Curative Effects Of Curcumin On Gonadotropin And Steroid Hormones In Female Rats Exposed To Fluoride Toxicity

Shashi A[1]; Pragati Kaushal
Department of Zoology and Environmental Sciences,
Punjabi University, Patiala -147002, India.
Corresponding Author

[1]Dr. Shashi Aggarwal, Professor
Department of Zoology and Environmental Sciences,
Punjabi University, Patiala -147002, India.
E-mail: shashiuniindia@yahoo.co.in
Contact No. +91 8054280827

Abstract

Background: Excessive fluoride in our body may result in a slow, progressive, crippling scourge known as fluorosis. However, it becomes toxic at higher doses and induces some adverse effects on number of physiological functions, including reproduction.

The reproductive tract is susceptible to disruption by fluoride at concentrations that are sufficient to produce other manifestations. High fluoride exposure is also associated with increased level of follicle stimulating hormone and Leutinizing hormone, decreased estrogen and testosterone level, disturbed androgen to estrogen ratios and estrogen receptor to androgen receptor ratios.

Objective: The aim of the present investigation was to study the reproductive alterations in fluoride exposed rats, and to demonstrate the correlation between the concentrations of fluoride in serum, and hormonal changes in fluoride intoxicated rats. The study was also conducted to observe the ameliorative effect of Curcumin on fluoride induced reproductive impairments in female rats.

Material and Methods:

Experimental protocol: Young female Wistar albino female rats weighing

150 -200g were randomly divided into six rats in each group. The rats in experimental groups received 300 and 600 mg NaF / kg b.w. / day by oral gavage for 40 days. The control animals were administered 1 ml deionized water / kg bw / day for the same duration. Rats treated with NaF for 40 days were post-treated with 200 mg / kg bw / day of Curcumin alone respectively. The blood samples were taken from each rat by the end of experiment. The blood samples collected in heparinized tubes were centrifuged at 3000 rpm for 10 minutes to separate the serum and kept at -20°C for the analysis of fluoride and gonadotropin (Follicle stimulating hormones and Leutinizing hormone) and steroid (Estrogen and Progesterone) hormones.

Statistical Analysis: The statistical analysis of the data was performed using

statistical package (SPSS) IBM, 20.

Results: The serum levels of follicle stimulating hormone (F = 41.254), leutinizing hormone (F= 36.307), estrogen (F= 53.300) and progesterone (F= 220.831) in test rats showed decrease after 40 days of fluoride exposure. Pearson's bivariate correlation and simple linear regression analysis demonstrated significant negative relationship between

levels of fluoride and FSH (r = 0.904, R^2 = 0 .816, Y = 1. 341 -

21.054 X) (P < 0.01 – 0.0001), LH (r = 0.892, R^2 = 0.796, Y = 1.403 - 19.822 (P < 0.001-0.01) X), estrogen (r = 0.86, R^2 = 0.739, Y = 60.271- 797.689 X) (P < 0.0001) and progesterone (r = 0.952, R^2 = 0.906, Y = 30. 761- 521.421) (P < 0.0001) after 40 days of fluoride exposure. Bonferroni multiple comparison test after ANOVA showed significant decrease in the serum levels of FSH, LH, estrogen and progesterone in all NaF treated groups post-treated with 200 mg/kg bw/day of Curcumin for 20 days. The present study demonstrated significant (P< 0.0001) accumulation of serum fluoride in test rats as compared to control decrease in FSH (P< 0.001), LH, estrogen and progesterone (P< 0.0001) in all NaF treated groups post-treated with 200 mg/kg bw/day of curcumin for 20

days.

Keywords: Curcumin, Estrogen, Female Wistar albino rats, Follicle stimulating hormone, Fluoride, Leutinizing hormone, Progesterone.

Introduction

Fluoride is widely spread natural pollutant with established toxic effects, and the potential relationship between long term fluoride exposure and fertility impairment has attracted concern [1,2,3,4]. The importance of reproductive health on offspring development has also prompted an epidemiological

investigation into the apparent connections between excessive fluoride exposure and male infertility and low birth rats [1,5]. Additionally, there are number of studies in the literature regarding the toxic effects of sodium fluoride on the male reproductive system [4,6,7].

However, the toxic effect of fluoride on the female reproductive system has rarely been reported. The reproductive tract is susceptible to disruption by fluoride at concentrations that are sufficient to produce other manifestations of toxicity. The intake of the fluoride ion by female rats and mice is reported to be fetotoxic [8,9,10]. Previous studies have shown that long-term exposure of female mice to sodium fluoride leads to adverse effects on the reproductive system and fertility [11]. A number of animal studies have indicated that adverse reproductive and developmental outcomes occur in individuals exposed to relatively high concentrations of fluoride [12].

Curcumin is a polyphenolic compound, derives from turmeric (Curcuma longa). It is considered as a source for food additive or dietary pigment and in the traditional medicine [13,14,15] clarified Curcumin as an antioxidant natural herb, a potent scavenger of many free radicals.

The aim of this study was to investigate the relationship between fluorosis and its effects on the female reproductive function and to study fluoride induced disruption of reproductive hormones in female rats. Additionally to investigate whether there is an ameliorating effect of Curcumin after fluoride administration.

