**ABSTRACT**

Background: Contamination of the environment (Air, water and soil) with antimony compounds may affect human health through the con-tinuous exposure to small doses over a long period. Therefore, the aim of the study was to evaluate the possible deleterious effects of con-tinuous oral exposure of antimony trioxide in rat testis.

Materials and Methods: Thirty three rats were randomly divided into four groups. Six rats were in control group and 9 rats in each three different test groups. Control group received no medication, test group A received 12 ppb, test group B received 500 mg/kg and test group C received 1000 mg/kg of antimony trioxide daily by oral route.

Result: The significant histological changes are distortion/broken epithelium, extensive edema (vacuolization), marked decreased in number of sperms and decreased in number of layer of spermatogonia in the seminiferous tubules and marked vascular degeneration in the highest dose group as compared to the control. The present results showed that, antimony trioxide is toxic to the testis of rat.

**Introduction**

Antimony is a silvery-white, brittle-solid metalloid present in the earth’s crust. Antimony compounds are widely used for producing semiconductors, infrared detectors and diodes. Because of its relative inflexibility in nature, it is usually mixed into alloys for further application, e.g., manufacture of lead storage batteries, solder, sheet and pipe metal, bearings, castings and pewter, etc. Antimony compounds have also been used for treating diseases such as parasitic infections in humans. On the other hand, antimony oxide is also used in fire-retardant formulations for plastics, rubbers, textiles, paper and paints.

Antimony, usually in the form of antimony trioxide, enters the environment mainly as a result of industrial activities such as coal burning or smelting of antimony-containing ores. Antimony can also be naturally present in the environment via erosion of rocks and runoff from soils. On the other hand, trace amounts of antimony in tap water may leach from household piping and non-lead solders under certain condition, e.g. after 7 days of contact. Antimony trioxide (ATO) is mainly used for the production of polyethene terephthalate (PET). Previous reports suggest that polyethylene terephthalate (PET) plastics used for water bottles leaches antimony. For drinking water, the WHO recommends 0.005 mg/L (ppm) as the provisional guideline value for antimony, and the USEPA (United States Environmental Protection Agency) has set 0.006 mg/L (ppm) as the maximum contaminant level for this metal.

Antimony is a potentially toxic trace element with no known physiological function and it is a regulated contaminant that poses both acute and chronic health effects in drinking water.

Some antimony compounds, including ATO are reported as genotoxic in vivo. During spermatogenesis, the process of sperm production in the testis, active DNA synthesis occurs. Thus, spermatogenesis is vulnerable to genotoxic effects. Gene mutation in sperm cells can cause them to die, resulting in transient or permanent sterility, and sometimes causes gene mutation in progeny.

As far as we know, there have been few studies on the effects of antimony compounds on the male reproductive system. Most of the available researches dealt with the biochemical effects of antimony on the different organs, however few of them described the histopathological changes induced by antimony in the testis and since there is only little information about the effects of antimony on the structure of testis and its possible toxicity. Therefore, the aim of the study was to evaluate the possible deleterious effects of continuous oral exposure of antimony in rat testis and to find out the possible carcinogenic effect of this heavy metal.

**Materials and Methods**

Thirty three rats were randomly divided into four groups. Six rats were in control group and 9 rats in each three different test groups. Control group received no medication, test group A received 12 ppb, test group B received 500 mg/kg and test group C received 1000 mg/kg of antimony trioxide daily by oral route.

**Preparation and administration of dose**

We purchased ATO from Sigma Aldrich, Germany. 0.5% carboxy methyl cellulose (CMC) suspension was prepared by adding 500 mg of CMC in 100 ml of distilled water. Different doses of antimony trioxide suspension with carboxy methyl cellulose were prepared to obtain concentrations of 12 ppb, 500 mg/ml & 1000 mg/ml. The test substance suspensions were freshly prepared every day for 3 month. The control animals were administered vehicle only. The test substance was administered orally (gavage) once daily for 3 month in 3 test groups. At the end of 1st and 3rd month, three animals from each test group were sacrificed and remaining 3 animals in each test groups were kept for recovery for another 1 month and then sacrificed.

At the end of 1st, 3rd and 4th months, rats were anaesthetized with the ether and placed on the dissection board. Abdomen was opened by giving the vertical incision after the small horizontal incision. Testis were removed and cleaned in distilled water and immediately fixed in the 10% buffered formalin and processed to get paraffin sections, and stained with Haematoxylin and Eosin stain.

