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FLUORIDE-INDUCED TOXICITY IN THE REPRODUCTION OF FEMALE ALBINO RATS (*Rattus norvegicus*)

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Abstract

The healthy adult female albino rats (*Rattus norvegicus*) weighing 150-200 gm were exposed to fluoride (5.8ppm) contaminated drinking water for 60 days. The fluoride water exposure to female rats for 60 days resulted in an irregular estrus cycle significantly, reduced organ weights, fertility (60%), litter per rat, and estradiol concentration. The protein, glycogen, ascorbic acid, and cholesterol concentrations of the ovary and uterus were altered in the FW group. The enzyme activity of acid phosphatase SGOT and SGPT declined, whereas alkaline phosphatase increased in serum as well as in reproductive organs studied. The histological observations revalued vacuolization in the stroma and increased atretic follicle in the fluoride-treated rat ovary. Histology of the uterus showed a reduction in the uterine gland and thickness of the myometrium part of the uterus, structural changes were observed in the uterine layers by fluoride treatment in female rats. The data suggests that fluoride water induced adverse effects on an internal milieu of the reproductive organs of female albino rats and it also affects the pituitary-gonadal axis or directly on the target organ of the animal.

Keywords: Ovary, uterus, estrus cycle, antiestrogen, fluoride water.

Introduction:

Fluoride is an inorganic anion of fluorine, naturally found in the biosphere with ubiquitous presence in the environment, and is the most electronegative and reactive among all elements. Fluoride is a widespread natural pollutant with established toxic effects, and the potential relationship between long-term fluoride exposure and fertility impairment has attracted concern (Long *et al*., 2009, Spittle, 2009). Additionally, there are several studies in the literature regarding the toxic effects of NaF on the male reproductive system (Sun *et al*., 2010, Wang *et al.*, 2009). Some animal studies have indicated that adverse reproductive and developmental outcomes occur in individuals exposed to relatively high concentrations of fluoride (Dhar and Bhatnagar, 2009). Most of these investigations, which were conducted with many different animal species, including rats, mice, and rabbits, found alterations in the levels of reproductive hormones, fertility, histological structures, and developmental outcomes (Collins *et al.,* 2001, Elbetieha *et al*., 2000). However, the toxic effects of fluoride on the female reproductive system have rarely been reported. The reproductive tract is susceptible to disruption by fluoride at concentrations that are sufficient to produce other manifestations of toxicity (Spittlea and Meiersb, 2007).

This study aimed to investigate the effects of fluoride water on female reproductive function and to examine the morphology of the ovaries and uteri in rats that had been exposed to fluoride-contaminated water.

Materials and Methods:

The fluoride-contaminated drinking water samples were collected from Watika village of Sanganer Tehsil and subjected to fluoride analysis using standard technique (APHA. AWWA, and WPCF, 1976). The water sample containing fluoride 5.8-ppm was used for the experiment in rats. Healthy adult female albino rats (*Rattus norvegicus*) weighing 150- 200 gm. were divided into two groups. In Group- I, control animals received only tap water (1.0 ppm) and in Group II, rats were exposed to fluoride 5.8 ppm contaminated water for 60 days. The animals were maintained under standard husbandry conditions on a standard diet (Ashirwad Ltd., Chandigarh) and water *ad libitum*. The animals were kept under air-cooled conditions and exposed to a 12-hour light/ dark cycle. The vaginal smear was checked daily, early in the morning. After the respective treatment half of the animals were kept for fertility tests and the rest of the animals were autopsied through cervical dislocation. The blood was extracted through cardiac puncture and utilized for serum estradiol level. The reproductive organs were excised, blotted free of blood, weighed, and used for biochemistry. The parameters studied were protein (Lowry *et al*., 1951) and cholesterol (Zlatkis *et al.,* 1953). The ovary and uterus were excised, blotted free of blood, weighed, and fixed in the boine's fluid for histological examination. Routine light microscopic techniques were used on the tissue sample. The paraffin-embedded blocks were cut to 5 um and stained with hematoxylin-eosin. The slides were then examined using a light microscope and photographed. The results were analyzed statically using the student's 't' test.

