

## EFFECT OF SODIUM FLUORIDE ON THE STRUCTURE AND FUNCTION OF THE THYROID AND OVARY IN ALBINO RATS (*RATTUS NORVEGICUS*)

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**ABSTRACT:** *Objective:* The objective was to investigate the effect of sodium fluoride (NaF) on the structure and function of the thyroid and ovary in albino rats. *Method:* Twenty-four female adult albino rats, 160–180 g body weight (bw), were divided into four groups of 6: the control group (I) received defluoridated water while the other 3 groups (II, III, and IV) received 5, 10, and 20 mg NaF/kg bw, respectively, for 45 days orally via the drinking water. The weights of the body, thyroid, and ovary were then examined together with the histology of the thyroid and ovary, and the levels of thyroid stimulating hormone (TSH), tri-iodothyronine (T3), thyroxin (T4), luteinizing hormone (LH), follicle stimulating hormone (FSH), estrogen (E), and progesterone (P). *Results:* **WEIGHTS:** Compared to the control group, a decrease in body weight and an increase in thyroid gland weight were present in both the 10 ( $p<0.05$ ) and 20 ( $p<0.001$ ) mg NaF/kg bw groups. **HISTOLOGY:** The control group rats showed the well known histological structure of the thyroid gland and ovary while the NaF-treated groups showed, in the thyroid, destruction of thyroid follicles, increased cell height, reduced colloid, and breakage of the epithelial layer. The changes in the NaF-treated groups in the ovary were disturbed ovarian follicles, dilated blood vessels, congestion of stroma, and necrotic granulosa cells with the higher dose treatment. **HORMONES:** Compared to the control group, there was an increase in TSH and a decrease in T3 and T4 in the 10 ( $p<0.05$ ) and 20 ( $p<0.001$ ) mg NaF/kg bw groups. For the reproductive hormones, compared to the control group, there was a decrease in LH, FSH, E, and P in the 10 ( $p<0.05$ ) and 20 ( $p<0.001$ ) mg NaF/kg bw groups. *Conclusion:* NaF administration causes hypothyroidism which affects the reproductive system of adult female albino rats (*Rattus norvegicus*).

**Keywords:** Albino rats (*Rattus norvegicus*); Organ weights; Ovarian hormones; Sodium fluoride; Thyroid hormones;

### INTRODUCTION

The intake of the fluoride ion (F) by female rats and mice is reported to be fetotoxic<sup>1-3</sup> and to reduce fertility in mice.<sup>4</sup> Besides the dental and osteal abnormalities,<sup>5-12</sup> non-skeletal changes due to chronic exposure to F have also been observed including gastrointestinal disturbances, neurological disorders, reproductive dysfunctions, apoptosis, excitotoxicity, genotoxicosis, and teratogenic effects.<sup>13</sup> The potential relationship between long-term F exposure and fertility impairment has attracted concern.<sup>14-16</sup> Several clinical investigations and animal experiments suggest that F has adverse impacts on male reproductive function.<sup>17,18</sup>

Hormones are the actual messengers in endocrine signalling. Thyroid hormones stimulate the O<sub>2</sub> consumption of most of the cells in the body, help regulate lipid and carbohydrate metabolism, and are necessary for normal growth and

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maturation, including in bone, brain,<sup>19</sup> ovaries, and endometrium.<sup>20</sup> Thyroid hormones act at all the levels of regulation within the reproductive system.<sup>21</sup> Granulosa cells and ovarian stromal cells express thyroid hormone receptors. Thus, thyroid hormones play an essential role in ovarian physiology.<sup>22</sup> Earlier it was detected that the ovarian surface epithelium is a physiologically important target for thyroid hormone.<sup>23</sup> Proper thyroid function is essential for healthy and normal reproduction.<sup>24</sup> Therefore, female reproduction is inhibited by both hyperthyroidism and hypothyroidism.<sup>25</sup> Hypothyroidism is the leading cause of impaired female fertility as it causes ovulatory dysfunction.<sup>26</sup>

The effect of the administration of sodium fluoride (NaF) on the structure and function of the female reproductive system in albino rats (*Rattus norvegicus*) has been extensively studied, including the effects on the developmental activity of the ovary and uterus, behaviour, the estrous cycle, and various reproductive mechanisms. The finding pertaining to the administration of NaF on the histopathology changes in uterus,<sup>27</sup> thyroid glands,<sup>28</sup> and estrous cycle and ovarian hormones<sup>29</sup> were reported earlier while the present paper deals with the relationship between sodium fluoride-induced hypothyroidism and ovarian histology and hormonal secretion.

