Asian J. Exp. Sci., Vol. 21, No. 2, 2007, 359-364

Sodium Fluoride Toxicity on Reproductive Organs of Female Albino Rats

J.D. Sharma, Mamta Solanki and Deepmala Solanki
Reproductive Physiology and Environment Toxicology Laboratory,
Department of Zoology, University of Rajasthan,
Jaipur-302004, India

Abstract : Fluorosis is a dreaded disease caused due to high fluoride (>1.5 ppm) in drinking water. In the present study normal cyclic female rats (*Rattus norvegicus*), weighing between 150- 200 gm, were kept on sodium fluoride (6ppm) water for 15 and 30 days. The results revealed that the fluoride water exposure to female rats caused irregular estrus cycle, reduced fertility rate, number of litters, weights of reproductive organs (ovary, uterus, vagina) and adrenal gland, concentration of protein (ovary, uterus, adrenal and liver), ascorbic acid (adrenal), enzyme activity of acid phosphatase (ovary and uterus), serum enzyme activity of acid phosphatase, where as alkaline phosphatase, SGOT, SGPT in serum, cholesterol (ovary, uterus, adrenal), glycogen (ovary, uterus, liver) and ascorbic acid content of ovary and uterus increased significantly as compared to control value. However haematological parameters were found to be within normal range. The data suggests that sodium fluoride water exposure for 15 and 30 days caused adverse effect on reproductive organs, leading to reduced fertility and number of litter in female albino rats.

Key words : Sodium Fluoride, Ovary, Uterus, Fertility, and Fluoride Toxicity

Introduction

As per P.H.E.D report, out of 37,889 villages and 45,311 habitations, 9741 villages and 6819 habitations, are enriched with excess fluoride content in ground water. The extent of severity was observed in Nagaur, Pali, Barmer, Jalore and Sikar district. The physico-chemical analysis of groundwater of villages of sanganer tehsil, Jaipur-District was carried out by Sharma *et al.* (2004, 2005), reported that villages of sanganer Tehsil contained very high fluoride concentration in drinking water, responsible various health problems. Prolonged ingestion of drinking water containing 1-3 ppm of fluoride ione causes deleterious effects on skeletal, dental (Choubisa, 1999) and soft tissues (Patel and Chinoy, 1998). Sodium fluoride has been tested in several species of laboratory animals, revealed no effects on reproductive function (Messer *et al*., 1973; Tao and Suttie, 1976; Hoffman *et al*., 1985; Pati and Bhunya, 1987) reported that exposure to sodium fluoride causes toxic effects on reproductive organs. In the light of above data, the present investigation has been undertaken to focus on the effects of sodium fluoride water on reproductive functions of female albino rats.

Materials and Methods

Healthy adult female albino rats (*Rattus norvegicus*) weighing between 150-200 gm.

* **Corresponding author :** J.D. Sharma, Reproductive Physiology and Environment Toxicology Laboratory, Department of Zoology, University of Rajasthan, Jaipur-302004. Email: *jaishree_ajay@yahoo.co.in*

were used for experiments. The animals were maintained under standard husbandry conditions on a standard diet (Ashirwad Ltd. Chandigarh) and water *ad libitum*. Further the animals were kept under air-cooled condition and exposed to 12 h light/ dark cycle. Group- I, control received only tap water, Group-II and III were exposed to sodium fluoride (6ppm) water for 15 and 30 days. The vaginal smear was checked daily, early in the morning. After the respective treatment half of the animals were kept for fertility test and rest of the animals were autopsied through cervical dislocation. The blood was extracted through cardiac puncture and utilized for haematology and serum biochemistry. The reproductive organs were excised, blotted free of blood, weighed and used for biochemistry. The parameters studied were protein (Lowry *et al*., 1951), glycogen (Montgomery, 1957), cholesterol (Zlatkis *et al*., 1953), ascorbic acid (Roe and Kuether, 1943), and enzyme activity of acid and alkaline phosphatase (Oser, 1979). A minimum of six replicates were done for each tissue and parameter. The results were analyzed statically using Student's 't' test.

