



Therapeutic Effect of Curcumin on Scanning Electron Microscopy of Rat Adrenal Gland in Experimental Fluorosis

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved and contributed equally to the manuscript.

Article Information

DOI: 10.9734/JPRI/2021/v33i41A32333

Editor(s):

- (1) Dr. Paola Angelini, University of Perugia, Italy.
(2) Dr. Ana Cláudia Coelho, University of Trás-os-Montes and Alto Douro, Portugal.

Reviewers:

- (1) V. R. Salunkhe, Rajarambapu College of Pharmacy, Shivaji University, India.
(2) Imane Zakariya, Morocco.

Complete Peer review History: <https://www.sdiarticle4.com/review-history/67383>

Original Research Article

Received 10 June 2021
Accepted 14 August 2021
Published 19 August 2021

ABSTRACT

Aim: The present study was designed to investigate the therapeutic role of Curcumin against fluoride induced toxicity on adrenal gland of rats by using scanning electron microscopy (SEM).

Methodology: Wistar albino rats were divided randomly into six groups. The group I was administered with 1 ml of deionized water/kg b.w./day orally for 40 days. The Groups II and III were given 300 and 600 mg of NaF/kg b.w./day for the same period, respectively. The group IV was given 200 mg/kg b.w. of Curcumin for 20 days. The Groups V and VI were treated with 300 and 600 mg of NaF/kg b.w./day for 40 days respectively and were post-treated with 200 mg of Curcumin for next 20 days. The animals were excised and adrenal tissue was taken out and processed for SEM.

Results: The results revealed that rats exposed to 300 mg/kg b.w./day of NaF showed rough edges, numerous microvilli and damaged surface with crystal depositions. Also, numerous granules were distributed all over the surface. The rats treated with 600 mg/kg b.w./day of NaF showed decellularized adrenal tissue along with network of collagen fibres. Moreover, adrenal gland surface displayed abrasions and distorted cuboidal cells. The filopodia were prominent on the surface and wall of cavity possessed rough outline. After post-treatment with Curcumin, fluoridated adrenal

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gland of rats showed normal structure, reappearance of cuboidal cells on the surface as well as less number of microvilli and filopodia.

Conclusion: The post-treatment with Curcumin possess therapeutic potential against NaF induced toxicity in adrenal gland of rats.

Keywords: Adrenal gland; Curcumin; rat; Sodium fluoride; scanning electron microscopy.

ABBREVIATION

ACTH: Adrenocorticotropic Hormone

NaF: Sodium fluoride

B.W: Body weight

g: gram

kg: kilogram

mg: milligram

SEM: Scanning electron microscopy

1. INTRODUCTION

Endemic fluorosis is a public health problem in India due to high fluoride concentrations in the ground water [1]. Accumulation of excess fluoride in the environment causes serious health risks to the plants, animals and humans [2]. The effects of fluoride at different levels have been studied experimentally in various laboratory animals, including rabbit, rat and mice. It causes impairment in digestive tract, liver [3], brain [4], kidneys [5] and endocrine organs. Extremely high intake of fluoride may alter the function of pancreas [6], parathyroid [7], thyroid [8] and also causes certain changes in the humoral profile [9].

The adrenal gland consists of two structurally and functionally distinct endocrine tissues named as cortex and medulla. The cortex is mesodermal in origin and produce steroid hormones. On the other hand, medulla is ectodermal in origin and secretes catecholamine i.e, epinephrine and norepinephrine that facilitate the acute mammalian stress on "flight or fight" response [10]. In modern environment, people are exposed to various stressful conditions. Stress causes the activation of both the hypothalamic-pituitary-adrenocortical axis and sympatho-adrenal system [11]. The stress tends to disturb the equilibrium between the living organisms and their surrounding environment. In response to stress, the levels of various hormones including glucocorticoid, catecholamine, growth hormones and prolactin changes [12]. Curcumin is diferuloyl methane, a natural yellow pigment in turmeric, isolated from the rhizomes (Fig. 1) of the plant *Curcuma longa* [13]. Its chemical formula is $C_{21}H_{20}O_6$ (Fig. 2).

Curcumin due to its various medicinal, biological and pharmacological activities is on high demand and huge market potential [14]. Many pharmacological studies have been conducted to demonstrate multiple biological properties of Curcumin. These studies have evaluated that Curcumin possess anti-inflammatory, anti-carcinogenic, anti-bacterial, antidepressant antioxidant and nephroprotective properties [15,16].