The effect of NaF on the reproductive organs and fertility impairment is not fully understood, and the data are conflicted. Therefore, the present study was planned to focus on the histological appearance and hormonal levels that occur in the ovaries and thyroid gland with fluorosis in order to clarify the pathological changes that can occur in the female reproductive system in association with hypothyroidism.

#### MATERIAL AND METHODS

*Animals for experimental study:* Twenty-four adult female albino rats (*Rattus norvegicus*), weighing 160–180 g, were included in this study. The animals were acclimatized in the animal house of the Department of Zoology, Rashtrasant Tukadoji Maharaj (RTM) Nagpur University, Nagpur, under laboratory conditions where they were housed under strict care and hygiene with a light: dark (12:12 hr) cycle, and fed with pellets and water twice daily to keep them in a normal healthy condition.

*Treatment:* The 24 rats were randomly divided into 4 groups of 6 animals. Group I, served as a control and was provided with defluoridated water while the remaining animals in groups II, III, and IV were treated with doses of NaF (Sigma Chemical Company, USA) at 5, 10, and 20 mg NaF/kg bw/day, respectively, orally in their drinking water for 45 days. The experimental protocol was approved by the Institutional Animal Ethics Committee (Register number 478/01/a CPCSEA) of the RTM Nagpur University, Nagpur, prior to the commencement of the study. During the experimental period the weight of the rats was checked. After the completion of the protocol the animals were sacrificed and tissues (ovary and thyroid) were excised, weighed, and processed for histopathology as described

earlier.<sup>28</sup> Blood samples were collected by cardiac puncture for hormonal assessment.

*Detection of hormones:* The blood samples from the different dose groups were centrifuged at 3000 rpm for 10 min to obtain the serum. The serum samples were used for further analyses of the estrogen (E), progesterone (P), luteinizing hormone (LH), and follicle stimulating hormone (FSH) levels by using an enzyme-linked immunosorbent assay (ELISA Kit). Simultaneously, the serum tri-iodothyronine (T3), thyroxin (T4), and thyroid stimulating hormone (TSH) levels were measured with a commercially available radio-immunoassay kit (RIA) according to the manufacturer’s recommended instructions.

*Histopathological analysis:* Dissected tissues of the ovary and thyroid gland were immediately fixed in Bouin’s fixative for 24 hr and then processed through a series of alcohol dilutions for dehydration, cleared in xylene, and embedded in the paraffin wax. Sections of 5 µm thickness were cut using a ultra microtome and stained with haematoxylin-eosin (HE). The stained sections were mounted in DPX, observed, and then photographed using a photomicrography unit (Digital camera Nikon COOLPIX 8400 attached to a light microscope, Nikon Eclipse E200) and magnified to the required size.

*Statistical analysis:* The data were statistically analyzed and expressed as mean±SEM. Statistical analysis of the variance between the control and experimental values was done by Student’s ‘t’ test using GraphPad software.

## RESULTS

*Evaluation of body, ovarian, and thyroid gland weights:* Compared to the control group, the bw of the rats was significantly reduced in the 10 (p<0.05) and 20 (p<0.001) mg NaF/kg bw/day groups but not, to a significant extent, in the 5 mg NaF/kg bw/day group. Significant decreases were also present in the ovarian weight in the 10 (p<0.05) and 20 (p<0.001) mg NaF/kg bw/day groups, compared to the control group, but not in the 5 mg NaF/kg bw/day group. In contrast, compared to the control group, the thyroid gland weight significantly increased in 10 (p<0.05) and 20 (p<0.001) mg NaF/kg bw/day groups but was not significantly changed in the 5 mg NaF/kg bw/day group (Table 1).

**Table 1.** Effect of sodium fluoride on body, ovary, and thyroid gland weight of albino rats. Values are expressed as mean±SEM (standard error of mean)

Groups	Initial body weight (g)	Final body weight (g)	Ovary weight (mg)	Thyroid weight (mg)
Control	180.00±1.50	182.71±3.66	40±2.6	12.62±0.31
5mg/kg bw	178.50±4.51	172.16±3.41	37±3.2	18.22±0.25
10mg/kg bw	180.50±3.27	169.00±6.22*	31±3.4*	21.96±0.18*
20mg/kg bw	175.80±3.18	156.62±3.45 <sup>†</sup>	26±2.0 <sup>†</sup>	26.02±0.16 <sup>†</sup>

Compared to the control group: \*p<0.05, <sup>†</sup>p<0.001.