Material and methods

Ethical aspects

Experimental protocols and procedures used in this study were approved by the Institutional Animal Ethical Committee of Punjabi University, Patiala (Animal maintenance and Registration No.107/GO/ReBi/S/99/CPCSEA/2017-31).

Experimental study design

Young female Wistar albino rats, weighing 150-200 g were housed in polypropylene cages with stainless grill tops and fed standard rat pellet diet (Hindustan lever limited, India) and water was given *ad libtium*. The group I and II rats were administered 300 and 600 mg of NaF/kg bw/day orally by gastric tube for 40 days. Thecontrol-I animals received 1 mL deionised water/kg bw/day for the same period. The fluoride treated animals were post-treated with 200 mg/kg bw/day of Curcumin for 20 days. The positive control group received Curcumin alone for 20 days. All the animals were sacrificed under ether anaesthesia.

Curcumin: Curcumin was purchased from Loba Chemie Pvt. Ltd, Mumbai (India).

Blood sampling and preparation of serum

The blood samples were taken from each rat by the end of experiment. The blood samples were collected into heparinized tubes, centrifuged at 3000 rpm for 10 minutes to separate the serum and kept at -20°C for the determination of hormones and fluoride.

Biochemical Analysis

1) Fluoride

The concentration of fluoride in the serum of control, fluoride and Curcumin treated rats were measured using an ion selective electrode (ELIT 8221) (Hardwood, 1969).

2) Hormonal Analysis

The serum levels of gonadotropins (follicle stimulating hormone and luteinizing hormone) and steroid (estrogen and progesterone) hormones in control, fluoride and Curcumin treated rats was determined using chemiluminiscent immuno assay kit. (Abbott, USA)

Statistical Analysis

The statistical analysis of the data was performed using statistical package (SPSS) IBM, 20. Data were analyzed using One way Analysis of Variance (ANOVA), Dunnett's and Post-hoc Tukey's HSD multiple comparison test and Bonferroni multiple comparison test. The results were considered significant at P<0.05.Therelationshipbetweenconcentrationoffluoride andhormoneswereanalyzedbyPearson's bivariatecorrelationmatricsandlinearregressiontest.

Results

1. Fluoride analysis

The serum level of fluoride in test rats showed significant (P < 0.0001) increase after 40 days (F=159.921) of fluoride treatment. The maximum elevation of 600% was registered in highest dose group given 600 mg NaF /kg b.w. / day (Fig. 1). Dunnett's T3 multiple comparison test after ANOVA showed significant (P <0.01-0.0001) increase in the serum level of fluoride between and within groups (95% CI = -0.021 to -0.0067; Mean difference = -0.0211 to -0.0143) after 40 days of fluoride exposure. Bonferroni multiple comparison test after ANOVA showed significant (P<0.01 - 0.001) decrease in the serum level of fluoride in all NaF treated groups (95% CI=0.003 to 0.020; Mean difference= 0.011 to 0.014) post-treated with 200 mg/kg bw/day of Curcumin for 20 day (Fig 2).

2. Hormonal profile

a) Gonadotropin hormones

i. Follicle stimulating hormone:

The serum level of follicle stimulating hormone (FSH) in test rats showed significant (P < 0.001) decrease after 40 days (F = 41.254) of fluoride treatment. The maximum decrease of 66.19% was registered in highest dose group given 600 mg NaF

/kg b.w. / day (Fig. 3). Dunnett's T3 multiple comparison test after ANOVA showed significant (P < 0.001 – 0.0001) decrease in the serum level FSH between and within groups. (95% CI = 0.0979 to 1.0822; Mean difference = 0.3900 to 0.3933) after 40 days of fluoride exposure. Bonferroni multiple comparison test after ANOVA showed significant (P < 0.01) decrease in the serum level of FSH in all NaF treated groups (95% CI= -0.545 to -0.398; Mean difference= -0.382 to -0.683) post-treated with 200 mg/kg bw/day of Curcumin for 20 days (Fig 4).

ii. Leutinizing hormone:

The level of leutinizing hormone (LH) in the serum of test rats showed significant (P < 0.0001) decrease after 40 days (F= 36.307) of fluoride treatment. The maximum decline of 55.76% was registered in highest dose group given 600 mg NaF / kg b.w. / day (Fig. 5). Dunnett's T3 multiple comparison test after ANOVA showed a significant (P < 0.01-0.001) decrease in the serum level of LH between and within groups (95% CI = 0.092 to 1.046; Mean difference = 0.500 to 0.221) after 40 days of fluoride exposure. Bonferroni multiple comparison test after ANOVA showed significant(P< 0.05 - 0.001) decrease in serum level of LH in all NaF treated groups (95% CI= -0.381 to -0.301; Mean difference= -0.240 to -0.503) post-treated with 200 mg/kg bw/day of Curcumin for 20 days (Fig.6).

b) Steroid hormones

iii. Estrogen :

The serum level of estrogen in test rats showed significant (P < 0.0001) decrease after 40 days (F= 53.300) of fluoride treatment. The maximum decrease of 52.76% was registered in highest dose group given 600 mg NaF /kg b.w. / day (Fig. 7). Dunnett's T3 multiple comparison test after ANOVA showed significant (P < 0.0001) decrease in the serum level of estrogen between and within groups. (95% CI = -2.638 to 38.728; Mean difference = 23.413 to 6.861) after 40 days of fluoride exposure. Bonferroni multiple comparison test after ANOVA showed significant (P < 0.005-0.1) decrease in the serum level of estrogen in all NaF treated groups (95% CI= -4.740 to -42.954; Mean difference= -23.847 to -24.258) post treated with 200 mg/kg bw/day of Curcumin for 20 days (Fig. 8).