Results and Discussion

The female rats exposed to sodium fluoride (6 ppm) for 15 and 30 days revealed that the reproductive organ weights of ovary, uterus, vagina and adrenal gland were declined significantly ($p<0.001$) as compared to control value (Table-1). Decreased in reproductive organ weights after NaF water treatment is reported. The prolonged and irregular estrus cycle may be due to disturb ovarian cyclicity. The positive mating was confirmed in all the groups studied. However, inhibition of fertility up to 33% and 67% was observed in treated group of rats as compared to control group.The number of litters were also reduced significantly ($p<0.001$) following fluoride water treatment for 15 and 30 days revending adverse effects of sodium fluoride water on female reproduction (Table-2). Disturbed estrus cycle in adult female mice following NaF (5 mg/kg body weight) treatment was observed by Patel and Chinoy (1998). Epidemiological studies have also shown that there is an association of decreasing total fertility rate with increasing fluoride levels in drinking water (Freni, 1994). The concentration of protein of ovary, uterus, adrenal and liver was diminished following NaF treatment significantly ($p<0.001$) (Table-3). Chinoy and Memon (2001) have also reported declined protein level in liver and gastrocnemius muscle of male mice following NaF exposure for 30 days. The serum glucose declined after NaF treatment for 15 days ($p<0.001$) (Table-4), but increased after 30 days treatment. Glycogen content is also increased in reproductive organs (ovary & uterus) and liver following sodium fluoride water treatment as compared to control value (Table-3). Similar observations have been reported by Chinoy and Memon (2001) in liver and gastrocnemium muscle. This accumulation of glycogen probably resulted from its decreased utilization, lead to hypoglycemic condition. The ascorbic acid content of ovary increased significantly ($p<0.001$), whereas in adrenal gland it declined significantly ($p<0.001$) revealed mobilization of ascorbic acid to over come from fluoride toxicity (Table-3).

The specific function of cholesterol in the ovaries to act as precursor molecule during steroidogenesis (Falck, 1959; Krum *et al*., 1964; Priedkalns and Waber, 1968) and accumulation of cholesterol in the ovary is indicative of altered steroid metabolism. In

the present investigation cholesterol concentration was increased significantly in ovary and adrenal ($p<0.001$) may attribute to altered steroidogenesis, thereby affecting reproductive functions. The enzyme activity of acid phosphatase diminished significantly ($p<0.01$) in ovary but alkaline phosphatase found to be elevated (Table-3). The significant ($p<0.001$) elevation in enzyme activity of acid and alkaline phosphatase in serum has been noted (Table-4). Alkaline phosphatase has been traditionally used as a lysosomal marker enzyme (Araki *et al*., 1995). Chen *et al*. (2005) investigated that the acid phosphatase activity decreased drastically with the increased fluoride concentration in the food of silkworm. Liver is an important organ for metabolism and detoxification of toxic substance. SGOT and SGPT are markers of liver function. The significant ($p<0.001$) elevation in enzyme activity of SGOT and SGPT following sodium fluoride treatment for 15 and 30 days was observed. Therefore, elevated activities of SGOT and SGPT may be due to liver dysfunction. These altered serum and tissue biochemistry of ovary and uterus may be due to changed in internal milieu of ovary and uterus, because of excess fluoride in water, which resulted in reduced fertility and reproductive functions of female albino rats.

Acknowledgement

Authors are thankful to Head, Department of Zoology, and University of Rajasthan for the necessary support.

Table-1 Body and organ weights of control and sodium fluoride treated rats.

Parameters		Control	Sodium fluoride (6 ppm)	
			15 days	30 days
Body weight (gm)	Initial	153.33±3.33	198.0±00.46	160.0±00.39
	Final	155.00 ^{ns} ±0.68	192.00 ^{ns} ±0.46	165.00 ^{ns} ±0.35
Organ weight (mg/100gm)	Ovary	25.71±0.29	18.04 ^{a} ± 0.23	16.53 ^{a} ±0.04
	Uterus	84.590±.50	74.63 ^{a} ±0.14	69.85 ^{a} ±0.12
	Vagina	43.00±0.34	34.60 ^{a} ±0.48	22.68 ^{a} ±0.43
	Adrenal	20.34±0.66	12.38 ^{a} ±0.06	15.22 ^{a} ±0.07

