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(RESEARCH ARTICLE)

Ultrastructural evaluation of oocytes during fluorosis in rat ovarian follicles

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Abstract

Fluoride is a well-known environmental pollutant and its effect on human health has long been of interest to biomedical researchers. Various studies have shown that fluoride causes adverse effects on the fertility. Wistar albino female rats weighing 150-200 g were randomly divided into six rats in each group. The rats in experimental groups treated with 300 and 600 mg NaF/kg bw/day by oral gavage for 40 days. The present investigation focuses on the ovary of rat treated with sodium fluoride and its amelioration by curcumin. The results revealed that the sodium fluoride exposure to female rats treated with 300 mg NaF/kg bw/day, the surface epithelium had less number of ruffles and blebs of the plasma membrane. There was abrasion of ovarian surface epithelium. In rats treated with 600 mg/kg bw/day NaF, the cuboidal shape of the surface epithelium were changed into elongated appearance. The cells were devoid of microvilli, blebs and ruffles. After administration of curcumin, many follicles in different stages of development were visible. The ovarian surface epithelium showed normal surface epithelium with improvement in the shape of cuboidal cells.

Keywords: Curcumin; Ovary; Scanning Electron Microscopy; Sodium Fluoride; Wistar Female Albino Rats

1. Introduction

Chronic fluorosis is a slow and progressive process causing symptoms related to several systems, particularly musculoskeletal and dental systems. The literature indicates that sodium fluoride may have toxic effects on the brains of suckling mice [1], may impair learning and memory in rats [2, 3, and 4]. Metabolic functional and structural damages caused by chronic fluorosis have been reported in many tissues, including gastrointestinal tract [5], pancreas [6], parathyroid gland [7], thyroid [8], adrenal [9, 10], skeletal muscle [11, 12], kidney [13] and heart [14]. Additionally, there are number of studies in the literature regarding the toxic effects of sodium fluoride on the male reproductive system [15, 16, and 17] but reports of its effect on female reproductive system are few.

Most of these investigations, which were conducted with a number of different animal species, including rats, mice and rabbits, found alterations in the levels of reproductive hormones, fertility, histological structures and developmental outcomes [18, 19].

The curcumin is a yellow pigment obtained from rhizomes of *Cucuma longa* and is commonly used as a spice and food colouring. Currently, curcumin is attracting strong attention as it is toxicologically very safe [20]. Some studies have also demonstrated that curcumin and its analogues exert a stimulatory effect on ovarian functions, because they promote proliferation and reduce apoptosis in murine ovarian cells [21, 22]. The aim of the present investigation was to examine the ultrastructural alterations in the ovary of fluoridated rat using scanning electron microscopy and ameliorating effect of curcumin after fluoride administration.

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2. Material and methods

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).

2.1. Design of Study

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 rats were administered 300 and 600 mg of NaF/kg bw/day orally by gastric tube for 40 days. The control 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. The ovary tissues were dissected out, washed in normal saline, and processed for scanning electron microscopic examination.

2.2. Scanning Electron Microscopy

For scanning electron microscopic viewing, the samples were fixed in 2.5% glutaraldehyde and 2% paraformaldehyde, washed in 0.1M sodium phosphate buffer (PH 7.4) for 12 hours at 4°C. After few washes in 0.1M phosphate buffer, the samples were dehydrated through graded acetones and dried by the critical point method. Dried samples were mounted on aluminium stubs. They were sputter-coated (SCD 050 super cool sputter system; Baltec Technology, Liechtenstein) with colloidal gold and observed under a Leo 435 VP scanning electron microscope (Cambridge, UK) at an operating voltage 15kV. Images were digitally acquired by using a CCD camera attached to the microscope.

3. Results and discussion

Scanning electron microscopic examination of ovary of control rat showed that the ovarian surface was characterized by outgrowths, the ovarian papilla and in growth of ovarian crypts (Figure 1). There were existence of cuboidal cells, which were shaped like cubes, and squamous cells on the surface of the germinal epithelium the surfaces of the superficial cells was covered with numerous microvilli, blebs and ruffles of the plasma membrane (Figure 2).