**Histopathological Method**

The tissue were dehydrated in an ascending grade of alcohol (ethanol), cleared in xylene and embedded in paraffin wax. Serial sections of 6 microns thick were obtained using a rotary microtome. The deparaffinized sections were stained routinely with haematoxylin and eosin. Photomicrographs of the desired sections were made for further observations.

**Results**

**Histological results:**

There were marked changes in testicular histology of antimony trioxide treated rats relative to control group.

**Control group:**

Histological observations revealed well defined and arranged...
spermatogenic tubules appeared as rounded or oval, surrounded by thin basal lamina which consists of two distinct populations of cells; the spermatogenic cells and the sertoli cells. The tubules were lined by stratified germinai epithelium. In between the tubules, the interstitial tissue present blood vessels with clusters of cells representing the leydig cells. Spermatogenic tubules showed normal spermatogonia (stem cells) which are in direct contact with epithelial basai lamina about 7-8 layer, spermatocytes and spermatids with sperms in the lumen in the testis of control rats (fig. 1).

Group A- At the end of 1st Month:
No difference was found in the histology of testis of group A at the end of 1 st month of treatment in rats as compared to the control group (fig. 2).

Group A- At the end of 3rd Month:
The histology of testis of group A at the end of 3rd month of treatment in rats revealed occasional broken epithelium, mild edema( cytoplasmic vacuolization ) and decreased in number of sperms in the lumen of seminiferous tubules and marked vascular degeneration as compared to control group (fig. 3).

Group A- At the end of 4th Month:
No difference was found in the histology of testis of group A at the end of 4th month of treatment in rats as compared to the control group (fig. 4).

Group B- At the end of 1st Month:
The histology of testis of group B at the end of 1st month of treatment in rats revealed mild to moderate edema in the seminiferous tubules and moderate vascular degeneration as compared to control group (fig. 5).

Group B- At the end of 3rd Month:
Light microscopic examination of testis of group B at the end of 3rd month revealed broken epithelium, mild edema and decreased in number of sperms in the lumen of seminiferous tubules as compared to the control. There was moderate vascular degeneration seen (fig. 6).

Group B- At the end of 4th Month:
The histology of testis of group B at the end of 4th month of treatment in rats revealed occasional broken epithelium, mild edema and decreased in number of sperms in the lumen of seminiferous tubules and moderate vascular degeneration as compared to the control (fig. 7).

Group C- At the end of 1st Month:
Light microscopic examination of testis of group C at the end of 1st month revealed broken epithelium, marked edema (cytoplasmic vacuolization) and decreased in number of sperms in the lumen of seminiferous tubules and mild vascular degeneration as compared to the control (fig. 8).

Group C- At the end of 3rd Month:
The histology of testis of group C at the end of 3rd month of treatment in rats revealed broken epithelium, extensive edema, marked decreased in number of sperms and decreased in number of layer of spermatogonia in the seminiferous tubules and marked vascular degeneration as compared to the control (fig. 9).

Group C- At the end of 4th Month:
The histology of testis of group C at the end of 4th month of treatment in rats revealed occasional broken epithelium, mild edema and decreased in number of sperms in the lumen of seminiferous tubules and moderate vascular degeneration as compared to the control (fig. 10).

Discussion
Reproductive hazards from metal exposure in males are one of the fastest growing areas of concern in toxicology today. The rapid industrialization and overgrowing urbanization, the toxic effects of heavy metals on male reproductive system have become a major health concern in the globe. It is well known that heavy metals are widely distributed in environment and some of them are known to exert toxic effects on multiple organs in different animals. Heavy metals produce cellular im-pairments at structural and functional level in male reproducti-

c system. These toxic effects are due to disturbances of the normal gene expression in the tissues. Humans are exposed to these metals from numerous sources, including contaminated air, water, soil and food. One of them is a antimony, which is a potentially toxic trace element. However, there is scarcity of studies regarding the genotoxic effect of antimony in animals. Therefore, the evaluation of toxic potentials of antimony is important for the risk assessment of human beings.

The current study found that exposure to antimony trioxide resulted in significant histopathological abnormalities in testis. At low doses in group A (12 ppb) there was no histological differences seen when compared to the control group. But high dose groups ( Group B and Group C) revealed significant histopathological abnormalities in testis as compared to the control group. In group B (500mg/kg) revealed deformity or broken epithelium, mild edema (vacuolization) and decreased in number of sperms in the lumen of seminiferous tubules. There was moderate vascular degeneration seen. Group C (1000mg/ kg) revealed broken epithelium, extensive edema, marked decreased in number of sperms and decreased in number of layer of spermatogonia (1-2 layers ) in the seminiferous tubules and marked vascular degeneration.