*Evaluation of reproductive and thyroid hormones:* Compared to the control group, the serum T3 and T4 levels were significantly lower in the 10 ( $p < 0.05$ ) and 20 ( $p < 0.001$ ) mg NaF/kg bw/day groups while the level of TSH was significantly increased in the 10 ( $p < 0.05$ ) and 20 ( $p < 0.001$ ) mg NaF/kg bw/day groups. No significant difference was present in the 5 mg NaF/kg bw/day group as compared to the control group (Table 2).

**Table 2.** Effect of sodium fluoride on thyroid hormones of albino rats.  
Values are expressed as mean  $\pm$  SEM (standard error of mean)

Groups	T3 (ng/mL)	T4 (ng/mL)	TSH ( $\mu$ IU/mL)
Control	0.93 $\pm$ 0.007	0.51 $\pm$ 0.007	0.94 $\pm$ 0.008
5mg/kg bw	0.86 $\pm$ 0.004	0.47 $\pm$ 0.004	1.0 $\pm$ 0.009
10mg/kg bw	0.74 $\pm$ 0.006*	0.42 $\pm$ 0.008*	1.23 $\pm$ 0.01*
20mg/kg bw	0.52 $\pm$ 0.007 <sup>†</sup>	0.31 $\pm$ 0.005 <sup>†</sup>	1.75 $\pm$ 0.01 <sup>†</sup>

Compared to the control group: \* $p < 0.05$ , <sup>†</sup> $p < 0.001$ .

Compared to the control group, the LH, FSH, and E levels were significantly lower in the 10 ( $p < 0.05$ ) and 20 ( $p < 0.001$ ) mg NaF/kg bw/day groups while no significant difference was present in the 5 mg NaF/kg bw/day group. Compared to the control group, the P concentration decreased significantly in the 20 ( $p < 0.05$ ) mg NaF/kg bw/day groups but was not significantly different in the 5 and 10 mg NaF/kg bw/day groups (Table 3).

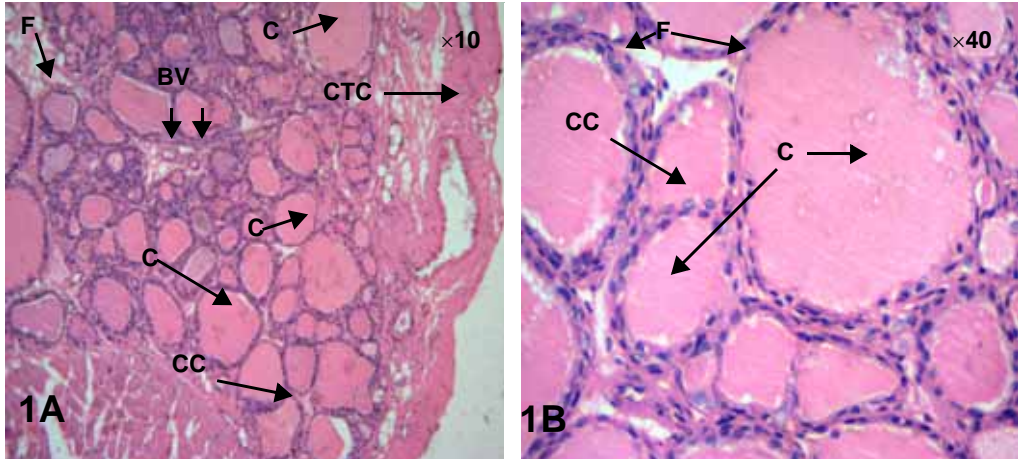
**Table 3.** Effect of sodium fluoride on serum gonadotropin and steroid levels of albino rats.  
Values are expressed as mean  $\pm$  SEM (standard error of mean)

Groups	LH (IU/mL)	FSH (IU/mL)	Estrogen (pg/mL)	Progesterone (ng/mL)
Control	1.35 $\pm$ 0.92	1.41 $\pm$ 0.48	1.38 $\pm$ 0.48	5.85 $\pm$ 1.48
5mg/kg bw	1.11 $\pm$ 0.56	1.57 $\pm$ 1.13	1.41 $\pm$ 0.82	5.08 $\pm$ 0.96
10mg/kg bw	0.78 $\pm$ 0.36*	1.08 $\pm$ 0.23*	0.86 $\pm$ 1.50*	4.82 $\pm$ 0.45
20mg/kg bw	0.40 $\pm$ 0.18 <sup>†</sup>	0.93 $\pm$ 0.48 <sup>†</sup>	0.75 $\pm$ 0.40 <sup>†</sup>	4.02 $\pm$ 0.34*

Compared to the control group: \* $p < 0.05$ , <sup>†</sup> $p < 0.001$ .