Results:

The fluoride water (5.8 ppm) exposure to female rats for 60 days revealed that the reproductive organ weight (ovary, uterus, vagina) and vital organ (adrenal gland, kidney, heart, and liver) weight declined significantly ($p<0.001$) as compared to control value (Table-1). However, the estrus cycle was prolonged and irregular, indicating disturbed ovarian cyclicity. The meta estrous and diestrous were seen for longer periods in a vaginal smear in the treated group as compared to the control group. The duration of the estrous cycle was four days in control rats, whereas in the fluoride water-treated group it was extended six to eight days. However, the positive mating was confirmed in the groups studied revealing the normal reproductive behavior of animals. The fertility rate was reduced to 60% and the number of litters per rat also declined significantly ($p<0.001$) as compared to the control value (Table 2).

The protein concentration of the ovary and uterus was diminished significantly ($p<0.001$) following fluoride water ingestion for 60 days (Table 3). Whereas the glycogen content of the ovary, uterus, and liver increased, the significance ($p<0.001$) was observed in liver glycogen as compared to the control group (Table 3). The cholesterol concentration of the ovary and uterus was increased significantly ($p<0.001$) following fluoride water exposure. In the ovary, the enzyme activity of acid phosphatase diminished whereas alkaline phosphatase was found to be elevated significantly ($p<0.001$) in the fluoride water-treated group. The haematological parameters (RBC, Hb%, and HCT) were found within the normal range but leucocyte count increased significantly ($p<0.001$) as compared to control rats. The serum glucose level, and enzyme activity of acid phosphatase, SGOT, and SGPT decreased significantly ($p<0.001$), the significant increase ($p<0.001$) (Table-4) in enzyme activity of alkaline phosphatase following fluoride water treatment for 60 days to *albino* female rats.

The light microscopic observation of the histology of the control rat ovary illustrates normal histology (Fig.- 1). The mature Graafian follicle has a primary oocyte surrounded by a zone of pellucida and a cluster of follicular cells. These cells project into the antrum, called cumulus oopherus. The Graafian follicle has a stratified layer of follicular cells known as the membrane granulose. The connective tissue stroma surrounding the follicle also differentiates into theca interna and theca externa (Fig.- 2). The histology of fluoride water (5.8 ppm) for 60 days treated rat's ovary showed a reduced number of graffian follicles (Fig.-3), increased atretic follicles, and vacuolization was observed in cortex part of the ovary (Fig.- 4).

The transfer section of the uterus of the control rat showed normal features such as well distinguishing three layers of perimetrium, myometrium, and endometrium (Fig.-5-6). The uterine histoarchitecture was altered in the fluoride water (5.8 ppm) experimental group as compared to the control group. In the experimental group, the rat uterus showed vacuolization in the myometrium, the uterine glands were very few, irregular, and showing degenerative changes in the treated group as compared to the control group (Fig.7-8).

Discussion:

In the present study, the reproductive organ weights of the ovary and uterus declined significantly following fluoride water treatment. The maintenance of reproductive organs depends on estrogen and progesterone hormones; these hormones are regulated by pituitary gonadotrophins and hypothalamic-releasing factors (Lerner, 1969). The reduction in the weight of the ovary and uterus was also observed in our early study (Sharma *et al.*, 2006, 2007).

The fluoride water-treated female showed an irregular estrus cycle, wherein metaestrus and dioestrus stages were prolonged. This may be due to disturbed ovarian cyclicity by fluoride water treatment. As ovarian cyclicity was changed there was an alteration in the production of the potent female hormone estrogen, hence affecting the internal milieu of the ovary, consequently altering ovarian physiology. Sequential changes of the vaginal smear in different phases of the estrus cycle of treated rats are closely associated with simultaneous secretary patterns of gonadal steroids (Gupta *et al.*, 1980). Ovarian hypofunction and anoestrous vaginal smear appear to be due to the absence or decrease of circulating gonadotrophins (Behrman, 1969). These disturbances in the reproductive cycle are related to the diminution of ovarian steroidogenesis.