iv. Progesterone:

The serum level of progesterone in the test rats showed significant (P < 0.0001) decrease after 40 days (F= 220.831) of fluoride treatment. The maximum decline of 72.99% was registered in highest dose group given 600 mg NaF /kg b.w. / day (Fig. 9). Bonferroni multiple comparison test after ANOVA showed significant(P < 0.005- P < 0.0001) increase in the serum level of progesterone in all NaF treated groups(95% CI= -5.184 to -7.789; Mean difference= -3.282 to -8.66) post-treated with 200 mg/kg bw/day of Curcumin for 20 days (Fig.10).

Correlation Analysis

Pearson's bivariate correlation and simple linear regression analysis demonstrated significant (P < 0.001) negative relationship between levels of serum fluoride and concentration of FSH (r = 0.904, R^2 = 0 .816, Y = 1. 341 -21.054 X; Fig 11) and LH (r = 0.892, R^2 = 0.796, Y = 1.403 - 19.822 X; Fig 12) after 40 days of fluoride treatment.

Pearson's bivariate correlation and simple linear regression analysis demonstrated significant (P< 0.001) negative relationship between levels of fluoride and estrogen (r = 0.86, R^2 = 0.739, Y = 60.0271-797.689 X; Fig 13) as well as progesterone r = 0.952, R^2 = 0.906, Y = 30. 761- 521.421 X; Fig 14) after 40 days of fluoride treatment.

Discussion

The present study demonstrated significant elevation in the level of serum fluoride in fluoridated rats as compared to control. Similar increase in the serum concentration of fluoride has also been reported earlier in rats [4,17,18,19,20].

Fluoride is known to cross the placenta from the maternal blood to the growing foetus. There was reported accumulation of fluoride in placenta on the maternal and fetal surfaces in women exposed to high fluoride concentrations in water and food in endemic fluorosis areas of Nalgonda, Aandra Pradesh [21].

Human studies conducted by Shashi and Bhardwaj [22] demonstrated elevated serum levels of fluoride in patients selected from seven endemic fluorosis areas of Punjab. The elevated levels of fluoride in serum of subjects from areas with more than 2.0 ppm fluoride in water has also been reported [23]. Shashi and Ghodda [24] reported higher levels of fluoride in erythrocytes of subjects affected with dental and skeletal fluorosis.

The present study demonstrated that FSH and LH secretion were significantly inhibited in the sodium fluoride exposed groups. FSH and LH are synthesized in the anterior pituitary gland via gonadotroph cells [25].

The secretion of FSH and LH is regulated by gonadotropin releasing hormone from the hypothalamus. The main function of FSH is to stimulate ovarian growth, promote follicular development and estradiol synthesis.

The LH played critical role in follicular maturation, ovulation, corpus luteum development, and intervenes in the synthesis of steroid hormone [26]. Estrogen modulates steroidogenesis, promotes granulosa cell proliferation, and maintains follicular development [27].

During the present study, the ovarian secretion of estrogen was markedly decreased in the sodium fluoride treated rats. The disruptions in the level of these hormones were more pronounced in rats treated with 600 mg NaF for 40 days. NaF may interfere with ovarian function indirectly by acting at the level of the hypothalamus or the pituirary gland or both [28].

Presently, treatment with NaF resulted in a reduction in the synthesis of the gonadotropin hormones, luteinizing hormone and follicle stimulating hormone, and in the steroid hormones estrogen and progesterone which is consistent with the results of similar studies in rats [17,29].

The present study revealed that reduced secretion of estrogen from fluoride treated ovary following reduced FSH secretion may cause a negative feedback resulting in hormonal imbalance. Moreover, the reduction of estrogen and progesterone is related to a decreased number of healthy follicles [30]. In the present study, it was found that there was significant decrease in the concentration of serum estrogen ($P< 0.0001$) with concomittent increase in the concentration of fluoride.

During the present study, it was found that there was significant negative relationship with the levels of serum fluoride and FSH significant ($P< 0.001$) ($r= 0.904$, $R^2= 0.816$, $Y= 1.341-21.054X$), LH significant ($P< 0.0001$) ($r= 0.892$, $R^2= 0.796$, $Y= 1.403-19.822X$), estrogen significant ($P< 0.001$) ($r=0.86$, $R^2= 0.739$, $Y= 60.271-797.689X$) and progesterone significant ($P< 0.0001$) ($r=0.952$, $R^2= 0.906$, $Y= 0.031-0.001X$) with concomitant increase in the concentration of fluoride. The findings are in agreement with the study of Dhurvey*et al*. [31] who reported decline of serum FSH, LH, estrogen and progesterone in the NaF treated rats.

The serum concentration of FSH and LH decreased in a dose-dependent manner in female mice after fluoride exposure for 30 days compare with control. The gonadotropin hormones also lowered the secretion of estrogen and progesterone which stimulate follicular cell development and responsible for secondary sexual characteristics and reproductive cyclicity [32].