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

Table 2 - Estrus cycle, fertility and litter per rat in control and sodium fluoride treated rats

Parameters	Control	Sodium fluoride (6 ppm)	
		15 days	30 days
Estrus cycle	Regular	Irregular	Irregular
Fertility (%)	90-100%	66.66%+ve	33.33%+ve
Litter per rat	8.00±0.20	2.33 [a] ±0.28	1.66 [a] ±0.39

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

Parameters	Organs	Control	Sodium fluoride (6 ppm)	
			15 days	30 days
Protein (mg/gm)	Ovary	228.76± 4.67	210.52 [b] ± 0.63	103.14 [a] ± 0.45
	Uterus	241.9± 6.86	200.76± 0.96	92.67± 0.69
	Adrenal	249.85± 7.88	209.42 [a]± 0.45	58.12 [a] ± 0.69
	Liver	317.80± 9.49	242.11 [a]± 0.61	121.54 [a] ± 0.13
Glycogen (mg/gm)	Ovary	8.74± 0.55	17.39 [a] ± 0.09	11.07 [b]± 0.02
	Uterus	11.66 ± 0.33	16.95 [a] ± 0.06	8.84 [a] ± 0.11
	Liver	12.91 ± 1.54	16.08 ± 0.18	16.99± 0.11
Ascorbic acid (mg/ gm)	Ovary	1.82± 0.14	2.59 [a] ± 0.02	3.57 [a] ± 0.01
	Uterus	1.85± 0.05	2.08± 0.02	1.08 [a] ± 0.01
	Adrenal	6.28± 0.18	3.21 [a] ± 0.02	1.55 [a] ± 0.04
Cholesterol (mg/gm)	Ovary	9.99± 0.34	19.32 [a] ± 0.08	19.53 [a] ± 0.15
	Uterus	14.70± 1.07	17.50± 0.08	15.62± 0.10
	Adrenal	18.17± 0.62	37.50 [a] ± 0.15	41.41 [a] ± 0.14
Acid phosphates (mg^{ip} /gm/hr)	Ovary	1.81± 0.14	0.56 [b]± 0.02	0.64± 0.04
	Uterus	1.48± 0.14	1.32± 0.01	1.60± 0.04
Alkaline phosphates (mg^{ip} /gm/hr)	Ovary	1.92±0.14± 0.14	1.50 [b]±0.02	2.23± 0.04
	Uterus	1.63± 0.14	2.72 [a] ± 0.06	3.00 [a] ± 0.04

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

Table 4 - Showing hematology and serum biochemistry in control and treated rats

Parameters		Control	Sodium fluoride (6 ppm)	
			15 days	30 days
Haematology	WBC (Per mm^3)	5666.66±198.29	7763.00 [a] ±91.57	6370.00±86.02
	RBC (Million/mm^3)	7.85±0.42	8.13±0.10	7.53±0.09
	Hb (gm %)	13.30±0.85	14.40±0.12	12.63±0.09
	HCT (%)	40.50±2.56	44.83±0.63	40.13±1.29
Serum biochemistry	Glucose (mg/dl)	134.80±0.92	123.83 [a] ±1.23	146.50 [a] ±1.28
	Cholesterol (mg/dl)	56.33±0.62	60.33 [b] ±0.61	58.67±0.55
	Total protein (g/l)	7.23±0.04	8.13±0.06	7.30±0.06
	Albumin (g/l)	3.63±0.02	3.43±0.04	3.57±0.03
	Acid phosphatase (ug^{ip}/hr)	19.83±0.29	8.77 [a] ±0.11	8.40 [a] ±0.81
	Alkaline phosphatase (ug^{ip}/hr)	207.66±2.21	413.67 [a] ±0.49	244.00 [a] ±3.61
	SGOT (u/l)	329.50 [a] ± 5.04	226.47 [a] ±0.53	364.70± [a] 3.91
	SGPT (u/l)	123.83 ±0.64	151.20 [a] ±1.42	167.47 [a] ±0.29

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

References

Araki N., Yokata S., Gakashima Y. and Ogawa K. (1995) : The distribution of cathepsin D in two type of lysosomal or endosomal profiles of rat hepatocytes as revealed by combined immunocytochemistry and acid phosphatase enzyme cytochemistry; *Exp. Cell. Res*., **217**, 469-476.