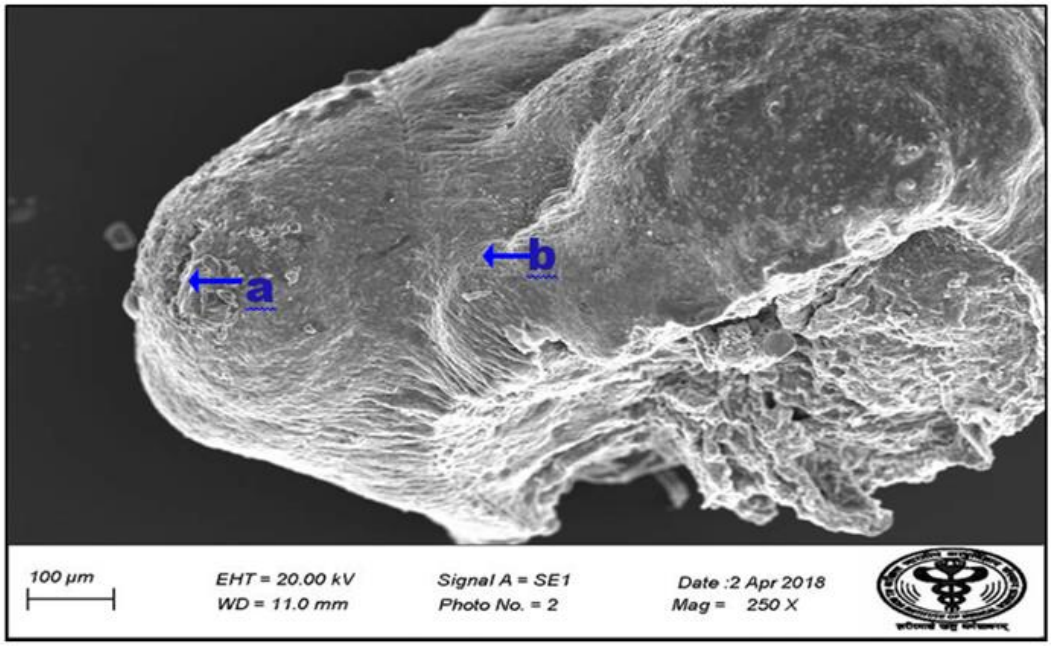

Figure 1 Scanning electron micrograph of ovarian surface epithelium in ovary of control rat showing ovarian papillae (a) and crypts (b) X 250.

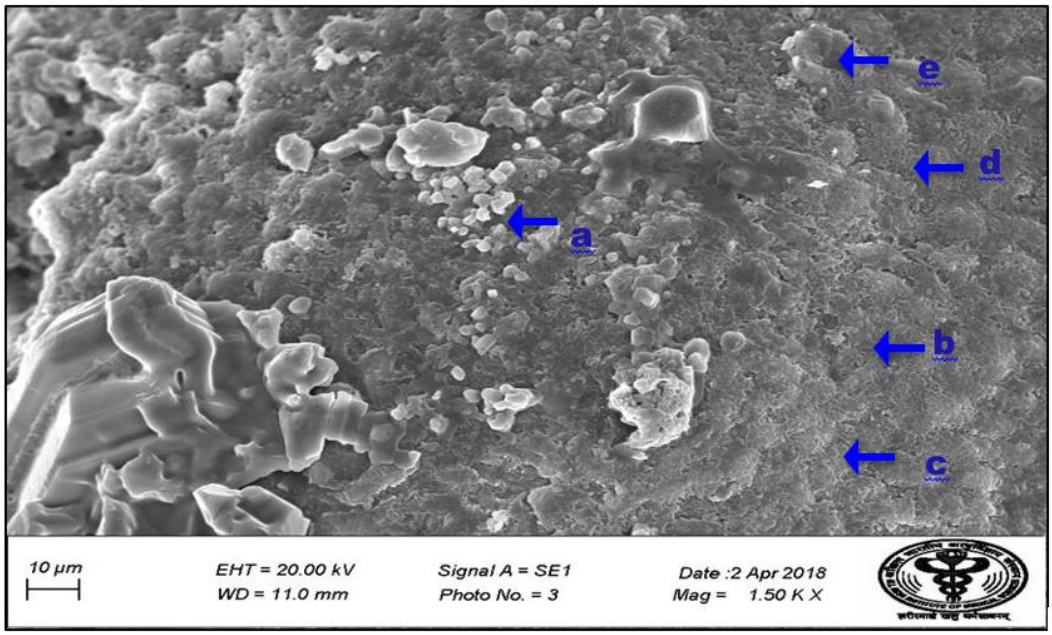

Figure 2 Scanning electron micrograph of ovary of control rat showing cuboidal (a) and squamous cells (b) of the germinal epithelium. Surfaces of the superficial cells covered with numerous microvilli (c), blebs (d) and ruffles (e) of the plasma membrane. X 2500.