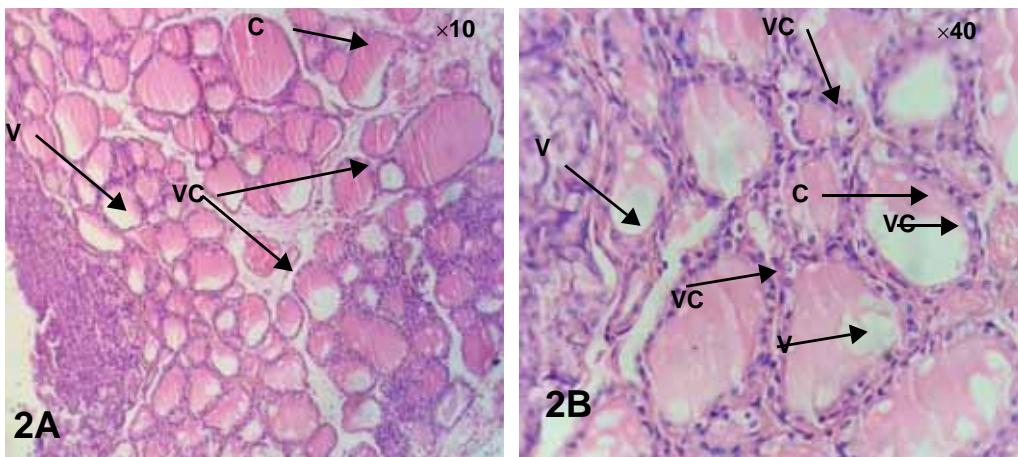
*Histology of thyroid:* As shown in Figure 1, the well known histopathological structure of the thyroid gland was seen in the control group. The glands were surrounded by connective tissue capsules creating follicular lobules separated by

thin connective tissue. The thyroid follicles were of various sizes and their lining was mainly with cubical follicular cells containing rounded nuclei. The centres of the thyroid follicles contained acidophilic homogenous colloid. Blood capillaries were present between the follicles, the epithelial cells were of a normal height, and there were no interfollicular spaces.



**Figures 1A and 1B.** Control thyroid gland sections (1A:  $\times 10$ , 1B:  $\times 40$ ) showing the glands were surrounded by a connective tissue capsule (CTC) and formed follicular lobules with blood vessels (BV). Several thyroid follicles (F) are shown of variable sizes which are lined by a single uniform layer of flattened to cuboidal cells (CC) with nuclei. The lumen of the follicles contains abundant colloid (C) material. (Sections stained with HE).

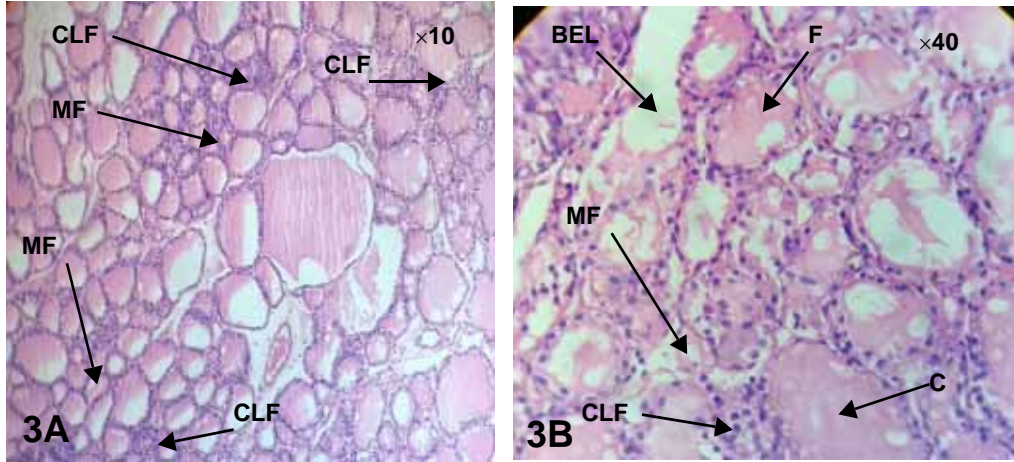
In the 5 mg NaF/kg bw/day group there was a decrease in the size of the thyroid follicles and an increase in the height of their lining epithelium with a vacuolated cytoplasm. Some follicles showed vacuolated colloid while others were devoid of colloid (Figure 2).