Keeping in view all these valuable properties, the present study elucidated the therapeutic role of Curcumin against fluoride induced ultrastructural changes in adrenal gland by using SEM.

2. MATERIALS AND METHODS

2.1 Experimental Design

Young Wistar albino rats weighing between 150-200 g were housed in polypropylene cages with stainless steel grill tops and fed with standard rat pellet diet (Hindustan lever Limited, India) and water was given *ad libitum*. After one week of acclimatization, animals were divided randomly into six groups (six rats in each group). The group I was administered with 1 ml of deionized water/kg b.w./day for 40 days. The Groups II and III were given 300 and 600 mg of NaF/kg b.w./day for same period, respectively. The group IV was given 200 mg/kg b.w. of Curcumin only for 20 days. The Groups V and VI were treated with 300 mg and 600 mg of NaF/kg b.w./day for 40 days respectively followed by 200 mg of Curcumin for next 20 days. At the end of experimental period, overnight fasting rats were sacrificed under anaesthesia. The adrenal tissue were taken out, washed in normal saline and further processed for SEM.

2.2 Scanning Electron Microscopy

For scanning electron microscopic viewing, adrenal tissue 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 and post-fixed in 1% osmium tetroxide for 2 hours. After few washes

in 0.1M phosphate buffer, the samples were dehydrated through grades of acetone 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 at All India Institute of Medical Sciences, New Delhi, India.

2.3 Chemical Purchased

Sodium fluoride and Curcumin were purchased from Loba Chemie Pvt. Ltd, Mumbai, India.

3. RESULTS

3.1 Group I (Control)

The scanning electron microscopic examination of adrenal gland of control rat exhibited adrenal cortex and medulla. Adrenal cortex showed zona

glomerulosa, zona fasciculata and zona reticularis. (Fig. 3).

3.2 Group II (300 mg NaF/kg b.w./day)

In rats treated with 300 mg/kg b.w./day of NaF for 40 days, the adrenal surface had rough edges and covered with numerous pores. The cell surface had large bulging with numerous microvilli. Scattered pits were also observed on the membrane surface (Fig. 4).

The surface was damaged and crystals of variable sizes deposited on it (Fig. 5). Three dimensional networks of intermediate filaments associated with lipid droplets and wandering cell were observed (Fig. 6). Numerous secretory granules were also noticed on the cell surface (Fig. 7).

3.3 Group III (600 mg NaF/kg b.w./day)

The pathological changes in adrenal gland of rats treated with 600 mg/kg b.w./day of NaF for 40 days were more prominent where clumping of surface epithelium was observed (Fig. 8).



Fig. 1. Rhizome of *Curcuma longa*

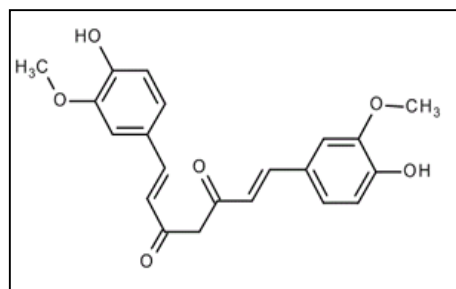


Fig. 2. Chemical structure of Curcumin

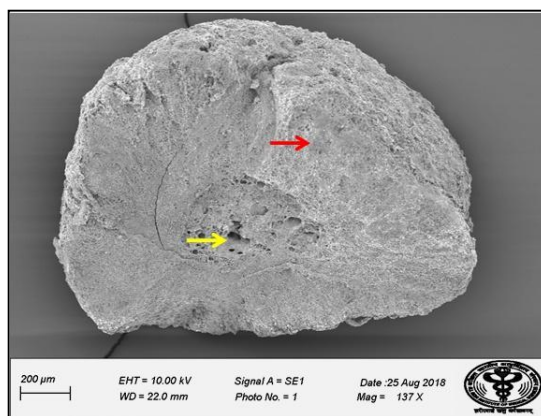


Fig. 3. Scanning electron micrograph of adrenal gland of control rat showing adrenal cortex (↑) and medulla (↑). X 137