In the animals treated with 300 mg fluoride/kg bw/day for 40 days, the surface epithelium had less number of ruffles and blebs of the plasma membrane (Figure 3). There was abrasion of ovarian surface epithelium and cuboidal shape was distorted (Figure 4).

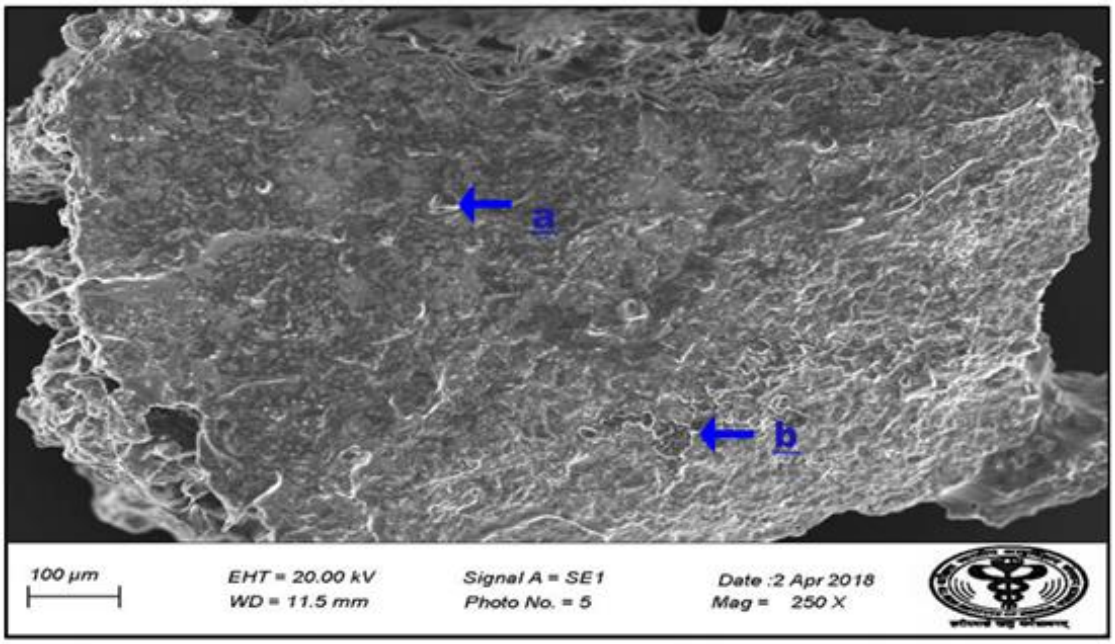

Figure 3 Scanning electron micrograph of surface epithelium in ovary of rat treated with 300 mg fluoride for 40 days showing less number of ruffles (a) and blebs (b) of the plasma membrane and absence of microvilli. X 250.

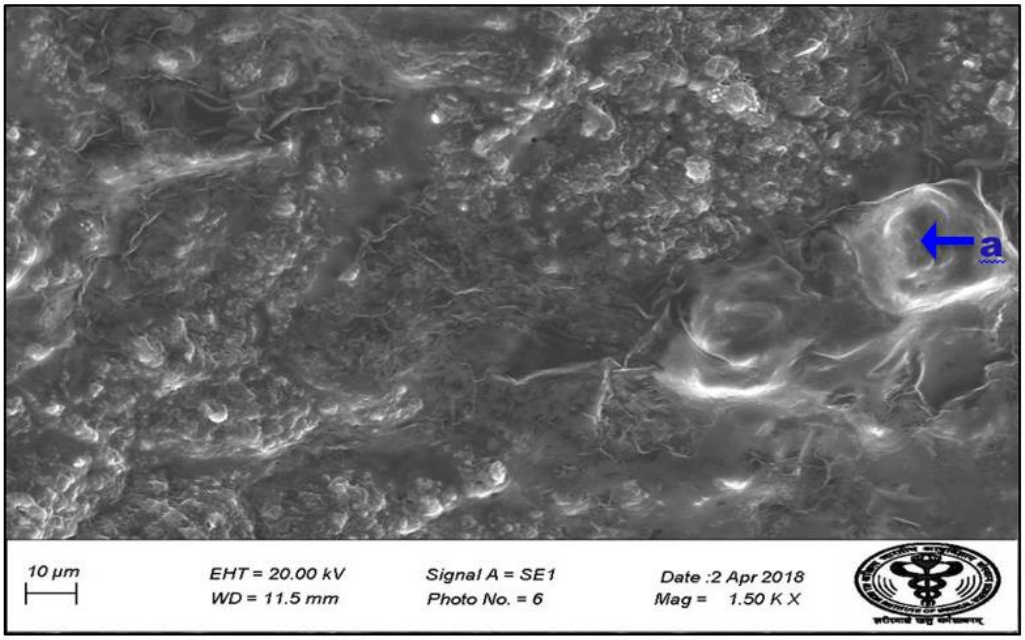

Figure 4 Scanning electron micrograph of surface epithelium in ovary of rat treated with 300 mg fluoride for 40 days showing abrasion of surface epithelium and cuboidal shape (a) is distorted. X 1500.