**Figures 2A and 2B.** Thyroid gland sections (2A:  $\times 10$ , 2B:  $\times 40$ ) of a rat treated with sodium fluoride in a dose of 5 mg NaF/kg bw/day showing thyroid follicles (F) with an increase in the height of their lining epithelium and a vacuolated cytoplasm (VC). The follicles had a low colloid (C) and contained many peripheral vacuoles (V). (Sections stained with HE).

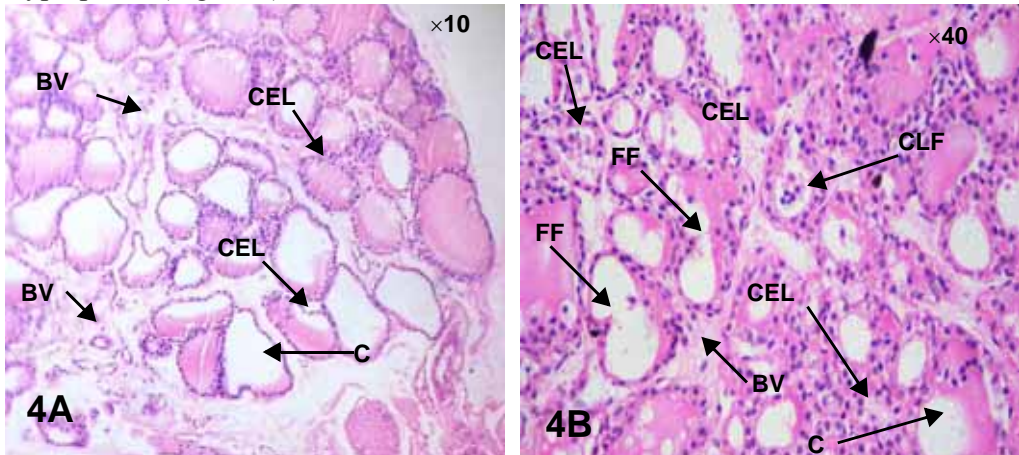


In the 10 mg NaF/kg bw/day group the thyroid section showed follicles with a reduced colloid percentage. The thyroid follicular cells were desquamated forming clusters in the lumen and some follicles had a breakage of the epithelial layer (Figure 3).



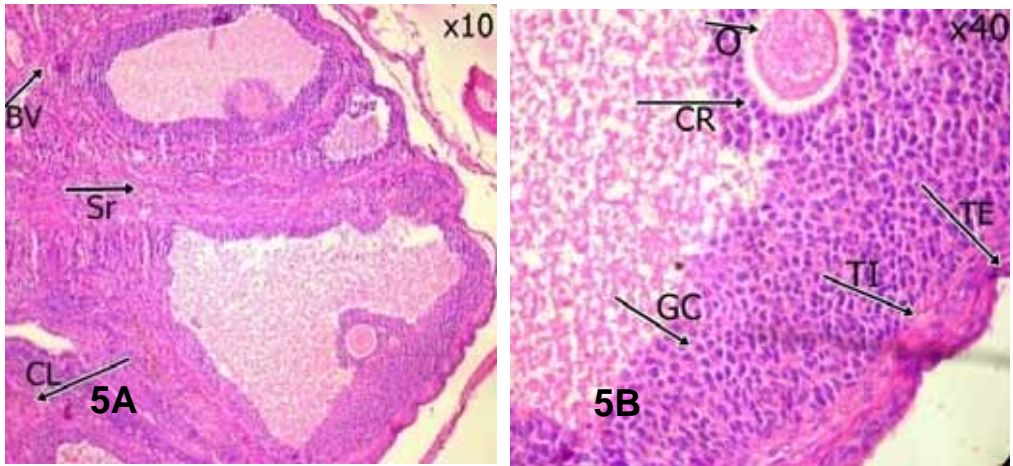
**Figures 3A and 3B.** Thyroid gland sections (3A:  $\times 10$ , 3B:  $\times 40$ ) of a rat treated with sodium fluoride in a dose of 10 mg NaF/kg bw/day showing thyroid follicles (F) with reduced colloid (C) and micro follicles (MF) whose narrow lumens are visible. Follicular cells were desquamated forming clusters in the lumen of follicles (CLF). Breakage of epithelial layers was present (BEL). (Sections stained with HE).