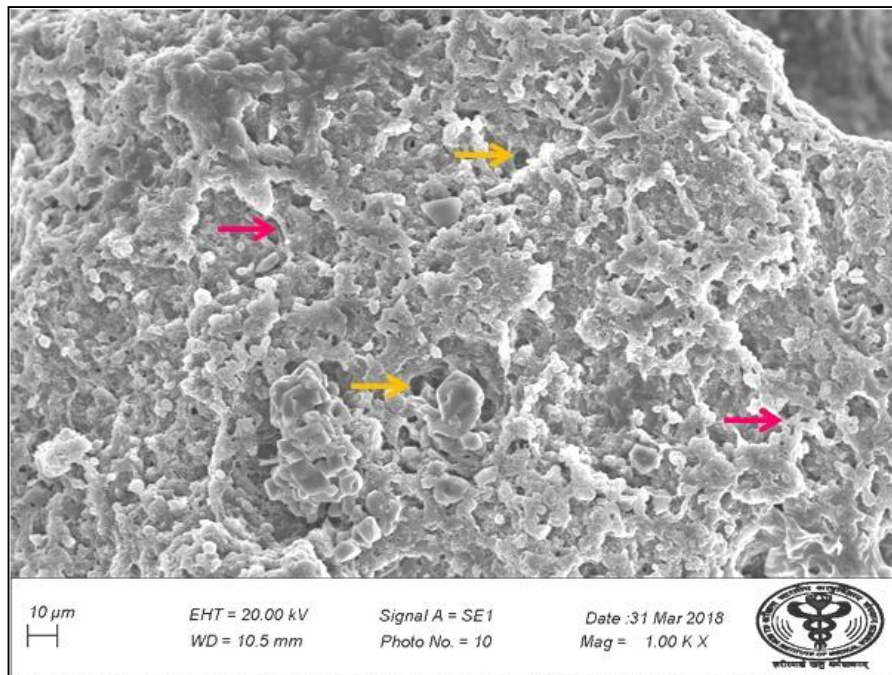


Fig. 4. Scanning electron micrograph of adrenal gland of rat treated with 300 mg/kg b.w./day of sodium fluoride for 40 days showing numerous microvilli (↑) and scattered pits (↗). X1000

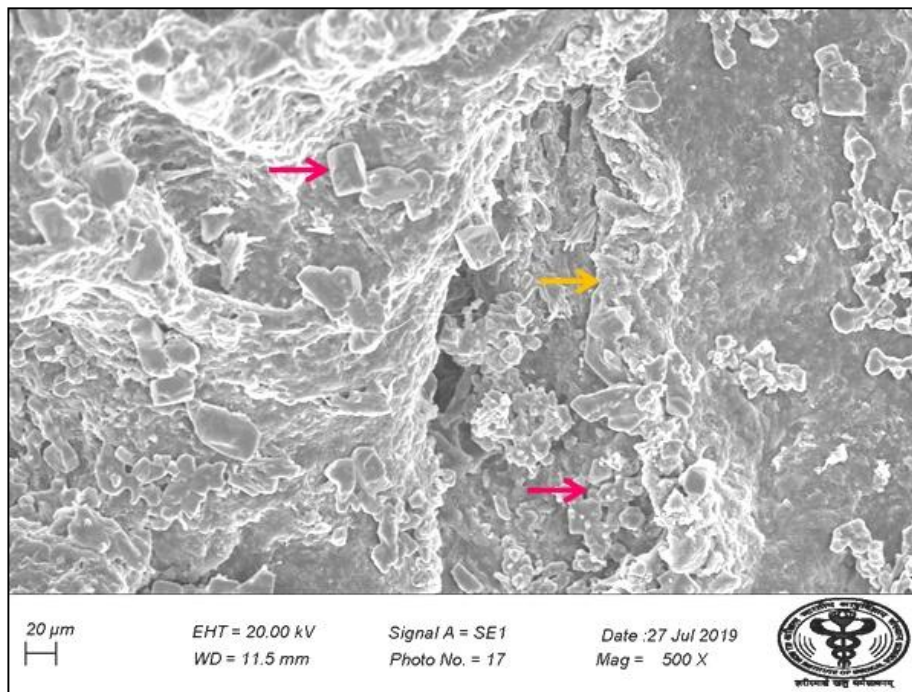


Fig. 5. Scanning electron micrograph of adrenal gland of rat treated with 300 mg/kg b.w./day of sodium fluoride for 40 days showing crystals of variable sizes (↑) with damaged surface (↗) X500

There was decellularization of adrenal tissue, distorted cuboidal cells and network of collagen fibres (Fig. 9).

