ORIGINAL ARTICLE

Inimical Effects of Sodium Fluoride on Ovarian Weight and Relative Tissue Weight Index of Adult Albino Rats

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ABSTRACT

Objective: To determine the impacts of sodium fluoride on ovarian weight and relative tissue weight index (RTWI) of adult Wistar albino rats.

Study Design: Comparative study.

Place and Duration of Study: This experimental study was performed at the Department of Anatomy, at Shaikh Zayed Postgraduate Medical Institute, Lahore from 25th November to 24th December 2016.

Materials and Methods: Forty-four adult female albino Wistar rats were selected randomly for this study. They were segregated into 4 groups, each comprised of eleven rats. Group A was control, group B was low dose experimental, group C was medium dose experimental and group D was high dose experimental. The control group received distilled water whereas low, medium and high dose experimental groups received 100mg/L, 200mg/L and 300mg/L sodium fluoride solution respectively. The animals were weighed before and after experiment. At 31st and 32nd day, dissection was done, ovaries were removed and evaluated for ovarian tissue weight and RTWI.

Results: The mean weight and RTWI of paired ovaries among the experimental groups were decreased. The differences among groups were statistically significant.

Conclusion: The present study confirms that sodium fluoride has detrimental dose dependent effects on ovarian weight and RTWI of adult albino rats.

Key Words: *Dental Health, Dental Caries, Sodium Fluoride, Wistar Rats.*

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Introduction

Sodium fluoride is a chemical compound that is used to fluoridate drinking water.[1,2] It occurs naturally in plutonic nepheline syenite rocks, which are present in different areas of world.[3,4] Fluoride concentration varies in different water sources while in drinking water, optimum fluoride level ranges 0.7-1.2ppm. The average daily consumption of fluoride from different sources by an adult female is 3mg/day and by an adult male is 4mg/day, which lies in the optimum fluoride intake range of 2-4mg/day according to WHO criteria.[5,6]

Sodium fluoride is an insecticide that acts as stomach poison for plant feeding insects.[7] It has bactericidal action for oral microbes that maintain dental health and prevent dental caries.[8] It is also used in toothpastes, mouthwashes, lozenges and varnishes. The ionic fluorides not only repair the enamel of teeth but also make enamel acid resistant so that damage through acid production by microbial agents is reduced. It leads to remineralization of teeth enamel.[9,10] It has many applications as cleaning agent, wood preservative, catalyst, ceramic pigment and paint dryer etc.[7,11,12]

Ingested fluoride accumulates in tissues and organs and then released slowly.[13,14] Dental fluorosis with tooth discoloration occurs due to fluoride excess leading to mottled enamel.[15] Another fluoride induced bone disease i.e., skeletal fluorosis with increased risk of bone fracture and osteosarcoma occurs in cases of fluoride excess.[16,17] Long term exposure of fluoride leads to multiorgan damage

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including lungs, liver, kidneys, spleen, brain and reproductive organs.[18-21] In an animal study, ovarian and uterine weight along with overall body weight were reduced due to long term exposure to sodium fluoride (NaF) to rats.[22]

As NaF is used in fluoridation of drinking water, toothpastes and mouthwashes and is consumed by humans of all age groups including young females, the present experiment was planned to determine the probable impact of sodium fluoride solution on gross parameters like ovarian weight and relative tissue weight index (RTWI) of adult albino rats.

Materials and Methods

This experimental research was performed at the Department of Anatomy of Shaikh Zayed Postgraduate Medical Institute, Lahore from 25th November to 24th December 2016.

Forty-four adult female albino Wistar rats were kept at room temperature of 23 ± 2 ˚C. They were acclimatized for 7 days and had access to water and food with ad lib feeding.

After acclimatization, these animals were divided randomly by lottery method into four groups and kept separately. In the beginning of experiment, the body weights of all rats were noted. NaF salt in powder form was taken from Pakistan Council of Scientific and Industrial Research (PCSIR) chemical laboratory, mixed with distilled water to form aqueous solution of NaF at concentrations of 100mg/L, 200mg/L and 300mg/L for low, medium and high dose groups respectively. The animals of control group were supplied with distilled water while NaF solution was given to experimental groups by orogastric intubation. The duration of dosage was 30 days.

The albino rats of group A were given 1ml of distilled water, while group B, C and D were given 1 ml of aqueous solution of sodium fluoride at doses of 0.1 mg/ml, 0.2 mg/ml and 0.3 mg/ml once daily respectively.