Similarly, the 20 mg NaF/kg bw/day group showed fusion of the follicles from breakage of the epithelial layers, congested blood vessels in the septa, flat epithelial cell lining, and some cells exfoliated in the lumen. The colloid was vacuolated and the thyroid follicles were highly disrupted with follicular hyperplasia (Figure 4).



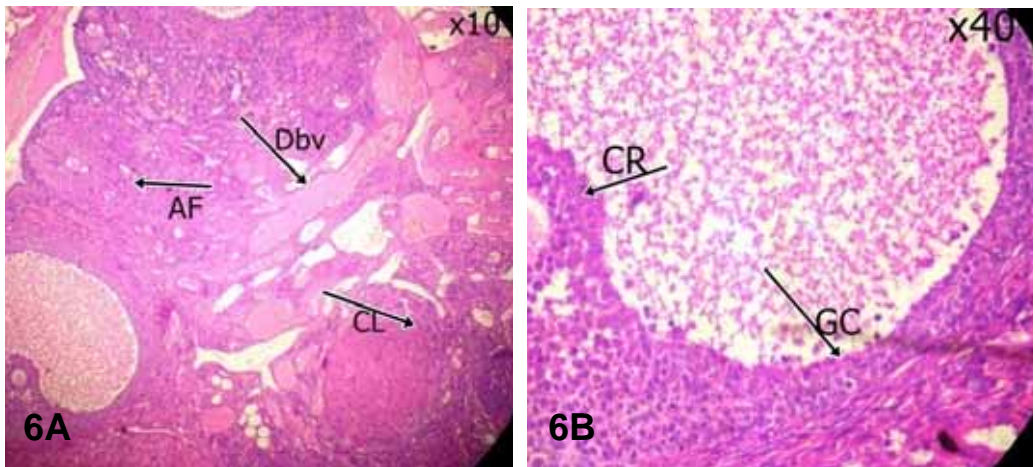
**Figures 4A and 4B.** Thyroid gland sections (4A:  $\times 10$ , 4B:  $\times 40$ ) of a rat treated with sodium fluoride in a dose of 20mg NaF/kg bw/day showing fusion of the follicles (FF), congested blood vessels (BV) in the septa, flat epithelial cell lining, and some cells exfoliated in the lumen (CEL). Vacuolated colloid (C) and highly distorted thyroid follicles with follicular hyperplasia were present. (Sections stained with HE)

**Histology of ovary:** The preantral follicles and antral follicular development were clearly observed together with the blood vessel network. Several secondary follicles, Graffian follicles (GF), and corpora lutea (CL) were found. The Graffian follicles showed three different layers of follicular cells, the theca externa (TE), the theca interna (TI), and the membrane granulosa cells (GC) which were normal and healthy. These granulosa layers were filled with follicular fluid. The theca interna had a vascularized mode of cells and the theca externa had a fibrous type of cells. The shape of these cells varied from fusiform to epithelioid within the collagen fibre surroundings. As shown in Figure 5, a clear follicular cavity was remarkable in the control ovarian sections.



**Figures 5A and 5B.** Control ovary sections (5A:  $\times 10$ , 5B:  $\times 40$ ) showing the normal ovarian histology characterized by the theca externa (TE), the theca interna (TI), ooplasm (O), the corona radiata (CR), the stroma (Sr), the blood vessels (BV), granulosa cells (GC), corpora lutea (CL), and follicles at various stages of development. (Sections stained with HE).

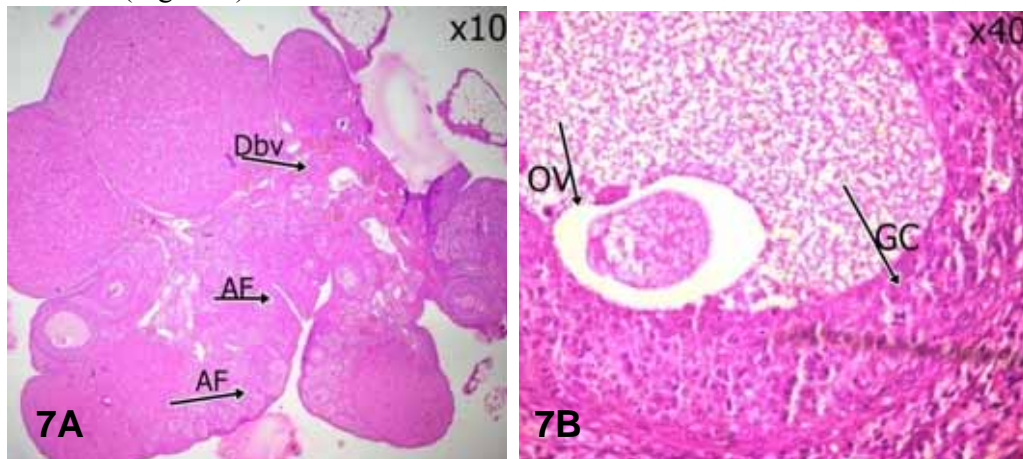
The follicles from the 5 mg NaF/kg bw/day group showed atretic follicles (AF) and dilated blood vessels (Dbv) when compared with the control group (Figure 6).