The edges of the surface showed roughness. The cell surface showed irregular protrusions. The fluoride toxicity induced development of long branched filopodia (Fig. 10).

The walls of the cavity showed rough outlines and fibrous structure (Fig. 11).

3.4 Group IV (200 mg Curcumin/kg b.w./day)

The scanning electron micrograph of adrenal gland of rat treated with 200 mg/kg b.w./day of Curcumin only, for 20 days showed normal adrenal cortex and medulla similar to control rat (Fig. 12).

3.5 Group V (300 mg/kg b.w./day of NaF+200 mg/kg b.w./day of Curcumin)

The adrenal gland showed normal adrenal capsule, cortex and medulla in this group (Fig. 13). The restoration of cuboidal cells and the less number of microvilli were observed on the surface (Fig. 14).

3.6 Group VI (600 mg/kg b.w./day of NaF+200 mg/kg b.w./day of Curcumin)

Scanning electron microscopic examination of adrenal gland of rats in this group showed smooth surface with few rough appearances. The walls of the cavity revealed improved structure with smooth outline (Fig. 15).

The adrenal surface exhibited less number of filopodia (Fig. 16).

The reappearance of the cuboidal cells and improved surface epithelium was noticed (Fig. 17).

4. DISCUSSION

Similar to previous reports, the present SEM study displayed three dimensional architecture of adrenal gland [17,18]. During the present study investigation the adrenal gland in fluoridated rats showed rough edges with numerous pores and rough and outlines on the walls of blood vessel cavity. These findings are in agreement with the study by Kemoklidge *et al.* [19] who performed SEM on the rat adrenal gland after surgical laser exposure. The crater had rough edges, molten inner surface covered with pores and small wrinkles. Rough outlines in the cavity of wall were also observed.

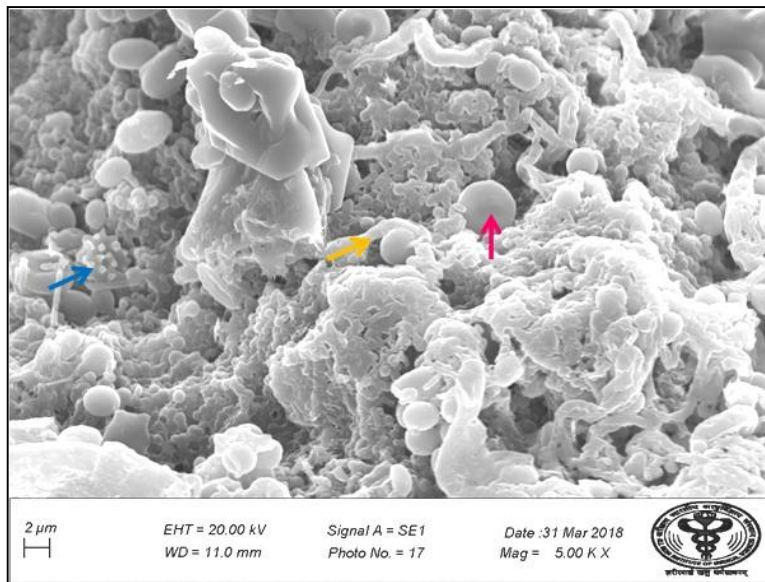


Fig. 6. Scanning electron micrograph of adrenal gland of rat treated with 300 mg/kg b.w./day of sodium fluoride for 40 days showing lipids droplets (↑) and filaments attached to lipid droplet (↑) and wandering cell (↑). X5000