Rehman*et al*. [33] also reported significant ($P<0.05$) decline in estrogen and progesterone against the control group in ewes and goats. The present study demonstrated that fluoride induced structural and physiological changes which reduces the fertility and disturb the reproductive hormones levels through inhibiting the ovarian hormonal function and cellular integrity.

Furthermore, the results suggest that Curcumin restored the hormone profile in the sodium fluoride treated groups of rats. These results are in agreement with an earlier study [34] which reported that Catechin administration alone increased ($P< 0.05$) plasma levels of FSH, LH, estrogen and progesterone in rats treated with sodium arsenate.

References

1. D Ortiz-Perez, M Rodriguez-Martinez, F Martinez, VH Borja-Aburto, J Castelo, JI Grimaldo, E Cruz, L Carrizales and F Diaz-Barriga. Fluoride-induced disruption of reproductive hormones in men. *Environ Research*. 2012;93:20-23.

2. H Long, Y Jin, M Lin, Y Sun, L Zhang and C Clinch. Fluoride toxicity in the male reproductive system. *Fluoride*. 2009;42:260-276.

3. Spittle. B Halting the inertia of indifference: fluoride and fertility revised (editorial). *Fluoride*. 2009. 42(3):159-161

4. Shashi, A., and Khan, I. Efficacy of *Boerhaavia Diffusa* L. on disruption of gonadotropins and testosterone in fluoride intoxicated male rats. *Asian J Pharma Clinical Res*. 2017. 10(12): 68-73.

5. SC Freni. Exposure to high fluoride concentration in drinking water is associated with decreased birth rate. *J Toxicol Environ Health*. 1994;42: 109-112.

6. Z Sun, R Niu, K Su, B Wang, J Wang, J Zhang and J Wang. Effects of sodium fluoride on hyperactivation and Ca2+ signaling pathway in sperm from mice: An in vivo study. *Archives Toxicol*. 2009; 84: 353-361.

7. J Wang, Y Zhang, H Zhang, K Zhang, Z Zhang and J Li. Toxic effects of fluoride on reproductive ability in male rats: sperm motility, oxidative stress, cell cycle and testicular apoptosis. *Fluoride*. 2009;42:174.

8. Ahmad, S. Al-Hiyasat, Ahmaed, M., Elbetieha, A.M. and Homa Darmani. Reproductive toxic effects of ingestion of sodium fluoride in female rats. *Fluoride*, 2009; 33(2): 79-84.

9. Y Li, M Sun, D Wu and X Chen. The toxicity of the combination of selenium, fluoride and arsenic on rat embryos. *Fluoride*. 2002;35(2):133.

10. Q Chang and Z Li. Toxic effect of fluoride on reproduction of female mice and development of their offspring. *Guangdong Yixue*. 2002;23(4):353-355.

11. H Darmani, AS Al-Hiyasat and Elbetieha. Effects of sodium fluoride in drinking water on fertility in female mice. *Fluoride*. 2001;34(4): 242-249.

12. V Dhar and M Bhatnagar. Physiology and toxicity of fluoride, Indian Journal of dental Research: *Official Publication of Indian Society for Dental Research*. 2009; 20: 350-355.

13. R Perrone, F Ardito, G Giannatempo, M Dioguardi, G Troiano, L Lo Russo, L Laino and L Lo Muzio.Biological and Therapeutic activities, and anticancer properties of Curcumin. *Experimental Therapeutic Med*. 2015; 10:1615 1623.

14. R Pattanayak, P Basak, S Sen and M Bhattacharya. Interaction of kras-g quadruplex with natural polyphenols: A spectroscopic analysis with molecular modeling, *Int J Biol Macromolecules*. 2016; 89: 228-237.

15. ASI Elsayed. The Curcumin as an antioxidant natural herb, with emphasize on its effects against some diseases. *Int J Applied Biol Pharma Techno*. 2016;7(1):26-40.

16. Hardwood, J.E. The use of an ion selective electrode for routine fluoride analysis on water sample. *Water Res*. 1969; 3(4).273-280.

17. Y Zhou, Y Qiu, J He, X Chen, Y Ding, Y Wang and X Liu.The toxicity mechanism of sodium fluoride

on fertility in female rats. *Food Chem Toxicol*. 2013; 62: 566-572.

18. Zhang, Z.,Zhou, B., Wang, H., Waang, F., Song, Y., Liu, S. and Xi, S. Maize purple plant pigment protects aagainst fluoride induced oxidative damage of liver and kidney in rats. *Int J Environ Res Public Health*. 2014; 11. 1020-1033.

19. SK Das, SN Karmakar and DS Roy. Efficacy of ascorbic acid as protective substance on sodium fluoride induced reproductive impairment. *European J Biomed Pharma Sci*. 2016;3(11):265-271.

20. El-Metwaally, A.E., H.A. and Ali, A.H. Immunohistopathologic study on the ameliorative effect of propolis against fluoride cytotoxicity on rabbit buck fertility. *Alexandria Journal Vetenaryscciences*. 2017. 53(1): 156-166.

21. Sastry, G.M., Mohanty,S., and Rao, P. a Role of placenta to combat fluorosis (in fetus) in endemic fluorosis area. *National J Integrated Res Med*. 2010. 1(4):16-19.