Our result also showed the significant histopathological changes after the recovery period of 1 month including occasional broken epithelium, mild edema( cytoplasmic vacuolization ) and decreased in number of sperms in the lumen of seminiferous tubules and moderate vascular degeneration as compared to the control. It was observed that the severity of the pathological effects was dependent on the duration of treatment period. This could be due to the direct influx of antimony trioxide to the cells in the testis.

These results are in close agreement with Gurnani et al(1993). They found that 1/50 to 1/20 of LD50 of antimony trioxide fed to male white Swiss mice, induced chromosomal aberrations in bone marrow and sperm head (37, 38). The frequency of chromosomal abnormalities induced, in bone marrow preparations, was dependent of the dose given and the duration of exposure (Table 8). The highest dose, given for the longest period, was lethal. Effects on germ cells, indicated by sperm head abnormalities, were possible, but differences did not reach the level of statistical significance. But these findings are in contrast to the observations by Minoru Omura et al. According to Minoru Omura et al, ATO and ATP (antimony potassium tartrate) are non toxic to testis of rodents. They used slightly water-soluble antimony compound antimony trioxide (ATO) and the highly water-soluble antimony compound antimony potassium tartrate (APT) were examined. Daily doses of the compounds were 27.4, 12.0 and 1.200 mg/ kg body weight in the APT group, low-ATO group and high-ATO group, respectively. The corresponding daily doses of antimony were 10, 10 and 1,000 mg/kg body weight, in the APT group, low-ATO group and high-ATO group, respectively. Both compounds were administered by gavage: rats, 3 days per week for 4 weeks; mice, 5 days per week for 4 weeks. Neither compound reduced the weights of reproductive organs or accessory sex organs nor affected sperm parameters. Few marked histopathological changes were found in the testis of the treated animals with the study duration (Four Weeks) are shorter than the period needed to complete spermatogenesis in rats (approximately 8 weeks). The current study could not detect any manifestations of hyperplasia, dysplasia or malignant transformation of the testicular cells rats even high dose group (1000mg/kg) administrated for a period of 3 month. These finding is in accordance with other studies. A longer period of continuous administration of antimony may be required to confirm such malignant changes.

Conclusion:
In conclusion, the present results showed that, antimony trioxide is toxic to the testis of rats. The significant histological
changes are distortion /broken epithelium, extensive edema (vacuolization), marked decreased in number of sperms and decreased in number of layer of spermatogonia in the seminiferous tubules and marked vascular degeneration in the highest dose group as compared to the control.

Considering the results of the current study and correlating them to those of other investigators, it can be concluded that antimony has proven to be toxic on the structure and function of the testis of rats. On the other hand, hyperplasia, dysplasia and malignant changes could not be detected following continuous oral administration of antimony for three months.

Based on the results of the present study, it should be recommended to avoid the use of any drinking water contaminated with antimony compounds.

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**Fig. 1:** Section of testis control group rat showing normal histoarchitecture containing seminiferous tubules (SF) covered by basal lamina (BL).

**Fig. 2:** Section of testis of Group A at the end of 3rd month showing mild vascular degeneration.

**Fig. 3:** Section of testis of Group B at the end of 1st month showing mild edema (vacuolization) and vascular degeneration.

**Fig. 4:** Section of testis of Group B at the end of 3rd month showing broken epithelium, mild edema and decreased in number of sperms in the lumen of seminiferous tubules.

**Fig. 5:** Section of testis of Group B at the end of 4th month showing occasional broken epithelium, mild edema and decreased in number of sperms in the lumen of seminiferous tubules and moderate vascular degeneration.

**Fig. 6:** Section of testis of Group C at the end of 1st month showing broken epithelium, cytoplasmic vacuolization and decreased in number of sperms in the lumen of seminiferous tubules and mild vascular degeneration.

**Fig. 7:** Section of testis of Group C at the end of 3rd month showing broken epithelium, extensive edema, marked de-
creased in number of sperms and significantly decreased in number of layer of spermatogonia in the seminiferous tubules and marked vascular degeneration.

Fig. 8: Section of testis of Group C at the end of 3rd month showing broken epithelium, extensive edema, marked decreased in number of sperms, significantly decreased in number of layer of spermatogonia and detachment of spermatogonia from the basal lamina in the seminiferous tubules.

Fig. 9: Section of testis of Group C at the end of 4th month showing occasional broken epithelium, mild to moderate edema, decreased in number of sperms, decreased in number of layer of spermatogonia in the seminiferous tubules.

REFERENCE