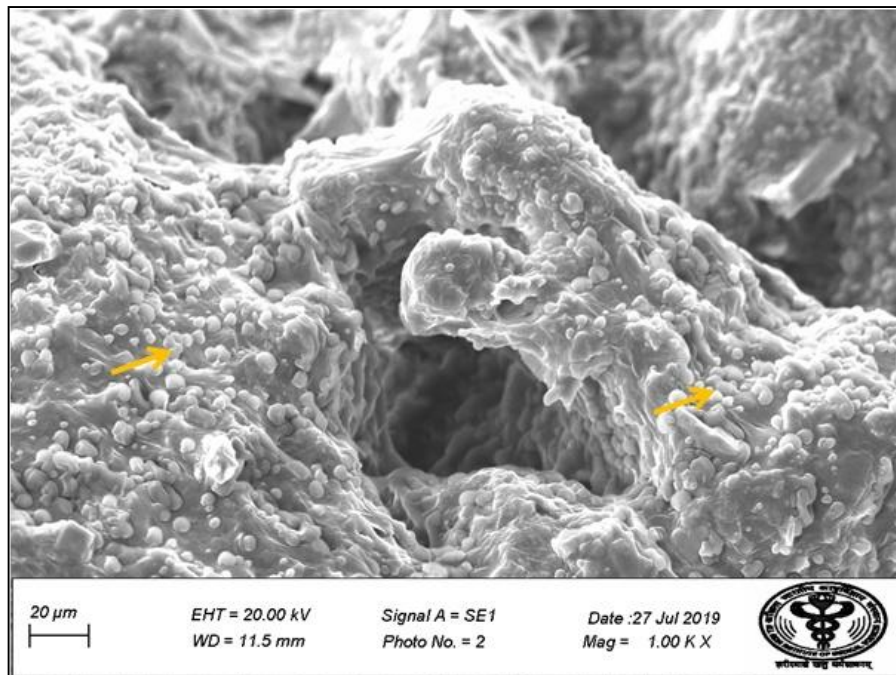


Fig. 7. Scanning electron micrograph of adrenal gland of rat treated with 300 mg/kg b.w./day of sodium fluoride for 40 days showing numerous secretory granules (↑) on the surface. X1000

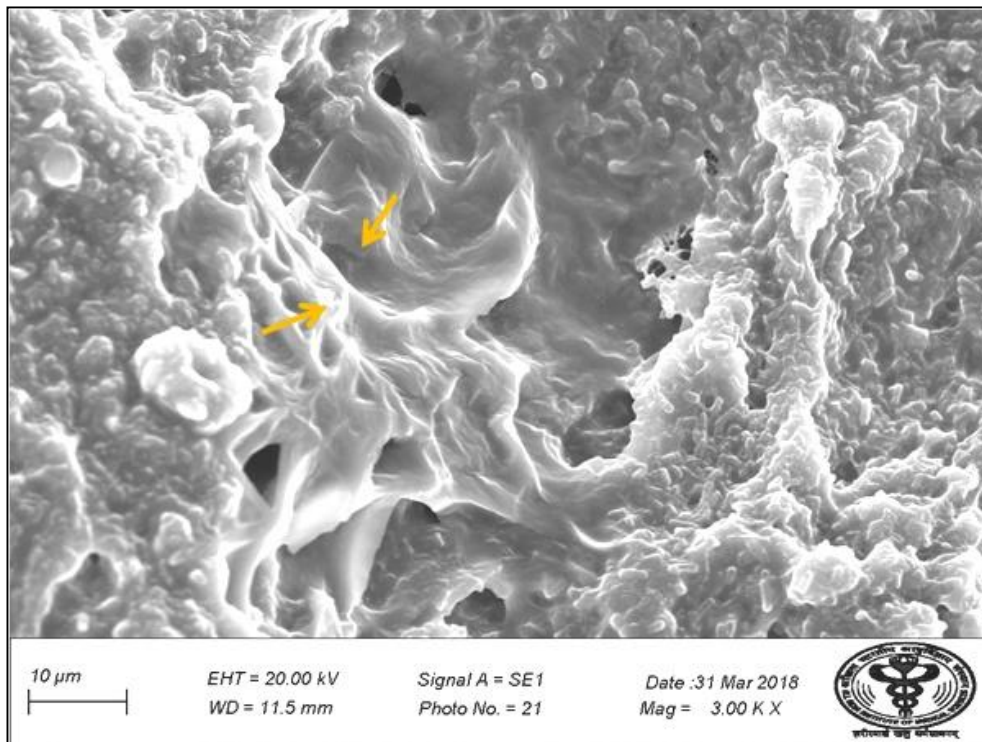


Fig. 8. Scanning electron micrograph of adrenal gland of rat treated with 600 mg/kg b.w./day of sodium fluoride for 40 days showing clumping of surface epithelium (↑). X3000