22. A Shashi and M Bhardwaj. Study or blood biochemical diagnostic indices for hepatic function biomarkers in endemic skeletal fluorosis. *Biol Trace Element Research*. 2011; 143: 803-814.

23. DS Chauhan, VK Sing, S Mishra, S Tripathi, M Tiwari and ATomar. Influence of fluoride exposure on hypothalamic pituitary gonadal axis hormones and semen quality. *Asian J Biol Life Sci. 2013.*3:201-206.

24. A Shashi and M Ghodda. Inhibitory effect of fluoride on Na+-K+ ATPase activity in human erythrocyte membrane. *Biol Trace Elemental Research*. 2015; 168:340-

25. TR Kumar, Y Wang, N Lu and MMMatzuk. Follicle stimulating hormone is required for ovarian maturation but not male fertility. *Nature Genetics. 1997;* 15: 201-204.

26. MG Hunter, RS Robinson, GE Mann and R Webb. Endocrine and Paracrine control of follicular development and ovulation rate in farm species. *Animal Rep Sci*. 2004; 82-83, 461-477.

27. AE Drummond and JK Findley. The role of estrogen in folliculogenesis. *Mol Cellular Endocrinol*. 1999; 151: 57-64.

28. YM Xu, SP Yuan and QW Xie. Effects of fluorosis on induced secretion of rat prolactin in vivo and in vitro, *Fluoride*. 1997; 30(3):173-178.

29. V Dhurvey and M Thakare. The effect of sodium fluoride intoxication on the estrous cycle and ovarian hormones in rats. *Fluoride*. 2016;49(3): 223-232.

30. R Badraoui, NB Abdelmoula, M Feki, NH Ben and T Rebai. Endocrine disruption and ovarian morphometric responses in rats following exposure to tertadifon, *General Comparative Endocrinol*. 2010;166: 268-272.

31. V Dhurvey, V Patil and M Thakare, M. Effect of sodium fluoride on the structure and function of the thyroid and ovary in albino rats (Rattusnorvegicus), *Fluoride*. 2017;50(2): 235-246.

32. P Sivasakthi and Kadalmani. Curative effects of ethanolic extract of medicinal plants on fluoride induced female infertility using mice model. *Int J Current Res Biosci Plant Biol*. 2017; 4(11):157-168.

33. GH Rehman, H El-Hallawany and RA Dohreig. Effect of excess fluoride on reproductive potentials in farm animals. *Alexandria J Vaterinary Sci*. 2018;57(2): 41-57.

34. A Hedayati, AA Sadaeghi and P Shawrang. The ameliorative effects of catechin on sexual hormones in female rats exposed to arsenic toxicity. Indian *J fundamental applied life sci*. 2015; 5 (1) 238-243.

Results

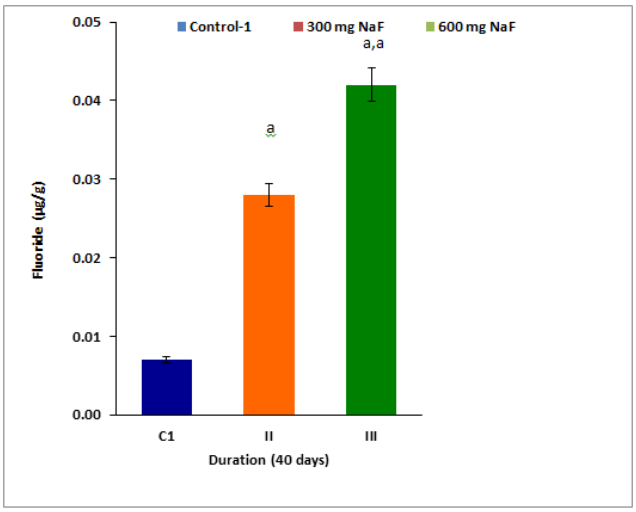

Fig. 1: Mean level of serum fluoride (µg/g) in control and fluoridated rats.

[a]P< 0.0001 Group II-III compared with respective control-I

[a,a]P< 0.0001 Group II compared with group III

(One way ANOVA followed by Dunnett's T3 multiple comparison test).

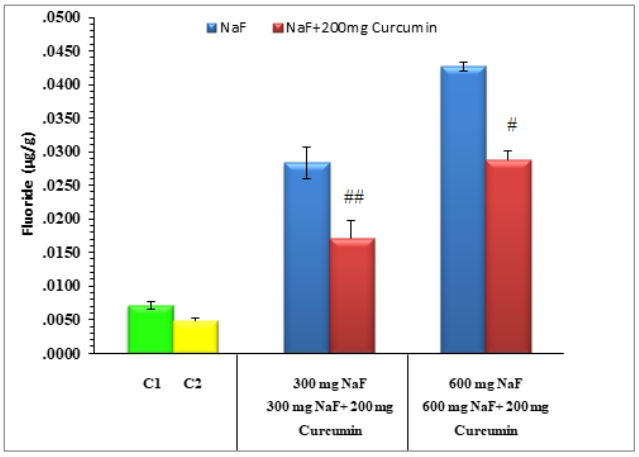

Fig. 2: The concentration of fluoride in fluoridated rats post-treated with Curcumin

Number of animals in each group = 6

C1- Control1- 1 mL deionized water

C2- Control2- 200 mg/kg bw/day Curcumin

#P< 0.01- ##P< 0.001 values are significantly different as compared to respective NaF treated groups.

