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Research Article

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

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ABSTRACT
Adult Mice (B.Wt. 30 gms & 40gms) were treated at a 5mg/kgB.Wt. and 10 mg/kg B.Wt. 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 kidney. The following features were evaluated: the overall cellularity of the glomerulus, the symmetry of the glomerulus and the thickness of the capillary walls space between the tubules . These changes were characterised by glomerular and tubulointerstitial injury, nephrosis, synechia, necrotic changes, cirrhosis and ischemia were noticed.Also reduction in visceral epithelial cell number was noticed. The size of the kidney slightly decreased and interstitium shrinked. Also a slight change in kidney weight was recorded. Body weight of the animal was slightly altered after challenge with Ethidium Bromide.

***Key Words**: Ethidium Bromide, Toxicity, Kidney, Histopathology, Albino Mice*

INTRODUCTION
Ethidium bromide is commonly used to detect nucleic acids in molecular biology. Since ultraviolet light is harmful to eyes and skin, gels stained with ethidium bromide are usually viewed indirectly using an enclosed camera, with the fluorescent images recorded as photographs. 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**.**
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, 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 are 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 & 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 & 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 kidney 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 kidney histoarchitechture.Every alternate section of the kidney was microscopically examined and appropriate areas were microphotographed and enlarged.Glomerular disintegration were also microphotographed to record the vulnarebility to Ethidium Bromide toxication.The behaviourable changes in mice were also observed .

The population of renal tubules and blood capillaries in each lobule was also quantitated approximately. The degenerating cells were identified on the basis of desquamation of cells ,nuclear pycnosis,chromatolysis and loss of shape.Necrotic and cirrhosis patches were seen and microphotographed. Serial sections were examined to assess glomerular structure.

RESULTS AND DISCUSSION

The kidney 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 kidney in all the groups of mice before and after the experiment were observed according to TABLE-I. The overall shape of the

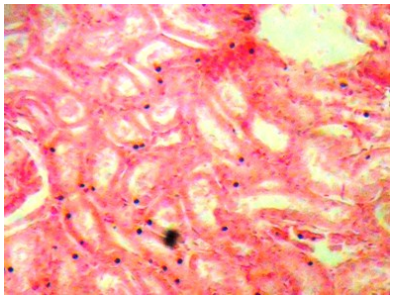

Figure1: Normal kidney seen in control mice with 5mgs/kg Bwt

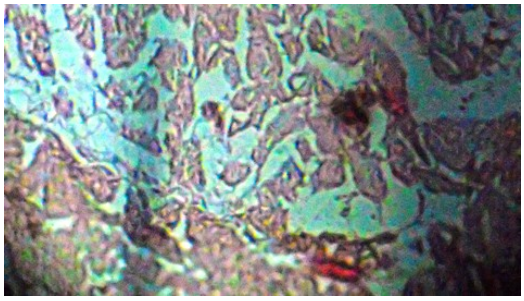

Figure 2: AbNormal kidney seen in mice treated

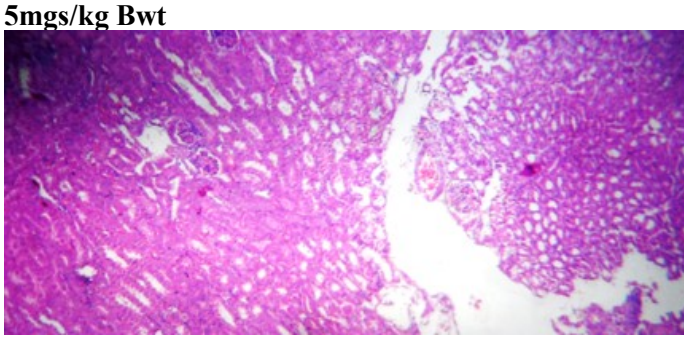

Figure 3: Haemorrhage in blood capillaries and necrotic patches

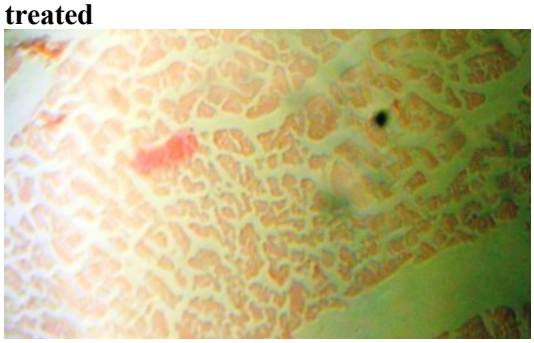

Figure4: Haemorrhage in blood capillaries and necrotic patches seen in mice of 30gms treated with 10mgs\kg Bwt patches seen in miceof 30gms treatewith10mgs\kg Bw

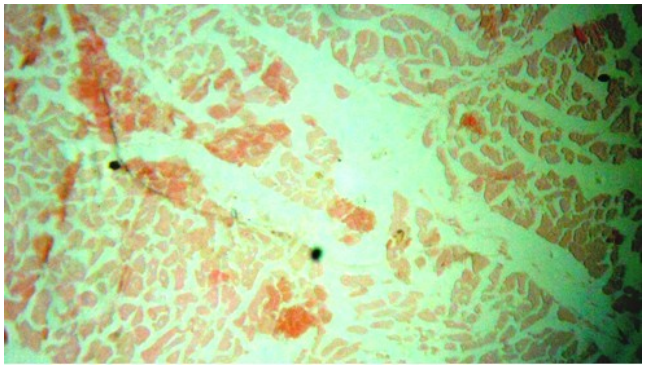

Figure 5: Necrosis and infilteration in mice of 40gms 40gms treated with 5mgs\kgBwt