These changes were most pronounced in animals treated with 600 mg/kg bw/day NaF for 40 days, where the cuboidal shape of the surface epithelium were changed into elongated appearance (Figure 5). The surfaces of the superficial cells had clumping. The surface epithelium had less blebs and ruffles of the plasma membrane of germinal epithelial cells (Figure 6). Cells were devoid of microvilli and blebs. Ovarian follicles at different stages of development were very less in number (Figure 7).

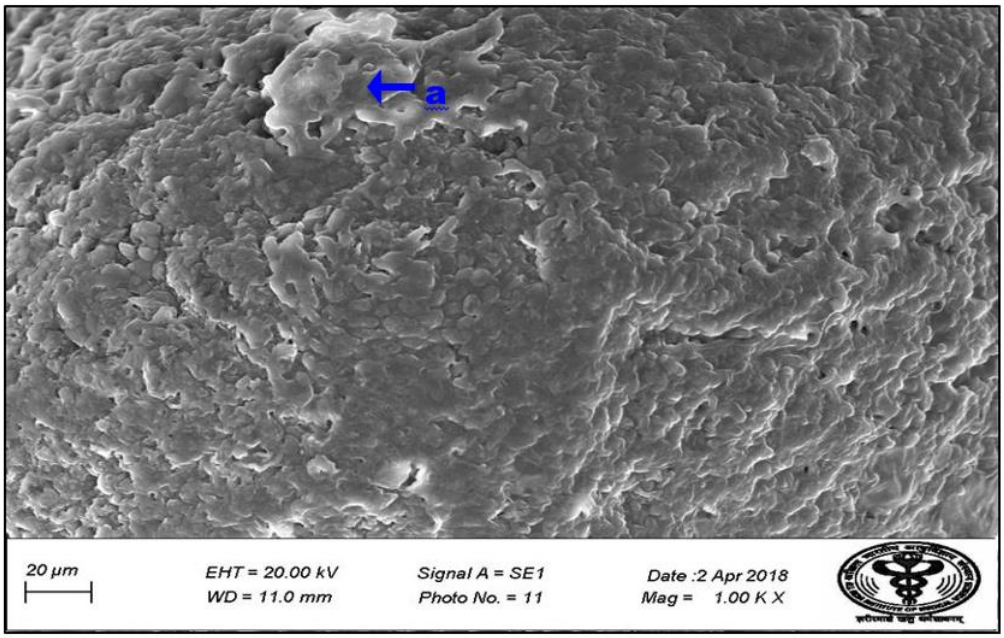

Figure 5 Scanning electron micrograph of surface epithelium in ovary of rat treated with 600 mg fluoride for 40 days showing that cuboidal shape of the surface epithelium was distorted and mostly showing elongated appearances (a) X 1000.