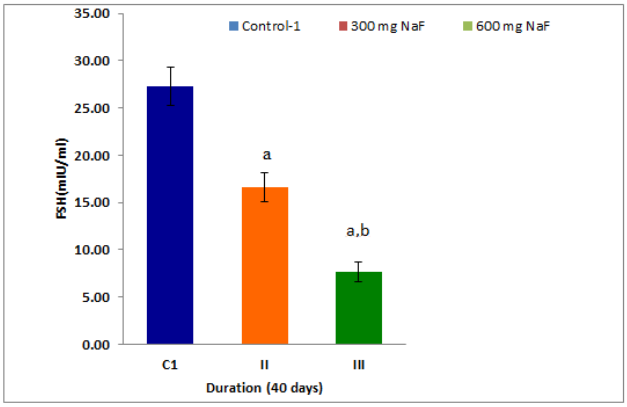

Fig. 3: Mean level of serum FSH (mIU/ml) in serum of control and fluoridated rats

aP< 0.001 Group II and bP< 0.0001 group III compared with control-1

a, bP< 0.001 Group II compared with group III

(One way ANOVA followed by Dunnett'sT3 multiple comparison test).

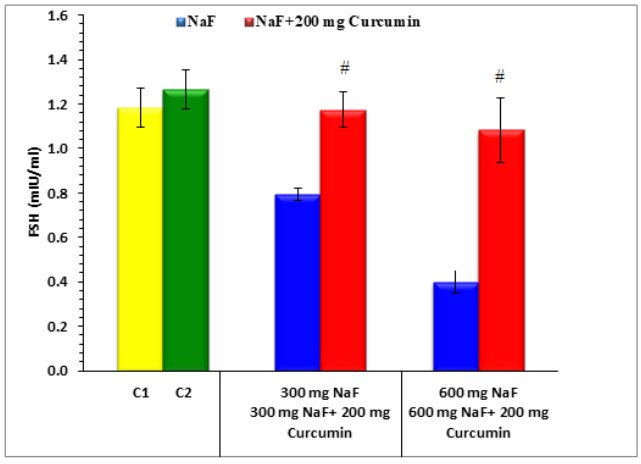

Fig. 4: The concentration of FSH (mIU/ml) in fluoridated rats post treated with Curcumin

Values are given as Maen±SD for 6 rats in each group.

C1- Contro1- 1 mL deionized water

C2- Control 2- 200 mg Curcumin

#P<0.01 values are significantly different as compared to respective NaF treated groups.

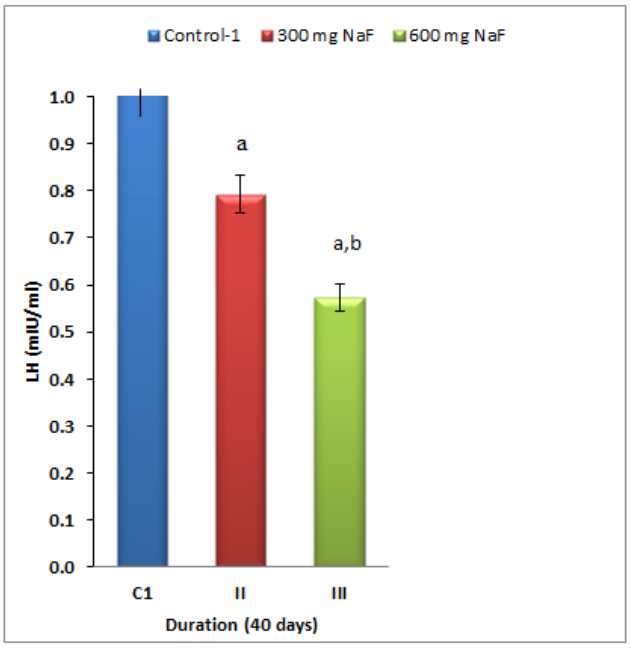

Fig.5: Mean level of serum LH (mIU/ml) in control and fluoridated rats

aP< 0.01 Group II andbP< 0.001 Group III compared with control-1

a, bP< 0.01 Group II compared with group III

(One way ANOVA followed by Dunnet's T3 multiple comparison test)

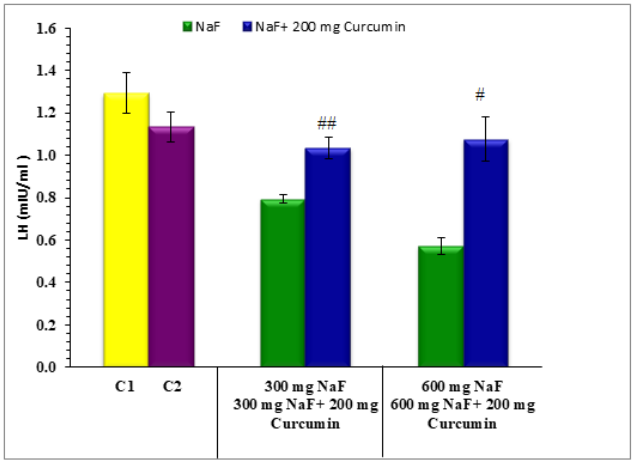

Fig.6: The concentration of LH (mIU/ml) in fluoridated rats post-treated withCurcumin.