**Figures 6A and 6B.** Ovarian sections (6A:  $\times 10$ , 6B:  $\times 40$ ) of a rat treated with sodium fluoride in a dose of 5 mg NaF/kg bw/day showing corpora lutea (CL), corona radiata (CR), granulosa cells (GC), atretic follicles (AF), and dilated blood vessels (Dbv). (Sections stained with HE).

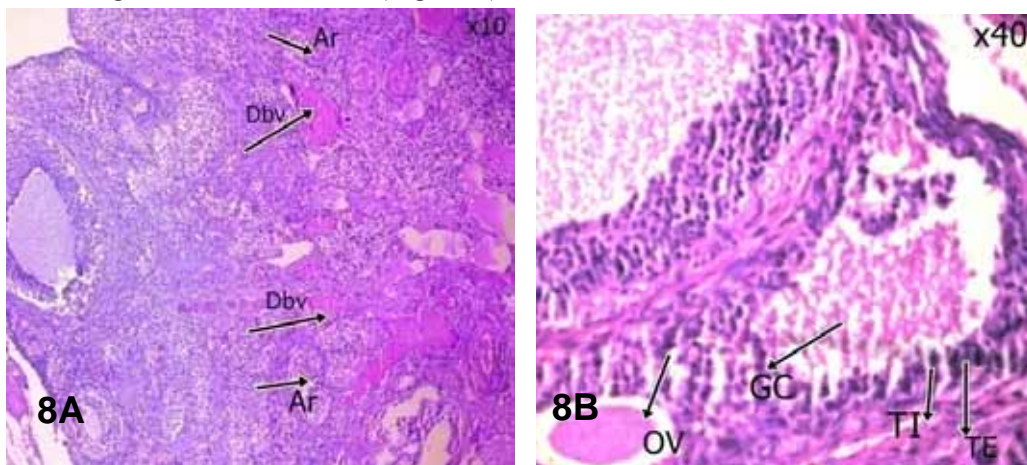


In the 10 mg NaF/kg bw/day group the region of the ovum and follicular antrum was well defined. However, the ooplasm showed marked degenerative changes without any indication of any residue of the nucleus or the nucleolus and the entire region of the corona radiata (CR) and the follicular antrum showed massive necrosis (Figure 7).



**Figures 7A and 7B.** Ovarian sections (7A:  $\times 10$ , 7B:  $\times 40$ ) of a rat treated with sodium fluoride in a dose of 10mg NaF/kg bw/day showing an atretic follicle (AF), an ovum (OV), dilated blood vessels (Dbv), granulosa cells (GC), and degeneration of the ovarian stromal cells. (Stained with HE).

Similarly, the 20 mg NaF/kg bw/day group also demonstrated degenerative changes in the ovum. There was a contraction and a shift of the ooplasm to one side with an associated non-visibility of the nucleus and the nucleolus and the absence of the zona pellucida. The area surrounding the ovum showed certain cavities. In the stroma of the ovary many vacant areas and atretic follicles (AF) were seen with some of these being full of fluid. The typical appearance of a follicle was totally lost. All the treated ovaries showed dilated blood vessels (Dbv) and congestion of the stroma (Figure 8).



**Figures 8A and 8B.** Ovarian sections (8A:  $\times 10$ , 8B:  $\times 40$ ) of a rat treated with sodium fluoride in a dose of 20 mg NaF/kg bw/day showing degeneration of the developing follicle ovum (OV), theca interna (TI), theca externa (TE), granulosa cells (GC), atretic follicles (AF), and dilated blood vessels (Dbv). (Stained with HE).