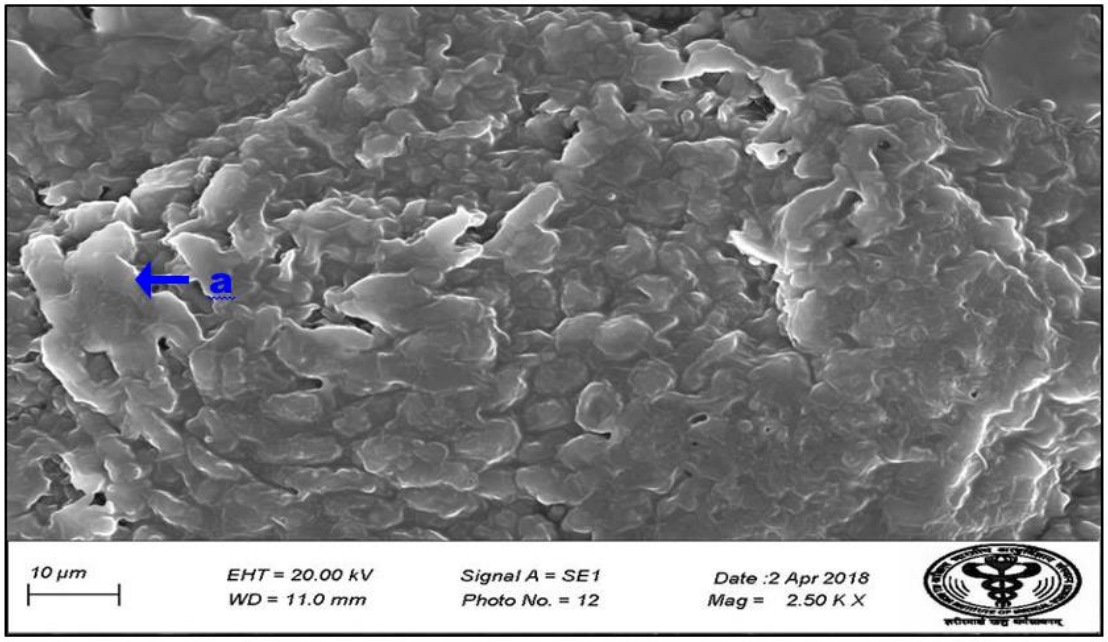

Figure 6 Scanning electron micrograph of germinal epithelium in ovary of rat treated with 600 mg fluoride for 40 days showing clumping of surface epithelium (a) have less microvilli, blebs and ruffles of the plasma membrane of germinal epithelial cells covering the ovary. X 2500.

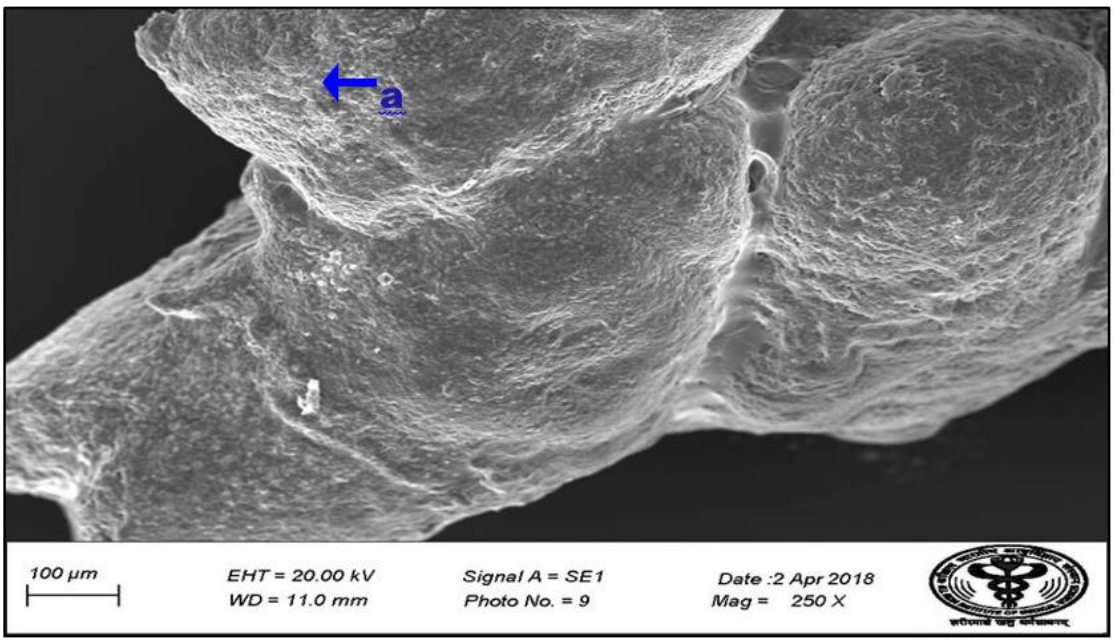

Figure 7 Scanning electron micrograph of surface epithelium in ovary of rat treated with 600 mg fluoride for 40 days showing less number of ovarian follicles (a) in developmental stages. X 250.

Administration of 300 mg/kg fluoride for 40 days followed by 200 mg/kg curcumin for 20 days revealed there were many follicles in different stages of development (Figure 8). The ovarian surface epithelium showed normal surface epithelium with improvement in the shape of cuboidal cells and less number of microvilli, blebs and ruffles of plasma membrane (Figure 9).

