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CHRONIC EFFECT OF ETHIDIUM BROMIDE ON OVARIAN HISTOARCHITECHTURE 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 BWt. Of Ethidium Bromide for 10 days in drinking water. Control animals were given equal dose of deionised water. Qualitative changes were studied in Ovary. Histopathological changes in Ovary, Primary Follicles, Secondary Follicles, Tertiary Follicles, Graffian follicles, etc. were evaluated. Significant changes were noticed. These changes were characterized by disorganization of follicles and degenerative changes were noticed. Slight change in ovarian weight was recorded. Body weight of the animal was slightly altered after challenge with Ethidium Bromide.

KEY WORDS

Toxicity, Ethidium Bromide, Ovary, Histoarchitectur, Albino Mice.

INTRODUCTION

Mammalian Ovary exhibits seasonal and Cyclic Changes. In non –primate species these are synchronised with estrous and in primates with the menstrual cycle. The mammalian ovary functions as a cytogenous gland, i.e., it has both endocrine and exocrine functions. Ethidium Bromide forms one of the sources of toxicants in the laboratories. Ethidium bromide is an intercalating agent commonly used as a fluorescent tag (nucleic acid stain) in molecular biology laboratories for techniques such as agarose gel electrophoresis. It is commonly abbreviated as "EtBr", which is also an abbreviation for bromoethane. When exposed to ultraviolet light, it will fluoresce with an orange colour, intensifying almost 20-fold after binding to DNA. Under the name homidium, it has been commonly used since the 1950s in veterinary to treat trypanosomosis in cattle, a disease caused by trypanosomes (1). The high incidence of antibiotic resistance makes this treatment impractical in some areas, where the related isometamidium chloride is used instead (2, 3, 5). Ethidium bromide may be a mutagen, carcinogen or teratogen although this depends on the organism and conditions (4, 6, and 8). The National Toxicology Program states it is nonmutagenic in rats and mice. (7, 9, 10]) Ethidium bromide (Homidium brand) use in animals to treat trypanosome infection suggests that toxicity and mutagenicity are not high. Studies have been conducted in animals to evaluate EtBr as a potential antitumorigenic chemotherapeutic agent.[11] Its chemotherapeutic use is due to its toxicity to mitochondria.[12] The above studies do not support the commonly held idea that ethidium bromide is a potent mutagen in humans, but they do indicate that it can be toxic at high concentrations. No study has been done on the histopathological changes in the ovary of Albino mice due to Ethidium Bromide toxicity. Hence the present study has been done.

MATERIAL AND METHODS

Adult males and females of swiss albino mice weighing 30 gms and 40 gms were used as a model in the present study. Two groups of mice were given Ethidium Bromide at a dose level of 5mgs\kg body wt. and 10 mgs\kg body wt. for 10 days. Experimental animals were given sodium fluoride orally through drinking water.

Animals of experimental and control group were sacrificed on tenth day of treatment by cervical

dislocation. The contralateral ovaries of experimental and control group of mice were fixed in formalin for 24 hrs. They were dehydrated, in graded EtOH series, cleared in xylene, in filterated 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 ovary histoarchitechture. Every alternate section of the ovary was microscopically examined and appropriate areas were microphotographed and enlarged.

RESULTS AND DISCUSSIONS

The ovary of control mice weighed 0.536 mgs (mean value). Necrosis, cirrhosis,and eschemia occurred but was not marked in control animals (FIG:1&2). The overall shape of the ovary was not altered but there was slight decrease in the weight of the organ of mice treated with 5mgs (FIG.3) and still more decrease in weight was observed in mice treated with 10 mgs\kg BWt, as compared with control (FIG.4). Haemorrhage in blood capillaries was prominent in mice treated with 5mgs and very prominent in mice treated with 10mgs\kg Bwt. But most of the follicles reached the maturity. The total number of atretic follicles in mice treated with 5mgs/kg Bwt was hence found to be high as compared with control. The overall shape of the ovary was not altered nor there did any significant change in organ weight as compare with control

Five days chronic ingestion of (10mgs\kg B.Wt.) Ethidium bromide reaveled pronounced degenerative changes in ovarian histoarchitechture. The sequence of atretic changes involved nuclear degeneration, characterized by Chromatolysis, rupture and dissolution of nuclear membrane. Granulosa cells associated with degenerating follicle types (bilaminar, Multilaminar and graffian follicle) showed desquamation, cytolysis and nuclear dissolution.Extensive vacuolation also occurred in these follicles. Only few follicles reached the maturity. In most of the females Graffian follicle failed to rupture, which led to failure in ovulation. This resulted in Sterility of the females.