The protein levels declined in tissues studied with fluoride water treatment. Jhala *et al* (2004) also reported a significant decline in protein levels in sodium fluoride (5mg/kg body weight) and arsenic (0.5-mg/kg body weight) treatment, fluoride likely affects the rate of cellular protein synthesis. In this study, the glycogen was accumulated in the ovary and uterus. The same result was reported by Chinoy and Memon (2001) after sodium fluoride (10 mg/kg bw) treatment with and without the combination of $AlCl_3$ (200 mg/ kg/bw) in male mice in the liver and gastrocnemius muscle. This accumulation of glycogen probably resulted from its decreased utilization, thereby affecting functions of the ovary and uterus, leading to hypoglycemic conditions. The increased cholesterol content in the ovary and uterus by the fluoride water treatment may contribute to altered steroidogenesis, thereby affecting reproductive functions. The specific function of cholesterol in the ovaries to act as a precursor molecule during steroidogenesis (Falck, 1959, Krum et *al.*, 1964) and accumulation of cholesterol in the ovary is indicative of altered steroid metabolism. Acid phosphatase has been traditionally used as a lysosomal marker enzyme (Araki *et al.*, 1995). However, in the present work decreased enzyme activity of acid phosphatase in the ovary and uterus. A similar finding was reported by Chen *et al.*, (2005) in silkworm, wherein the acid phosphatase activity decreased drastically as the fluoride concentration in the food increased. Decreased level of glucose in serum probably due to increased utilization of compound to meet the energy requirement following fluoride toxicity. The liver is an important organ for metabolism and detoxification of toxic substances. SGOT and SGPT are markers of liver function. Therefore, elevated activities of SGOT and SGPT may be due to liver dysfunction.

In the female rats exposed to fluoride-contaminated drinking water for 60 days alteration in histoarchitecture was observed as compared to the control ovary. Jhala *et al*. (2004) also reported that NaF (5 mg/ kg bw) treatment for 30 days in female mice ovary showed stroma vacuolization, atretic follicles, and pyknotic follicular cells. The decrease in the number of primary, secondary, and graffian follicles could be due to a lack of available proteins necessary for cell division, growth, and differentiation of germ cells during oogenesis or else inhibition of the hypothalamus (Yuan *et al.*, 1993) or pituitary by fluoride, and hence a decrease in follicle-stimulating hormone (FSH) secretion as reported for prolactin (Xu *et al.*, 1997) in rats.

The level of circulating estradiol increases progressively with the growth of follicles and the increase in the number of theca and granulose cells in control rats. However, the treatment of fluoride inhibited the production of

estrogen so that follicular development was arrested and the ovulatory period was also prevented. The treatment of fluoride-contaminated potable water caused an estrogen deprivation effect at the target organ, leading to alteration in the structure and function integrity of the ovary and uterus. Atretic follicles are degenerating preovulatory follicles; the degeneration of ovulatory follicles takes place when their growth and differentiation become disrupted (Hannah and Kenneth, 1980). The disruption in the growth and differentiation of preovulatory follicles takes place either due to the non-availability of steroidal hormones, which are essential for their maturation and differentiation, or due to the non-availability of local estrogen produced by granulose cells (Byskov, 1979). The decrease in the number of graffian follicles and increase in the number of atretic follicles in the treated rats when compared with those of the control group indicate that the fluoride-contaminated drinking water both promotes the degeneration of preovulatory follicles.

The fluoride-contaminated drinking water caused structural alterations in the uterus of rats. However, the epithelium cells lining the endometrium and endometrial glands are secretory whereas the fluoride water treatment destroys them. Chinoy and Patel (1998) observed ultrastructure and histopathological changes in the uterus of fluorotic mice. The structural and functional integrity of the uterus is dependent on the circulating level of hormones. Hence, any change in the hormonal level leads to estrogen deprivation condition in the reproductive organ. The ovarian hormones affect uterine physiology in many ways, especially uterine growth and muscular activity or tissue synthesis mechanism induced by the different hormones.

Conclusion:

The data suggest that drinking water contaminated with fluoride caused toxic effects on the internal milieu of the gonads, leading to altered homeostatic conditions of the animal. Thereby reducing organ weight, and estradiol level and altering the estrous cycle.

Table 1 Body and organ weights of control and fluoride-contaminated drinking water treated rats.

