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

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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, R² = 0.816, Y = 1.341 - 21.054 X) (P < 0.01 - 0.0001), LH (r = 0.892, R² = 0.796, Y = 1.403 - 19.822 (P < 0.001-0.01) X), estrogen (r = 0.86, R² = 0.739, Y = 60.271- 797.689 X) (P < 0.001) and progesterone (r = 0.952, R² = 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...
previous studies have shown that ones in been reported. The clarified Curcumin as.

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Chemie Pvt. Ltd, Mumbai (India).

animals group received Curcumin alone for 20 days. All the bw/day of Curcumin for 20 days. The positive control treated animals were post.

water/kg bw/day for the same period. The fluoride days.

600 mg of NaF/kg bw/day orally by gastric tube for 40

The group I and II rats were administered 300 and

grill tops and fed standard rat pellet diet (Hindustan

registered in highest dose group

administration.

ameliorating effect of Curcumin after fluoride exposure.

induced 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 libitum. 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. Therelationship betweenconcentrationoffluorideandhormones were analyzed by Pearson’s bivariateregulationmatricsandlinearregression test.

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

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Correlation Analysis

Pearson’s bivariate correlation and simple linear regression analysis demonstrated significant (P < 0.001) negative relationship between levels of 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.

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 pituitary 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 concomitant 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, R² = 0.816, Y= 1.341-21.054X), LH significant (P< 0.0001) (r= 0.892, R² = 0.796, Y= 1.403-19.822X), estrogen significant (P< 0.001) (r=0.86, R² = 0.739, Y = 60.271-797.689X) and progesterone significant (P< 0.0001) (r=0.952, R² = 0.906, Y = 0.031-0.001X) with concomitant increase in the concentration of fluoride. The findings are in agreement with the study of Dhurveyet 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].

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


Results

![Fig. 1](image1.png)

![Fig. 2](image2.png)
The table below summarizes the results of the experiment:

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>FSH (mIU/ml)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1</td>
<td>Control 1 - 1 mL deionized water</td>
<td>5.2 ± 0.3</td>
<td>P &lt; 0.01</td>
</tr>
<tr>
<td>C2</td>
<td>Control 2 - 200 mg/kg bw/day Curcumin</td>
<td>6.5 ± 0.4</td>
<td>P &lt; 0.001</td>
</tr>
</tbody>
</table>

Significant differences were observed in the FSH levels compared to the respective NaF treated groups.

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

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

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

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>LH (mIU/ml)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1</td>
<td>Control 1 - 1 mL deionized water</td>
<td>1.2 ± 0.1</td>
<td>P &lt; 0.01</td>
</tr>
<tr>
<td>C2</td>
<td>Control 2 - 200 mg Curcumin</td>
<td>1.5 ± 0.2</td>
<td>P &lt; 0.001</td>
</tr>
</tbody>
</table>

Significant differences were observed in the LH levels compared to the respective NaF treated groups.

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

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

Number of animals in each group = 6
C1- Control 1 mL deionized water
C2- Control 2 200 mg Curcumin

Values are significantly different as compared to respective NaF treated group.
Fig. 7: Mean level of serum estrogen (pg/ml) in control and fluoridated rats.

*P < 0.0001 Group II and III compared with control

*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 - Control 1 - 1 mL deionized water

C2 - Control 2 - 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

*P < 0.0001 Group II-III compared with control

*#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 with Curcumin

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

C1 - Control 1 - 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 showing correlation 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 regression between 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.