Chen Y., Du X., Hangzhau Y.J. and China P. R. (2005) : Cytochemical evidence for an anomalous dose response of acid phosphatase activity in the blood but not the midgut of fluoride treated silkworm larva. Bombyx mori. *Fluoride*. **38 (2)**, 133-138.

Chiony N.J. and Memon M.R. (2001) : Benificial effects of some vitamins and calcium on fluoride and aluminium toxicity on gastrocnemium muscle and liver of male mice. *Fluoride*; **34**, 21-33.

Choubisa S.L. (1999) : Some observation on endemic fluorosis in domestic animals in southern Rajasthan (India). *Vet Res Commun*, **23**, 457-465.

Falck, B. (1959) : Site of production of oestrogen in rat ovary as studied in micro- transplants. *Acta. Physiol. scanel*., **47**: 163, 94-101.

Freni S.C. (1994) : Exposure to high fluoride concentrations in drinking water is associated with decreased birth rates. *J. Toxicol and Environ. Health*., **42**, 109-121.

Hoffman D.J., Pattee O.H. and Wiemeyer S.N. (1985) : Effect of fluoride on screech owl reproduction: teratological evalution, growth, and blood chemistry in hatchlings. *Toxicol Lett*, **26 (1)**, 19-24.

Krum A., Morris M.D. and Leslie L.B. (1964) : Role of cholesterol in *vivo* biosynthesis of adrenal steroids. *Endocrino*., **74**, 543-547.

Lowry O.H., Rose H.N., Broug, J., Farr A.L. and Randall R.J. (1951) : Protein measurement with the folin phenol reagent; *J. Bio. Chem*., **193**, 265-675.

Messer H.J., Armstrong W.D. and Singer L. (1973): Influence of fluoride intake on reproduction in mice. J. *Nutrition*., **103**, 1319-1326.

Montgomery R. (1957) : Determination of glycogen; Arch. Biochem. Biophys., 367-378.

Oser B.L. (1979) : Hawks Physiological Chemistry. Publ. Mc Graw Hill, New York.

Ozha D.D., Mathur K.M.L. and Golani F.M. (2003): Scourge of High Fluoride in Ground Waters of Arid Rajasthan and Strategy of Its Mitigation. *Asian J. Exp. Sci*., **17(1&2)**, 43-49.

Patel D. and Chinoy N.J. (1998) : Ameliorative role of aminoacid on fluoride induced alteration in mice (part 2): Ovarion and uterine nucleic acid metabolism. *Fluoride*; **31**, 143-148.

Pati P.C. and Bhunya S.P. (1987) : Genotoxic effect of an environmental poutant sodium fluoride, in mammalian in *vivo* test system. *Caryologia*; **40**, 79-87.

Priedkalns J. and Waber A.F. (1968) : The Succinic dehydrogenase and lipid content of follicular and luteal cells of the bovine ovary. *Acta. Anat*., **71**, 542-564.

Roe J.H. and Kuether C.A. (1943) : The determination of ascorbic acid in whole blood and urine through the 2, 4- dinitrophenyl hydrazine derivative of dehydro ascorbic acid; J. *Biol. Chem*., **147**, 399- 407.

Sharma J.D., Sharma M.K. and Agrawal P. (2004): Effect of Fluoride Contaminated Drinking Water in Albino Rats *Rattus norvegicus. Asian J. Exp. Sci*., **18(1&2)**, 37-46

Sharma J.D., Sharma M.K., Jain P. and Sohu D. (2005) : Quality Status of Potable Water of Tehsil-Sanganer, District-Jaipur, Rajasthan, India. *Asian J. Exp. Sci*., **19(2)**, 113-118.

Toa S. and Suttie J.W. (1976) : Evidence for a lack of an effect of dietary fluoride level on reproduction in mice. J. *Nutrit*., **106**, 1115-1122.

Zlatkis A., Zak,B. and Boyle A.J. (1953) : A new method for the direct determination of serum cholesterol; J. *Lab. Chin.Med*., **41**: 476.