Parameters		Control	Fluoride water (5.8 ppm) for 60 days
Body weight (gm)	Initial	168.33 ±2.73	183.33 ±3.33
	Final	180.00 ±0.68	165.00 [ns] ±7.07
Organ weight (mg/100gm)	Ovary	34.09 ±0.09	19.78 [a] ± 0.07
	Uterus	75.21 ±0.15	47.07 [a] ±0.18
	Vagina	41.65 ±0.37	37.60 ±0.08
	Adrenal	16.6 ±0.66	18.59 ±0.09
	Kidney	365.28 ±0.83	249.78 [a] ± 5.76
	Heart	390.44 ±0.44	349.00 [a] ±6.65
	Liver	3675.29 ±32.39	3579.60 ±40.85

Values are mean ± SE, NS= Non – Significant, a= P<0.001

Table 2 Estrus cycle, Fertility, and Litter per rat in control and fluoride-contaminated drinking water treated rats.

Parameters	Control	Fluoride water (5.8 ppm) for 60 days
Estrus cycle	Regular	Irregular
Fertility (%)	90-100%+ve	60% -ve
Litter per rat	8.00 ±0.20	2.00 [a] ±0.20
Estradiol (ng/ml)	15.00 ±1.15	7.99 [a] ±1.23

Values are mean ± SE, a= P<0.001

Table -3 Concentrations of protein, glycogen, ascorbic acid, cholesterol, and enzyme activity of acid and alkaline phosphatase control and treated rats.

Parameters	Organs	Control	Fluoride water (5.8 ppm) for 60 days
Protein (mg/gm)	Ovary	228.76± 4.67	150.50 [a]± 5.21
	Uterus	241.9± 6.86	125.92 [a]± 5.11
Glycogen (mg/gm)	Ovary	8.74± 0.55	12.52 [a]± 0.59
	Uterus	11.66± 0.33	12.00± 0.93
	Liver	12.91± 1.54	20.11 [a]± 3.12
Ascorbic acid (mg/ gm)	Ovary	1.82± 0.14	2.25± 0.68
	Uterus	1.85± 0.05	2.32± 0.26
Cholesterol (mg/gm)	Ovary	9.99± 0.34	28.32 [a]± 1.15
	Uterus	14.70± 1.07	16.38 [a]± 1.21
	Liver	17.27± 0.87	11.10± 1.69
Acid phosphates (mg^{ip} /gm/h)	Ovary	1.81± 0.14	0.81± 0.10
	Uterus	1.48± 0.11	1.00± 0.12
Alkaline phosphates (mg^{ip} /gm/h)	Ovary	1.92± 0.14	3.92 [b]± 0.06
	Uterus	1.63± 0.12	1.91± 0.01

Values are mean ± SE
a= P<0.001 b= P<0.01

Table-4 Showing Hematology and Serum biochemistry in control and treated rate

Parameters		Control	Fluoride water (5.8 ppm) for 60 days
Haematology	WBC (Per mm^3)	5666.66±108.29	9924.33 [a] ±169.21
	RBC (Million/mm^3)	7.85±0.42	7.56±0.22
	Hb (gm %)	13.30±0.85	13.80±0.73
	HCT (%)	40.50±2.56	38.27±1.74
Serum biochemistry	Glucose (mg/dl)	134.80±0.92	98.7 [a] ±7.84
	Cholesterol (mg/dl)	56.33±0.62	57.0±4.32
	Total protein(g/l)	7.23±0.04	8.03±0.33
	Albumin(g/l)	3.63±0.02	3.40±0.24
	Acid phosphatase (ug^{ip}/h)	19.83±0.29	7.47 [a] ±2.98
	Alkaline phosphatase (ug^{ip}/h)	207.66±2.21	321.67 [a] ±4.11
	SGOT(u/l)	329.50 ± 5.04	233.87 [a] ±4.63
	SGPT(u/l)	123.83 ±0.64	107.1 [a] ±7.50

Values are mean ± SE
a= P<0.001

Table -5 Concentrations of protein, glycogen, and cholesterol control and treated rats.