On the 31st day, the rats from groups A and B were weighed and then euthanized for dissection. Anterior abdominal wall was opened by an incision extending from xiphoid process of sternum to symphysis pubis. After reflecting anterior abdominal viscera and identifying the uterine tubes, the ovaries were removed for detailed study. Both ovaries from Group A and B animals were weighed. Paired ovarian weight for all animals of those groups was calculated and noted in proforma. RTWI was also measured. The same procedure and calculations were repeated for animals of Group C and D on 32nd day of experiment.

Analysis of data was done using SPSS version 23.0 (Statistical Package of Social Sciences). Quantitative variables i.e., weight of paired ovaries and RTWI were calculated using mean and standard deviation (SD). One-way ANOVA was applied for comparison between groups.

Statistically significant *p*-value was ≤ 0.05.

Results

The mean weight of paired ovaries for the animals of group A (Control), group B (Low dose), group C (Medium dose) and group D (High dose) was 0.115 ± 0.010 gm, 0.109 ± 0.009 gm, 0.106 ± 0.006 gm and 0.100 ± 0.007 gm respectively (Fig 1).

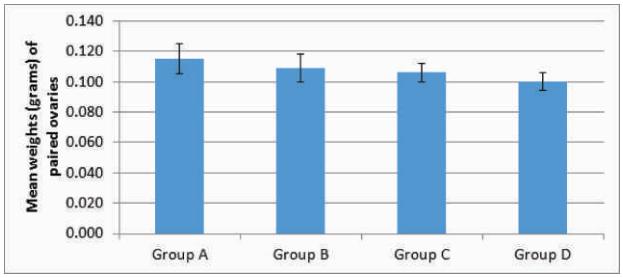

Fig 1: Bar chart showing comparison of mean weights (gm) of paired ovaries of albino Wistar rats after sodium fluoride solution dosage between various groups

One-way ANOVA used to determine the differences in mean weights of paired ovaries among groups, and the difference was found to be statistically significant (Table 1).

Table 1: Comparison of mean weights (gm) of paired ovaries of adult female albino Wistar rats after sodium fluoride solution dosage among different groups

	Sum of Squares	Degree of Freedom	Mean Square	f-test	*p*-value
Between Groups	0.001	3	0.000448	6.891	0.001 *
Within Groups	0.003	40	0.000065		
Total	0.004	43			

Statistically significant difference is indicated by *

Pair wise comparison showed difference of high dose group with control group was significant statistically. The comparison of high dose group with low dose group was also statistically significant. (Table 2).

The mean values of RTWI for animals of group A (Control), group B (Low dose) group C (Medium dose) and group D (High dose) groups were 0.054 ±

Table 2: Group wise comparison of mean weights (gm) of paired ovaries of adult female albino Wistar rats after sodium fluoride solution dosage among control and experimental groups

	Group wise Comparison					
	Serial No.	Groups (P)	Groups (Q)	Mean Difference (P-Q)	Standard Error	*p*-value
Weight of Paired Ovaries	1	Control	Low Dose	0.00591	0.00344	0.327
			Medium Dose	0.00873	0.00344	0.069
			High Dose	0.01536	0.00344	<0.001 *
	2	Low Dose	Medium Dose	0.00282	0.00344	0.845
			High Dose	0.00945	0.00344	0.042 *
	3	Medium Dose	High Dose	0.00664	0.00344	0.232

* Significant difference

0.004, 0.053 ± 0.004, 0.052 ± 0.003 and 0.049 ± 0.003. (Fig 2).

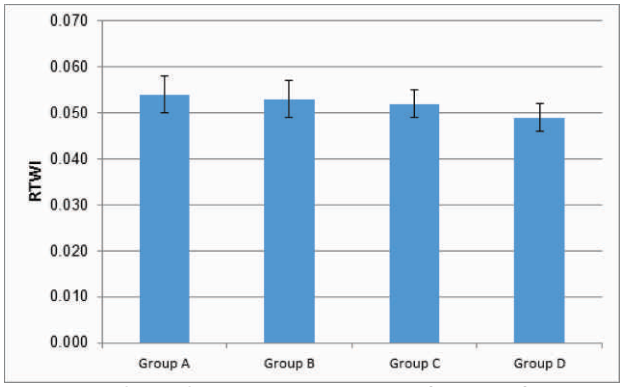

Fig 2: Bar chart showing comparison of RTWI of ovaries of adult female albino Wistar rats after sodium fluoride dosage between control and experimental groups

A significant difference was found in mean RTWI between groups. (Table 3).