Number of animals in each group = 6

C1- Control- 1 mL deionized water

C2- Control- 2- 200 mg Curcumin

##P < 0.05 - #P < 0.001values are significantly different as compared torespectiveNaF treated group

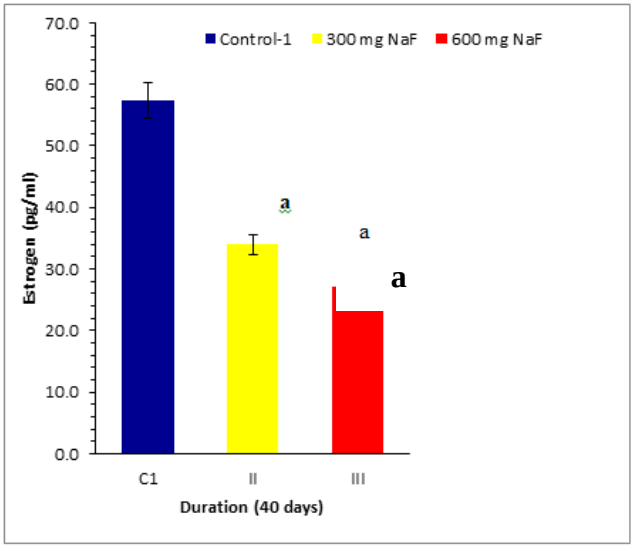

Fig. 7: Mean level of serum estrogen (pg/ml) in control and fluoridated rats.

[a]P< 0.0001 Group II and III compared with control-1

[ns]P=ns Group II compared with Group III

(One way ANOVA followed by Dunnett's T3 multiple comparison test)

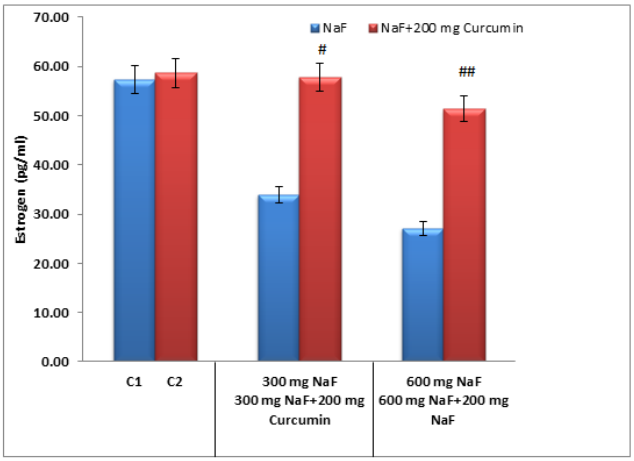

Fig.8: The concentration of estrogen (pg/ml) in fluoridated rats post-treated with

Curcumin

Number of animals in each group = 6

C1- Control1- 1 mL deionized water

C2- Control2- 200 mg/kg bw/day Curcumin

($P^{\#\#}$< 0.005- $^{\#}P$ < 0.1) values are significantly different as compared to respective NaF treated group.

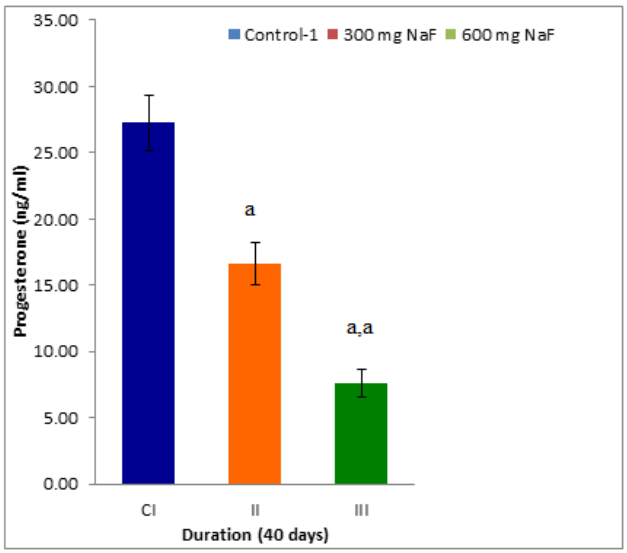

Fig 9: Mean level of serum progesterone (ng/ml) in control and fluoridated rats

[a]P< 0.0001 Group II-III compared with control

[a,a]P< 0.0001 Group II compared with group III

(One way ANOVA followed by Post-hoc Tukey's multiple comparison test)

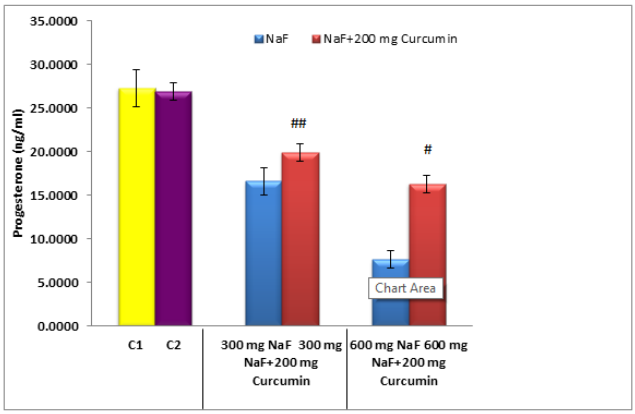

Fig.10: The concentration of serum progesterone (ng/ml) in fluoridated rats post-treated withCurcumin

Values are given as mean ± SD for 6 rats in each group.

