



ISSN : 0973-7057

Effect of fluoride contaminated drinking water on the histology of uterus of mice (*Mus musculus*)

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Received ,20th December, 2012 ;Revised: 28th January, 2013

Abstract : Fluoride is an environmental pollutant. Consumption of water containing excess fluoride for a long period of time result in fluorosis. In India, millions of people are affected with fluorosis by drinking naturally fluorinated water up to 38.5 ppm fluoride. Female mice (*Mus musculus*) weighing 25 to 35 gm were randomly distributed into three group of 10 animals each. Solution of sodium fluoride at the dose 20mg and 40mg/kgbw/day were administered orally to two group of mice for 60 days. The other group served as a control. Normal histology of the control uterus showed basically three layers, outer perimetrium, middle thick myometrium and inner endometrium. The endometrium consisted of uterine glands, blood vessels and stromal cells. The uterine layers were seen disrupted due to fluoride toxicity at the dose of 20mgNaF/kgbw /day for 60 days. In the other group of the mice exposed to 40mg NaF/kgbw/day the uterine layers were observed with loose cellular architecture. In higher magnification blood vessels, uterine glands and stromal cells altered their normal shape and size in endometrium layer. The function of uterus is to develop foetus during pregnancy. The lumen of uterus is the implantation site for blastocyte. Long term exposure of female mice to NaF resulted to the destruction of columnar epithelial cells lining the lumen. These changes may adversely affect the structure of uterus and cause pseudo-pregnancy. These finding may have direct bearing on the females drinking a long term fluoride contaminated water and suffering from fluorosis disease.

Key words : Fluoride, Toxicity, Mice, Uterus, Drinking Water, Fluorosis

INTRODUCTION

The well being of human depends on quality of drinking water. Consumption of water containing excess fluoride for a long period of time result in fluorosis. Fluoride intake by female rats and mice is reported to be fetotoxic¹⁻³ and reduce fertility⁴ in mice. The adverse effect of fluoride on the testes, epididymis and vas deferentia of rabbits and mice have been reported^{5,6}. Adverse reproductive effects of fluoride and their amelioration have been investigated earlier in rodents. These studies revealed that ingestion of sodium fluoride by female mice caused alterations in structure and metabolism in some of their organs⁷⁻¹⁰. An epidemiological study to assess whether fluoride could affect human birth rates using a U.S.

database of drinking water system showed an association of decreasing total fertility rate with increasing fluoride level¹¹. In India, millions of people are affected with fluorosis by drinking naturally fluorinated water with up to 38.5 ppm fluoride and depends on hand pumps and dug well water naturally fluorinated. A large majority of people in India consume water that has fluoride above the permissible upper limit of 1 ppm.

In the light of above data, the present investigation has been undertaken to focus on the effects of Sodium fluoride in water on the reproductive functions of female mice.

MATERIALAND METHOD

Female mice (*Mus musculus*) weighing 25 to 35 gm were randomly distributed into three group of 10 animals each and were housed in individual wire cages in the animal houses of our institute. A standard animal diet and

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drinking water was provided. All animals were acclimatized for at least one month before the day of dosing. Solution of sodium fluoride and doses 20mg and 40mg/kgbw/day were administered orally to two group of mice for 60 days. The other group served as a control. The mice was killed by cervical dislocation, abdominal cavity was opened and the uterus was dissected out. The organ was washed briefly in normal physiological saline. The specimen were cut into pieces and were kept in Carnoy's fixative for three hour. After the fixation the specimens were washed well and dehydrated using a graded series of alcohol. It was then cleaned in xylene and embedded in paraffin wax. The specimens were sectioned at 5 mm thickness and stained with the standard haematoxylin and eosin. The slides were observed under the microscope and photomicrographed.

RESULTS & DISCUSSION

Normal histology of the uterus in control mice showed basically three layers, outer perimetrium, middle thick myometrium and inner endometrium (fig.1& 2). The endometrium consisted of uterine glands, blood vessels and stromal cells. The uterine layers were seen disrupted due to fluoride toxicity at the dose of 20mgNaF/kgbw / day for 60 days. The perimetrium, myometrium and endometrium with irregular muscle striation and destruction of endometrium at several places (fig.3) were observed. The less number of stromal cells, blood vessels and uterine glands were observed in endometrium layer (fig.4) in comparison to the control group of mice.

In the other group of the mice exposed to 40mgNaF/kg bw/day for 60 days the uterine layers were observed to loose cellular architecture. The outer layer of perimetrium, middle myometrium and epithelial lining of the endometrium was seen reduced (fig 5). The endometrium was also seen with large lumen space. In higher magnification, the blood vessels, uterine glands and stromal cells were seen with altered and abnormal shape and size. The reduced uterine glands and less number of stromal cells were observed (fig.6) in comparison to the control group of mice. There were extensive damages in the columnar epithelial cells lining the lumen.