Table 3: Comparison of RTWI of ovaries of adult female albino Wistar rats after sodium fluoride solution dosage between various groups

	Sum of Squares	Degree of Freedom	Mean Square	f-test	*p*-value
Between Groups	0.000	3	0.000036	4.370	0.009 *
Within Groups	0.001	40	0.000014		
Total	0.001	43			

* Significant difference.

Group wise comparison showed difference of high dose group from control group statistically significant. (*p*-value = 0.008) while all remaining group wise differences were insignificant. (Table 4).

Discussion

Sodium fluoride is an ionic compound that gives sodium and fluoride ions in aqueous solution.[23] The fluoride ion has toxic effects on metabolic enzymes and alters the processes of DNA and RNA synthesis.[24,25] Its deficiency causes dental caries while its excess leads to dental and skeletal fluorosis.[15-17] Prolonged exposure to fluoride also adversely affect various organs including liver, kidneys, spleen, lungs, brain etc.[18-21]

Table 4: Group wise comparison of relative tissue weight index of ovaries of adult female Wistar albino rats after sodium fluoride solution dosage between control and experimental groups

	Group wise Comparison					
	Serial No	Groups (P)	Groups (Q)	Mean Difference (P-Q)	Standard Error	*p*-value
Relative Tissue Weight Index	1	Control	Low Dose	0.001182	0.001542	0.869
			Medium Dose	0.001545	0.001542	0.749
			High Dose	0.005273	0.001542	0.008 *
	2	Low Dose	Medium Dose	0.000364	0.001542	0.995
			High Dose	0.004091	0.001542	0.053
	3	Medium Dose	High Dose	0.003727	0.001542	0.090

* Significant difference

In this experiment, the mean weight of paired ovaries was decreased after giving sodium fluoride solution for 30 days in the low, medium and high dose groups as compared to the control group.

The difference of mean weight of paired ovaries was statistically significant among groups. The group wise comparison of mean paired ovarian weight showed that the differences of high dose group with control and low dose group were statistically significant. These results indicated that sodium fluoride causes decreased ovarian weight in increasing doses.

These above mentioned results are in accordance with the experiment carried out by Zhou Y, et al in which there was significant dose dependent lowering in ovarian organ coefficients in sodium fluoride treated groups as compared to the control group.[22] The ovarian weight decreases with increase in dose of sodium fluoride solution. Studies done on rats for 15 and 30 days by Sharma JD, et al and on mouse by Chawla SL, et al for 30 days also showed the decrease in ovarian weight after sodium fluoride exposure.[26,27] This was due to the fact that sodium fluoride in higher doses decreases nucleic acid synthesis in ovary and alters DNA synthesis to produce structural changes in ovarian tissue. Sodium fluoride also interferes ovarian steroidogenesis and proteogenesis, thus leads to decrease in ovarian weight.[28,29] Ovarian steroid hormones include estrogen and progesterone, which are synthesized from pregnenolone precursors. 3 β hydroxysteroid dehydrogenase and 17 β hydroxysteroid dehydrogenase enzymes, involved in intermittent

processes of estrogen and progesterone synthesis, are adversely affected by sodium fluoride resulting in decreased ovarian production of estrogens and progesterones and altered ovarian steroidogenesis.[30]

In the current research work, the fluctuation in weights of paired ovaries when compared with the body weights of the animals were calculated by RTWI. It showed the decline in weight of paired ovaries occurs with a relative reduction in the total body weight of the adult female albino Wistar rats in the low, medium and high dose groups after giving sodium fluoride solution when compared with the control group. When the mean RTWI was compared between the groups, the comparison was statistically significant. Group wise comparison of RTWI showed that the difference of high dose group with control group was significant.

These finding of reduced RTWI in experimental groups showed that sodium fluoride has more toxic effect on ovarian weight at higher doses as compared to damaging effects on overall body weight. The result is similar to the finding of Dhurvey V, et al on rats where relatively more decrease in ovarian weights in relation to decreasing overall body weights was observed and RTWI was decreased at higher doses of sodium fluoride solution in experimental animals.[31]

Conclusion

Fluoride rich diets and highly fluoridated water should be consumed cautiously as fluoride has toxic effects on reproductive organs mainly ovaries.

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