Parameters	Organs	Control	Fluoride water (5.8 ppm) for 60 days
Protein (mg/gm)	Ovary	228.76± 4.67	150.50 [a] ± 5.21
	Uterus	241.9± 6.86	125.92 [a] ± 5.11
Glycogen (mg/gm)	Ovary	8.74± 0.55	12.52 [a] ± 0.59
	Uterus	11.66 ± 0.33	12.00 ± 0.93
	Liver	12.91± 1.54	20.11 [a] ± 3.12
Cholesterol (mg/gm)	Ovary	9.99± 0.34	28.32 [a] ± 1.15
	Uterus	14.70± 1.07	16.38 [a] ± 1.21
	Liver	17.27± 0.87	11.10± 1.69

Values are mean ± SE, a= P<0.001, b= P<0.01

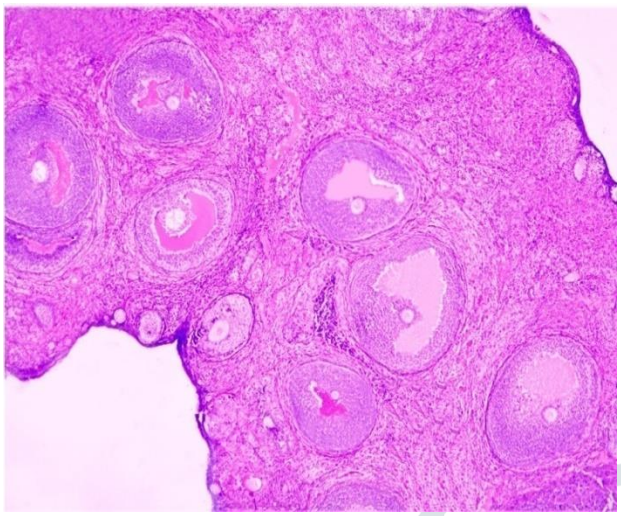

Figure:1 T.S. of the ovary of a control rat (x 40)

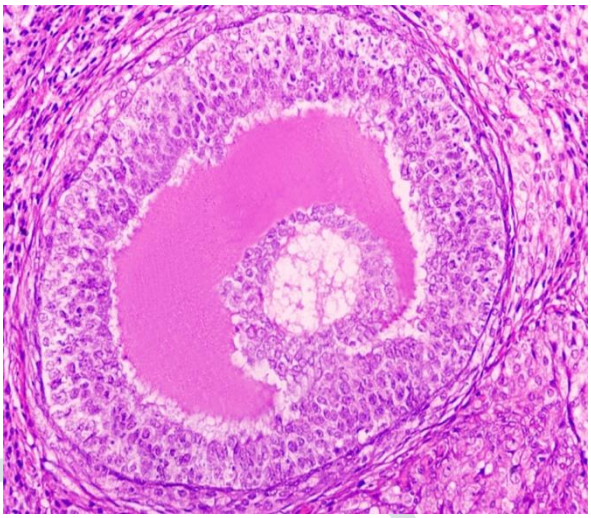

Figure:2 T.S. of the ovary of a control rat (x 200)

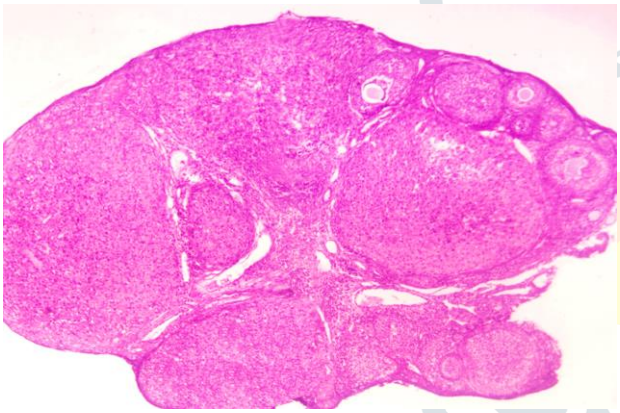

Figure:3 T.S. of the ovary of fluoride water (5.8 ppm) treated rat for 60 days (x 40)

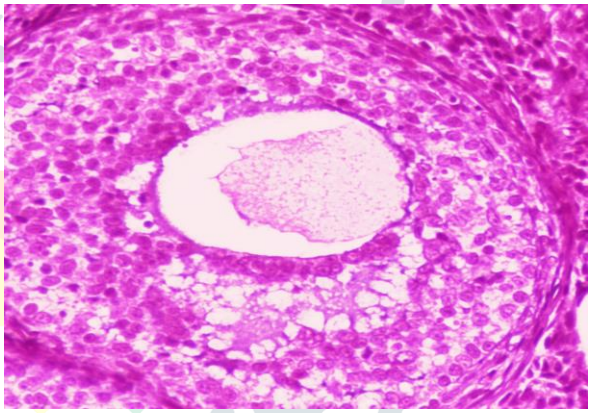

Figure:4 T.S. of the ovary of fluoride water (5.8 ppm) treated rat for 60 days (x 200)