C1- Control1- 1 mL deionized water

C2- Control 2- 200 mg/kg b.w./day Curcumin

[##]P < 0.0001 - [#]P < 0.005 values are significantly different as compared to respective NaF treated group.

Correlation analysis

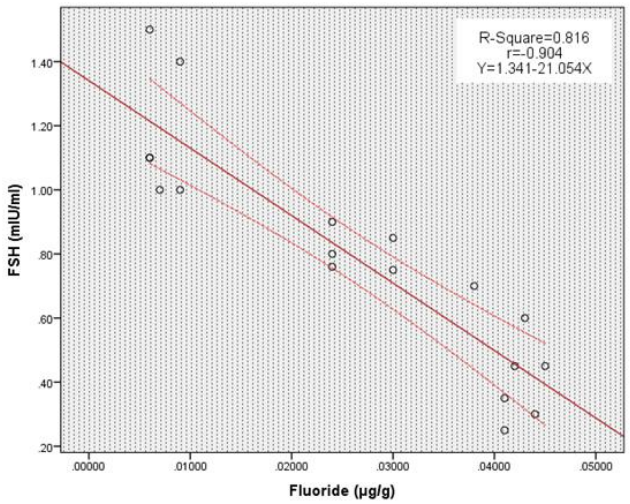

Fig 11: Scatterplot showing correlation and simple linear regression between levels of serum fluoride (µg/g) and FSH (mIU/ml) in test rats after 40 days of fluoride treatment

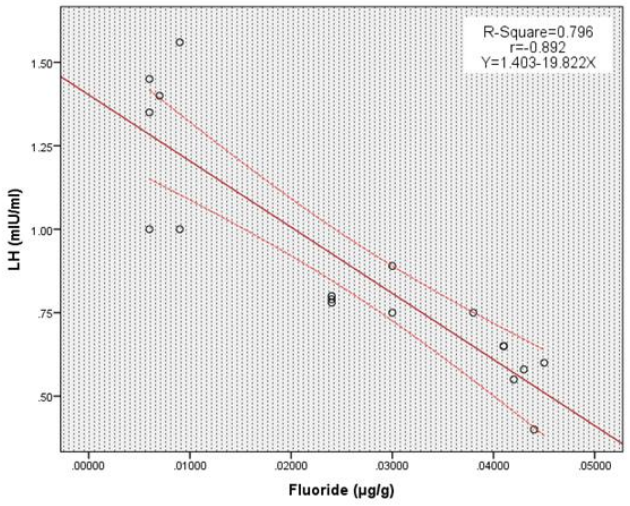

Fig. 12: Scatterplot showingCorrelation and simple linear regression between levels of serum fluoride (µg/g) and LH (mIU/ml) in test rats after 40 days of fluoride treatment.

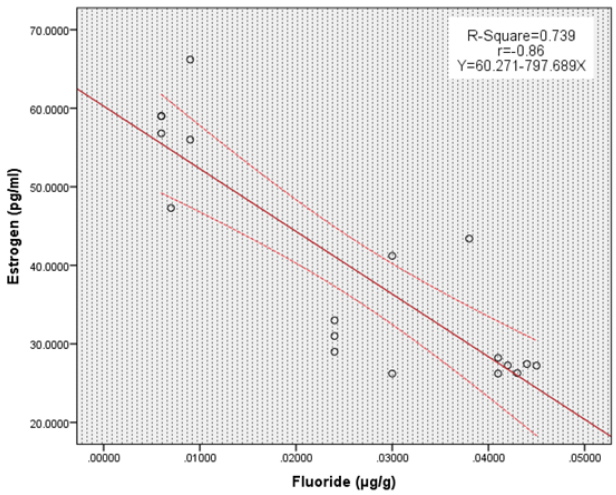

Fig 13: Scatterplot showing correlation and simple linear regressionbetween levels of serum fluoride (µg/g) and estrogen (pg/ml) in fluoridated rats.

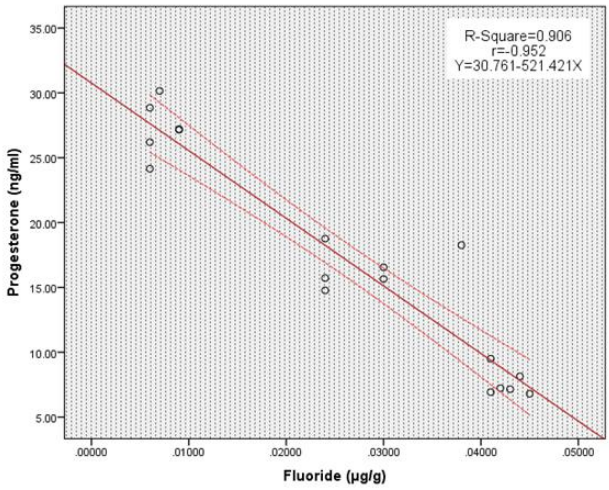

Fig 14: Scatterplot showing correlation and simple linear regression between level of serum fluoride (µg/g) and progesterone (ng/ml) in test rats after 40 days of fluoride treatment.