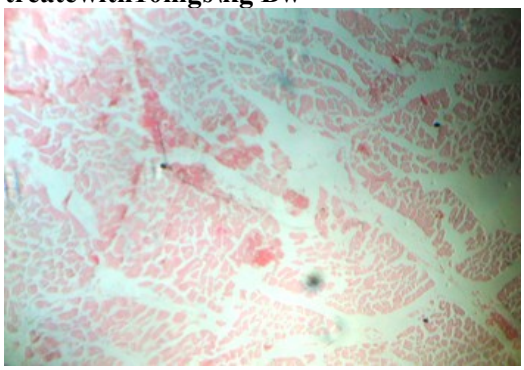

Figure 6: Necrosis and infilteration in mice of treated with 10mgs\kgBwt

kidney 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

(a) ***Control group****:* All animals showed normal behaviour and there was no mortality or lingering of animals .

(b)*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.

(C)**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 40gms

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.

Histological Observations of Kidney

Histopathological changes of Ethidium Bromide treated rats of 30gms BWt with a dose of 5mgs/kg body wt showed little degenerative changes characterized by glomerular and tubulointerstitial injury, nephrosis, synechia**,** necrotic changes, cirrhosis and ischemia. Ethidium Bromide treated mice with a body weight 30 gms, 10mgs\kgBWt showed that the normal renal cortex contains less glomeruli, other vessels, tubules and interstitium. When evaluating a renal specimen by light microscopy on an H&E stained section, the following glomerular features were evaluated: the overall cellularity of the glomerulus, the symmetry of the glomerulus (which of course is broken at the hilum if there is a section through it) and the thickness of the capillary walls. In slide 1 normal structure of kidney was seen. All the glomerular capillaries were of the same thickness, which is very thin (almost wispy). With normal cellularity, cell nuclei are not clustered or overlapping. Infiltration or, rarely, an angiotrophic lymphoma is seen. In the cortex but not the medulla, the tubules were almost back to back, i.e. the tubular basement membranes were almost touching. There is very little interstitium in the cortex, therefore, if there is space between the tubules, there is something wrong in the tubulointerstitial compartment (e.g. edema or fibrosis). Intrarenal arteries have very little intima, i.e. there is little or no space between the endothelium and the muscularis.. Visceral epithelial cells line the capillary walls. Parietal epithelial cells line Bowman's capsule, and are continuous with the proximal tubular epithelial cells. Endothelial cells line capillary lumens. Mesangial cells are in the middle (meso) between the capillaries (angis).

Table 1: Weight of kidney before and after experiment

GROUPS	Kidney Weight Before Experiment(av)	Kidney weight After Experiment(av)
Control	0.26gms	0.26gms
30grms(5mgs\)	0.23gms	0.22gms
30gms(10mgs\)	0.26gms	0.24gms
40gms(5mgs\)	0.29gms	0.28gms
40gms(10mgs\)	0.30gms	0.28gms

Table 2: Percent Degenerative changes in kidney cells

GROUPS	%Degenerative changes in Hepatocytes
control	0%
30grms(5mgs\)	30%
30gms(10mgs\)	55%
40gms(5mgs\)	20%
40gms(10mgs\)	30%

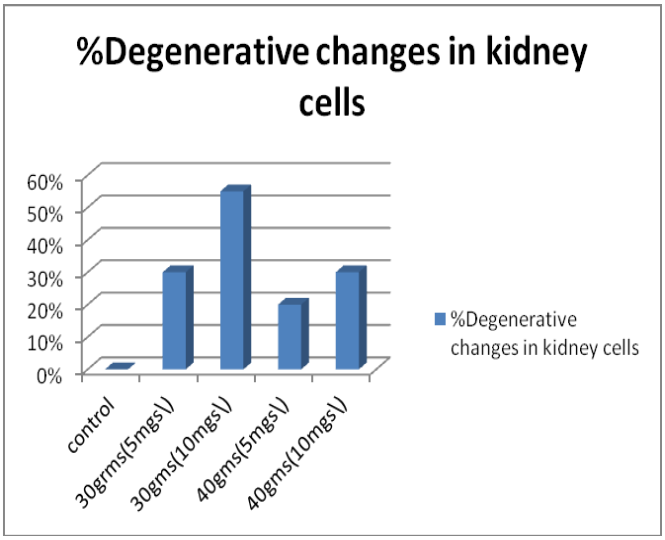

Figure 6: Graph showing percent degenerative changes in kidney cells

Slight change in kidney 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 kidney of Albino mice due to Ethidium Bromide toxicity. Hence the present study has been done.As seen in (Fig:2) the changes were charecterised by disorganization of lobules, Necrotic changes ,cirrhosis and ischemia were noticed. Quantitative changes were also characterised by significant decrease in the number of renal cells . The glomerulus and kidney cells began to degenerate along with the haemorrhage in blood capillaries (Fig. 2) but most of the glomerulus reached the maturity in mice with a BWt of 30gms treated with 5mgs\KgBWt..The percentage of degenerating glomerulus and kidney cells was hence found to be still high as compared with control in mice with a BWt of 30gms treated with 10mgs\KgBwt **(**Fig. 3) & (Fig. 4).In mice with 40gms BWt treated with 10mgs\KgBwt (Fig:4) and mice with 40gms treated with 5mgs\kgBWt. (Fig. 5) & (Fig. 6) dose showed lesser and still lesser disintegration respectively ..The overall shape of the kidney was not altered nor there was any significant change in organ weight as compared with control. Acute ethidium bromide lead to nephrosis and caused a notable reduction in visceral epithelial cell number. However, widespread glomerular injury is characterized by synechia.

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