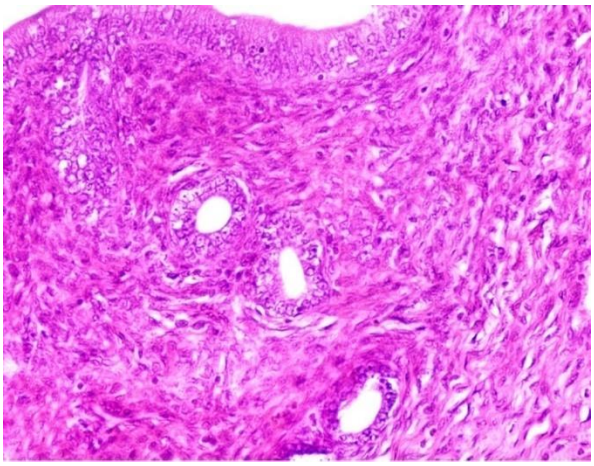

Figure: 5 T.S. of the uterus of a control rat (x 200)

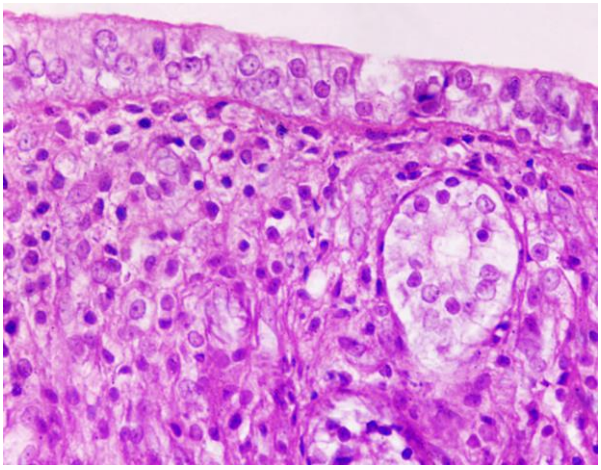

Figure: 6 T.S. of the uterus of a control rat (x 400)

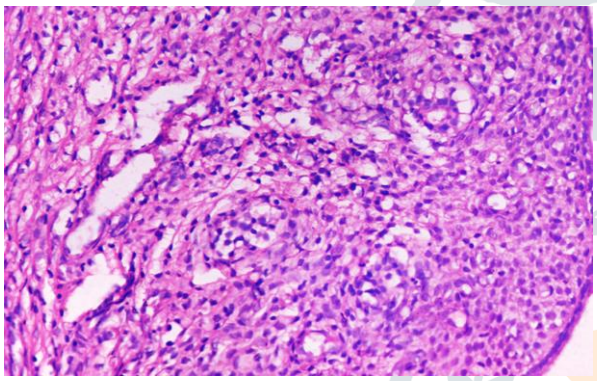

Figure:7 T.S. of the uterus of fluoride water (5.8 ppm) treated rat for 60 days (x 200)

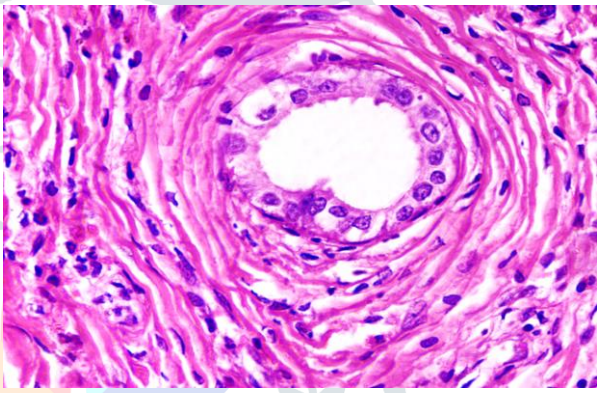

Figure: 8 T.S. of the uterus of fluoride water (5.8 ppm) treated rat for 60 days (x 400)

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