Green I enhance the genotoxicity of UV-irradiation and chemical mutagens in *E.coli*. *Mutation Research* **492**(1-2) 91–7. PMID 11377248.

Philip A Skau and Ivar Folstad (2005). *Behavioral Ecology* **16**(2) 410-416.

Quillardet P and Hofnung M (1988). Ethidium bromide and safety-readers suggest alternative solutions. *Trends in Genetics* **4**(4) 89-90.

RChapple C and William D (2011). Steers Reviews: http://books.google.co.in/books/about/Practical_Urology.html?id =A9m8TkdCUqEC Springer [Accessed on: 13-Jul-2011 569].

Von Wurmb-Schwark N, Cavelier L and Cortopassi GA (2006). A low dose of ethidium bromide leads to an increase of total mitochondrial DNA while higher concentrations induce the mtDNA 4997 deletion in a human neuronal cell line. Mutation Research **596**(1-2) 57–63.

Wang L, Xu T, Lei W-W, Liu D-M and Li Y-J (2011). Cadmium-Induced Oxidative Stress and Apoptotic Changes in the Testis of Freshwater Crab, Sinopotamon henanense. PLoS ONE **6**(11) e27853.