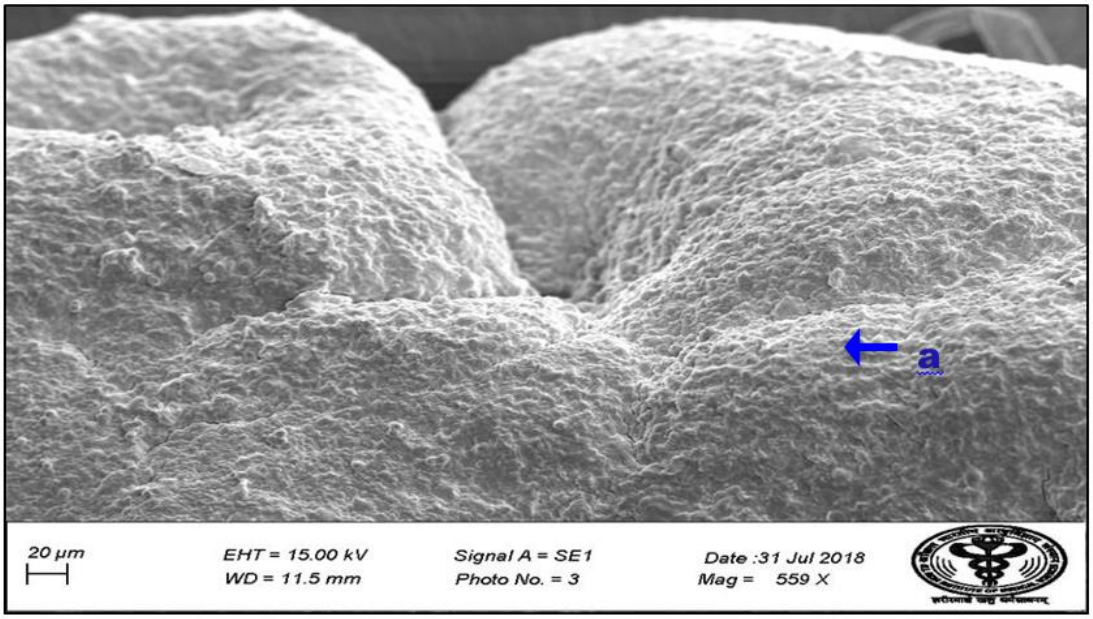

Figure 8 Scanning electron micrograph of germinal epithelium in ovary of rat treated with 300 mg fluoride for 40 days followed by 200 mg Curcumin for 20 days showing normal follicles (a) at different stages of development. X 559.

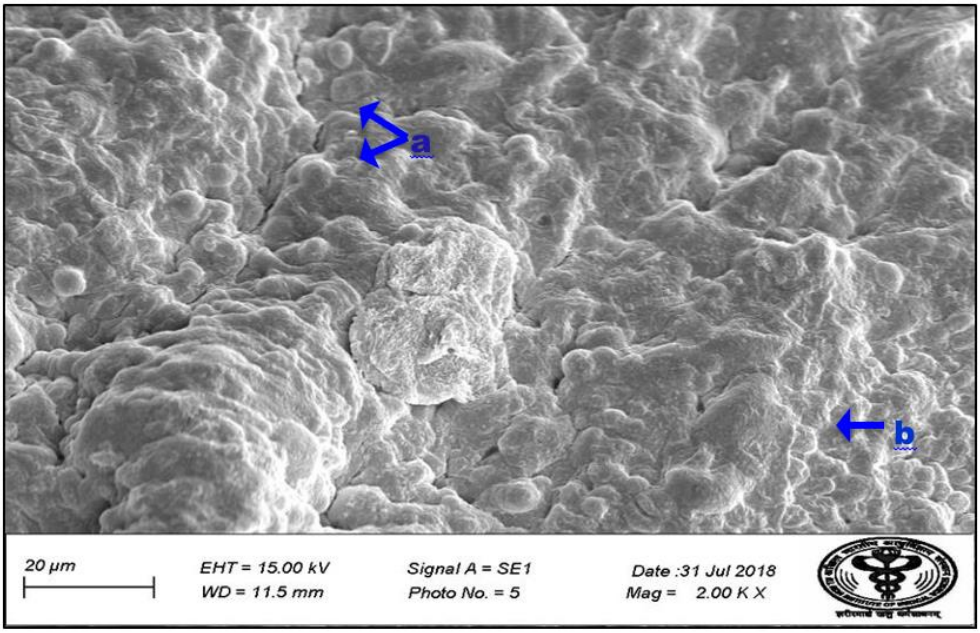

Figure 9 Scanning electron micrograph of surface epithelium in ovary of rat treated with 300 mg fluoride for 40 days followed by 200 mg Curcumin for 20 days showing restoration in cuboidal shaped cells (a) and also contain less microvilli (b), blebs and ruffles of plasma membrane. X 2000.