## DISCUSSION

As reported earlier by us, a significant reduction was found in the body and ovarian weights of the NaF-treated rats as compared to the control group. Similar results have been reported in rats and mice and may be due to an adverse effect of F on metabolism or physiology.<sup>1,29,30</sup> We found an increased thyroid gland weight in our study. Several investigators have found that F administration to experimental rats may cause an enlargement of the thyroid gland.<sup>31</sup> TSH stimulates the growth of the gland and therefore the gland becomes enlarged.<sup>32</sup> Significant increases in the thyroid weight in the mother and offspring mice have been found after F ingestion.<sup>33</sup>

Thyroid function ultimately depends on an appropriate iodine supply to the gland. The structure of F is similar to iodine because both belong to halogen group and fluoride is more chemically active than iodine. Therefore, F binds to the iodine receptor on thyroid gland, inhibits Na/K-ATPase activity, and decreases the iodine level in the gland.<sup>34</sup> This mechanism disturbs the activity of the enzyme that catalyses the conversion of T4 into active T3 leading to interrupted thyroid physiology along with a decrease in circulating thyroid hormones.<sup>33,35,36</sup> The data reported here, of a significant increase in TSH and a decrease in the concentrations of the T3 and T4 hormones, confirms that NaF ingestion may cause hypothyroidism. The results are in agreement with similar studies,<sup>37,38</sup> which found hypothyroidism in human populations with increased F exposure.

We found that the treatment with NaF resulted in a reduction in the production of the gonadotropin hormones, luteinizing hormone (LH) and follicle stimulating hormone (FSH), and in the steroid hormones estrogen and progesterone which is consistent with the results of similar studies in rats.<sup>39,40</sup> NaF may interfere with ovarian function indirectly by acting at the level of the hypothalamus or the pituitary gland or both.<sup>41</sup> A reduced secretion of estrogen from the ovary following reduced FSH secretion may cause a negative feedback resulting in hormonal imbalance. Moreover, it is possible that a reduction of estrogen and progesterone is related to a decreased number of healthy follicles.<sup>42</sup> The present study resulted in the clinical conditions of hypothyroidism and the changes which were found to occur in the ovary of the female albino rats. Hypothyroidism also decreases estrogen and progesterone since the conversion of progesterone to estrogen occurs under the influence of FSH by the expression of aromatase activity in the follicles. Thyroid hormone receptor  $\alpha$  and  $\beta$  messenger RNA (mRNA) is expressed in oocytes, and granulosa and cumulus cells.<sup>43,44</sup> The thyroid hormones may affect oocyte maturation, aromatase activity, estrogen secretion, and the functional differentiation of granulosa cells.<sup>45,46</sup>

The present work found variable histopathological changes in the thyroid gland. The gland has a strong capacity for absorbing and accumulating fluoride.<sup>47</sup> Some thyroid follicles showed a reduction of colloid in their lumen and some follicles had a disrupted cell lining with most of the epithelial cells being swollen with vacuolated cytoplasm. Our finding of an increased height and hypertrophy of the follicular epithelial cells is consistent with the results of earlier reports.<sup>33,48</sup> The

groups treated with 10 and 20 mg/kg bw of NaF showed a breakage of the epithelial layer of the follicles and a fusion of the follicles. The toxicity of NaF-induced morphological changes in the thyroid gland increased with increasing exposure duration leading to generalised damage to the gland and disturbed functioning including changes in hormonal secretion and the leakage of hormones through damaged cellular membranes.<sup>33</sup> These changes may be due to an increased level of TSH, which is responsible for the proliferative activity of follicular cells.<sup>48</sup>

In the present study, many histopathological changes were seen in the ovary of the albino rats after treatment with NaF. The number of ovarian follicles decreased and most of them degenerated leading to an increase in atretic follicles. These results are in conformity with another study<sup>30</sup> which attributed the decrease in number of primary, secondary, and Graffian follicles to reduced availability of the proteins necessary for cell division, growth, and the differentiation of germ cells during oogenesis or to an inhibition of the hypothalamus by F.<sup>49</sup> In fluoride-induced hypothyroidism the follicles becomes atretic. The ovarian follicles are not well developed in hypothyroid animals because the thyroid hormones may have a direct effect on the growth of ovarian follicles.<sup>50</sup>

### CONCLUSION

The results of current study suggest that ingestion of NaF by adult rats causes adverse effects on the thyroid and ovary. The effects on the thyroid gland cause hypothyroidism which may lead to the development of atretic follicles and the proliferation of interstitial cells because thyroid hormones play an important role in the regulation of folliculogenesis.

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