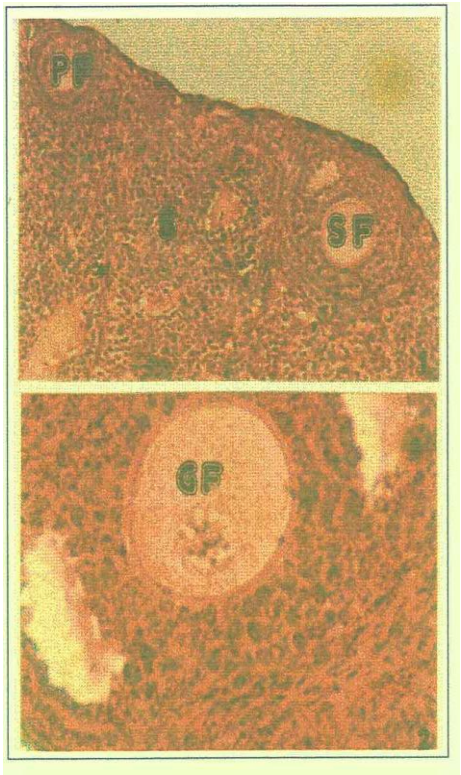

FIG: 1 Low power photograph showing primaryPF)(and secondary follicles(SF) in control Ovary. Haematoxilin -Eosin staning (10X)

FIG: 2 High power photomicrograph showing Graffian (GF) and Atretic Follicles (AF) in a control ovary. Haematoxiline –Eosin staining (40X)

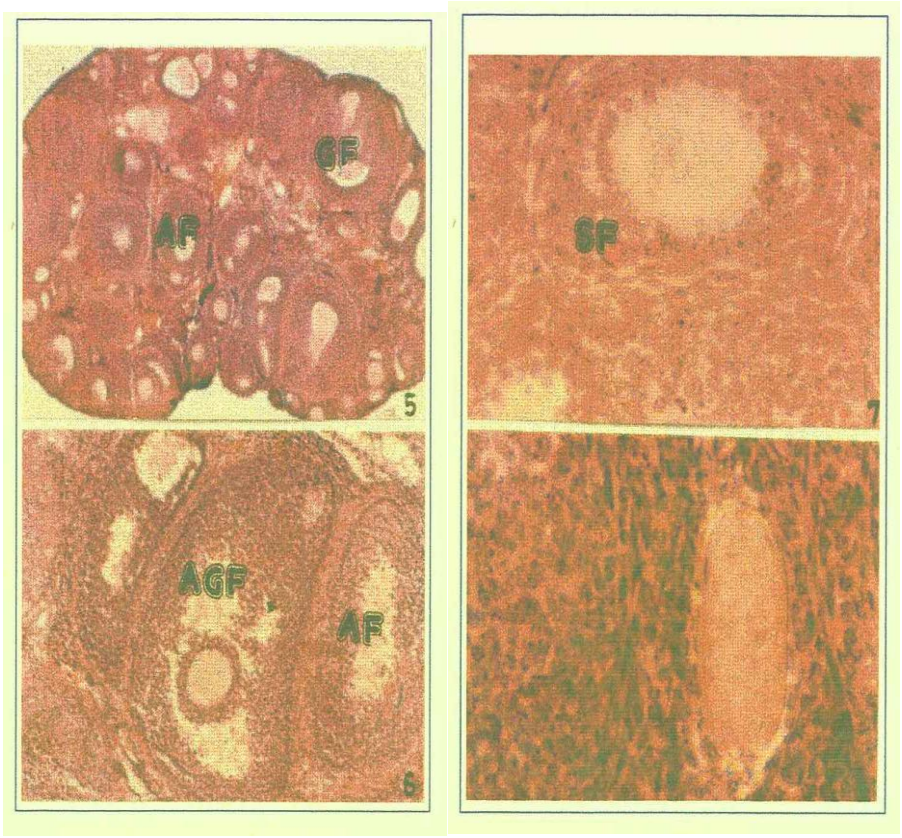

FIG. 3: High power photomicrograph of Mice Ovary after 5mg/kg Bwt.dose of Ethidium bromide. NOTE-Secondary follicle (SF) and minor changes in Granulosa cells(40X).Well developed follicle with fertilization cone.Slight degeneration seen in follicular cells of Graffian Follicle(GF).Atretic Follicles seen clearly.

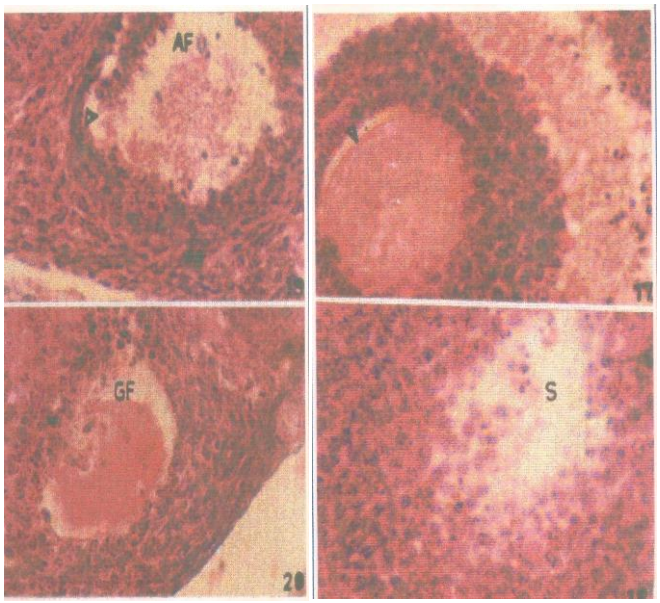

FIG.4: High power photomicrograph of an ovary demonstrating various degenerative changes after 10mg/kg Bwt.dose of Ethidium Bromide.

NOTE- Cell necrosis,infilteration of interstistial cells and vacuolation of corpus leuteum

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