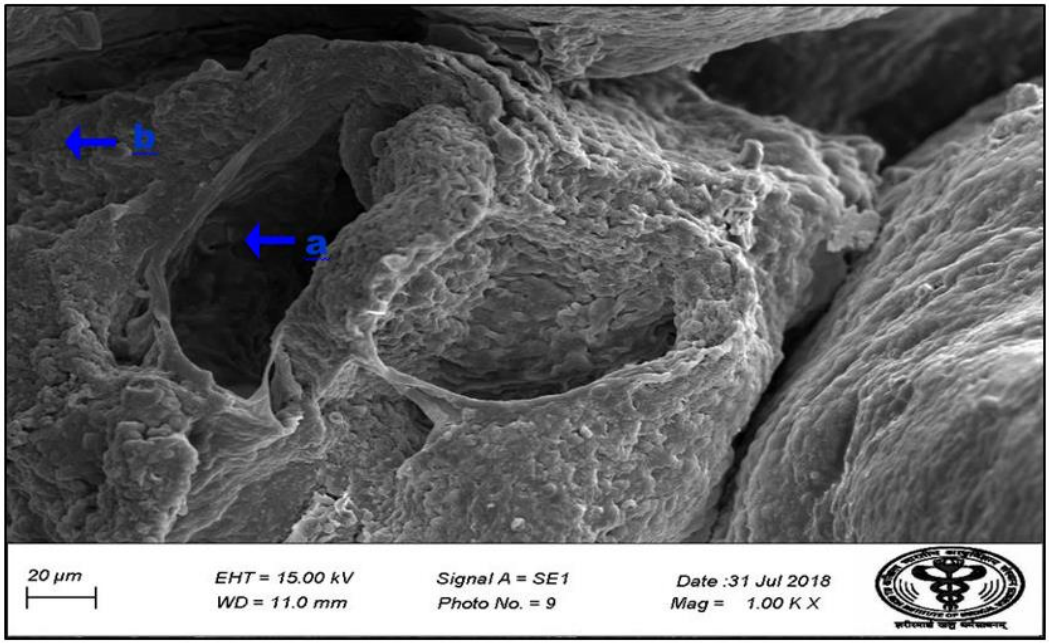

Figure 10 Scanning electron micrograph of ovarian follicle in ovary of rat treated with 600 mg fluoride for 40 days followed by 200 mg Curcumin for 20 days showing fluid filled cavity, antrum (a) surrounded by granulosa cells (b). X 1000

The animals treated with 600 mg/kg fluoride followed by 200 mg/kg curcumin showed well developed antrum, the fluid filled cavity surrounded by granulosa cell layer (Figure 10). There was preservation of cuboidal epithelial cells, microvilli, blebs and ruffles of the plasma membrane were less in number (Figure 11). There was preservation of ovarian follicles with improved shape of germinal epithelium (Figure 12).