The present investigation demonstrated that oral administration of fluoride to mice for 60 days induces infertility in mice and has hazardous and lethal effect on reproduction if accumulated in the body. It is evident that in fluoride toxicity the histological structure of uterus is altered and uterine function is adversely affected which is supported by other experimental results¹². There was overall alteration in the uterine layer but columnar epithelial cells of the endometrium lining the lumen was observed more damaged during the present study. The function of uterus is to develop foetus during pregnancy. The lumen of uterus is the implantation site for blastocyte. Long term exposure of female mice to NaF resulted to the destruction of columnar epithelial cells lining the lumen. It has been reported that nuclear enlargement of luminal epithelial cell is associated with the dose of oestradiol¹³. The cell membrane of stroma found in the endometrium has progesterone receptor which is not found in luminal epithelial cells¹⁴. The damages in the stromal cells due to toxic effect of fluoride seen during the present study may result in non proliferation of stromal cells as well as luminal epithelial cells.

These abnormal changes in the structural morphology of the uterus may adversely affect its physiology and cause pseudo-pregnancy. These findings may have direct bearing on the females drinking a long term fluoride contaminated water and suffering from fluorosis disease.

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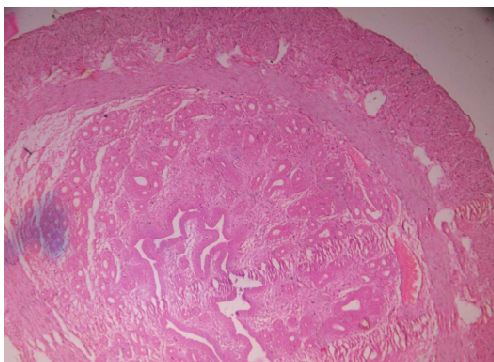


Fig.1.Photomicrograph of the section of uterus of control mice showing perimetrium, myometrium and endometrium (100 X).

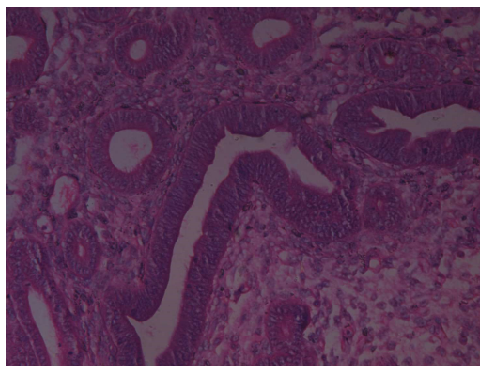


Fig.2. Photomicrograph of the section of uterus of control mice showing the endometrium containing large number of stromal cells, blood vessels, uterine glands and well organized lumen (400 X).



Fig.3. Photomicrograph of the section of uterus of mice treated with 20mg NaF for 60 days showing the perimetrium, myometrium and endometrium with irregular muscle striation and destruction of endometrium at several places (100 X).

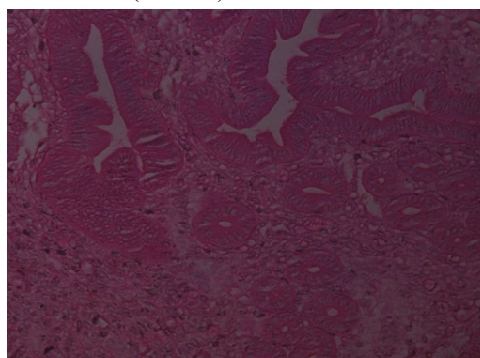


Fig.4. Photomicrograph of the section of uterus of mice treated with 20mg NaF for 60 days the endometrium showing alterations in shape and size of the lumen and less number of stromal cells, blood vessels and uterine glands (400 X).

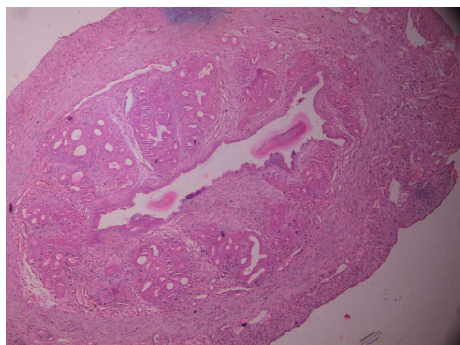


Fig.5. Photomicrograph of the section of uterus of mice treated with 40 mg NaF for 60 days showing reduced perimetrium, myometrium and epithelial lining of the endometrium (100 X).

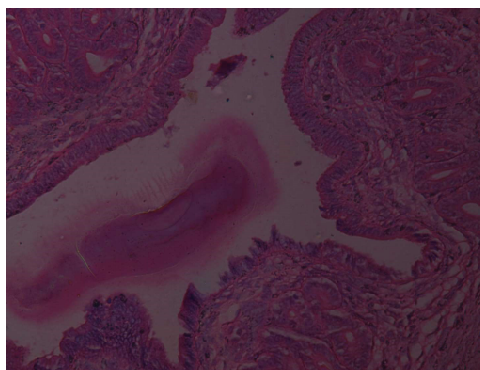


Fig.6. Photomicrograph of the section of uterus of mice treated with 40mg NaF for 60 days the endometrium showing abnormal uterine glands, less number of stromal cells. The epithelial lining of the endometrium is extensively damaged (400 X).

Biospectra : Vol. 8(1), March, 2013

An International Biannual Refereed Journal of Life Sciences

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