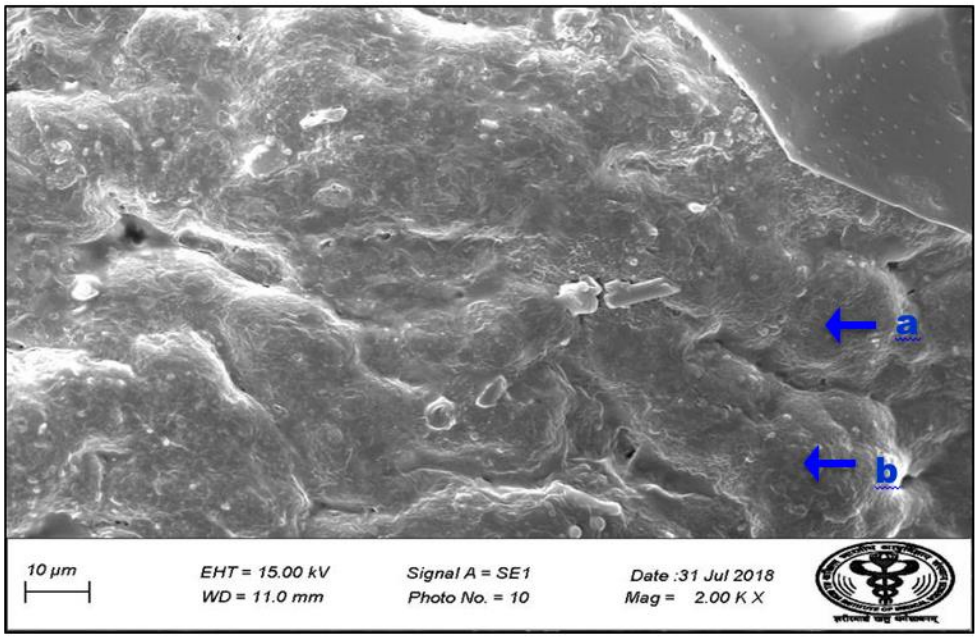

Figure 11 Scanning electron micrograph of germinal epithelium in ovary of rat treated with 600 mg fluoride for 40 days followed by 200 mg Curcumin for 20 days showing preservation of cuboidal cells (a) and less number of microvilli (b), blebs and ruffles of the plasma membrane. X 2000.

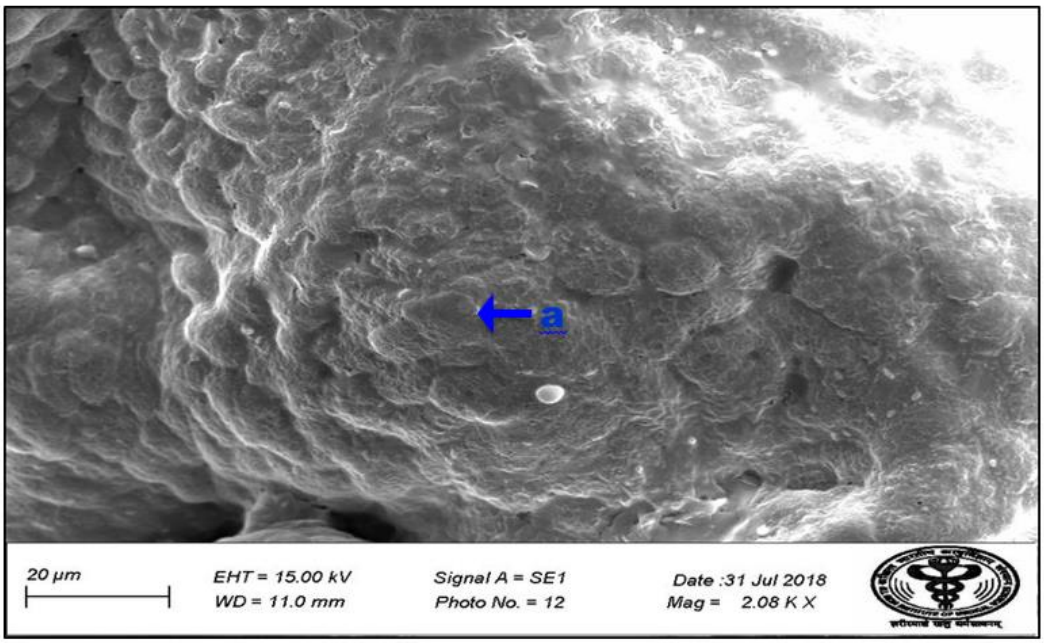

Figure 12 Scanning electron micrograph of germinal epithelium in ovary of rat treated with 600 mg fluoride for 40 days followed by 200 mg Curcumin for 20 days showing preservation of ovarian follicle (a) and germinal epithelium. X 2000.

In the present study, the scanning electron microscopic examination of the control rats revealed normal ovarian surface epithelium with ovarian papillae and crypts and normal ovarian follicles. Two distinct cell types viz., cuboidal and squamous were observed in ovarian surface epithelium. These have also been observed previously in mouse [23].

The NaF treated animals showed many pathological alterations in ovaries including distorted cuboidal shape of the epithelium, and clumping of the ovarian surface epithelium, reduced microvilli, blebs and ruffles. These findings are in agreement with the study of Kumar and Kumari [24] who observed abrasion and clumping of surface epithelium, distorted cuboidal shape of epithelium and absence of microvilli and blebs in NaF treated mice.

Curcumin administration causes greater degree of restoration in ovarian surface epithelium, follicular development, shape of cuboidal and squamous cells of surface epithelium, increased microvilli, blebs and ruffles of the plasma membrane. There was preservation of antral follicle with antrum and granulosa cells.

4. Conclusion

It is concluded that sodium fluoride induced alterations in the cellular ultrastructure of the granulosa and theca cells alongwith abrasion of surface epithelium. The administration of curcumin and had curative effects on the ovarian follicles in experimental fluorosis.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The author declare that there was no conflict of interest.

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