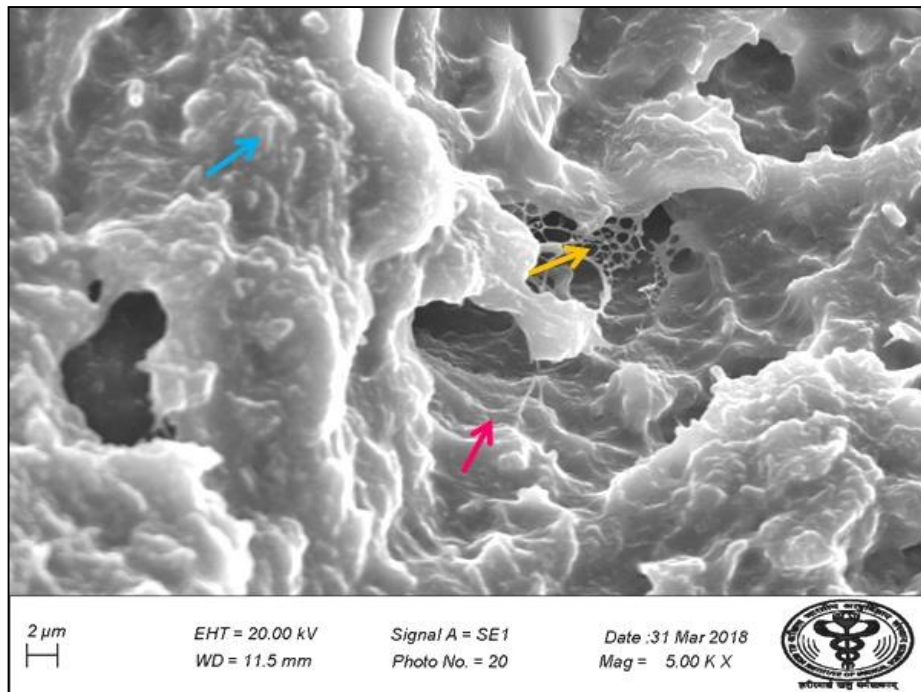


Fig. 9. Scanning electron micrograph of adrenal gland of rat treated with 600 mg/kg b.w./day of sodium fluoride for 40 days showing decellularized adrenal tissue (↑), distorted cuboidal cells (↑) and network of collagen fibres (↑). X5000

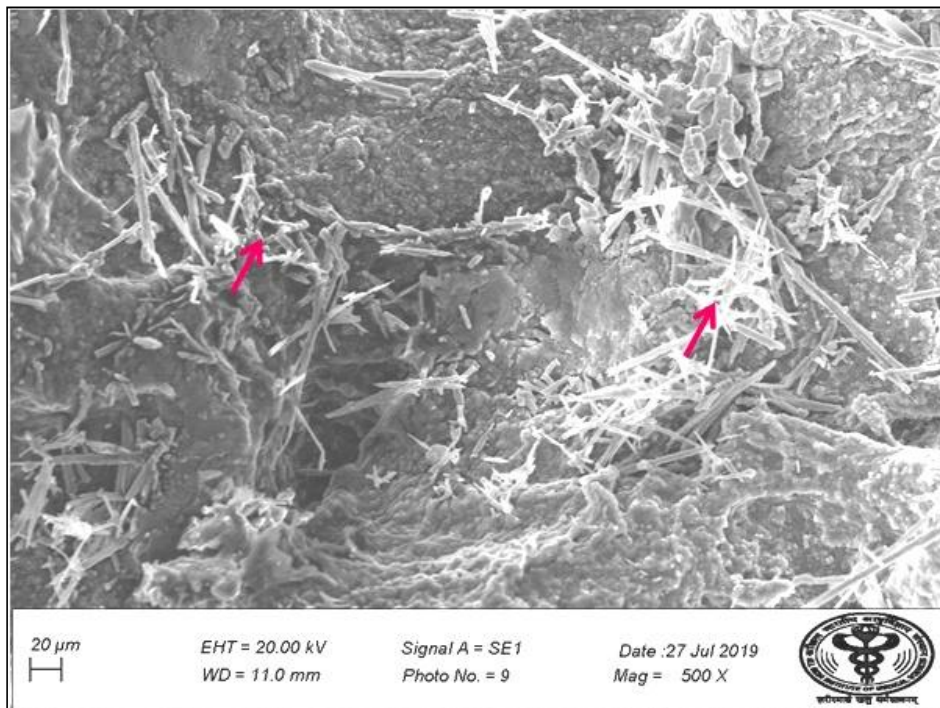


Fig. 10. Scanning electron micrograph of adrenal gland of rat treated with 600 mg/kg b.w./day of sodium fluoride for 40 days showed many filopodia (↑). X500

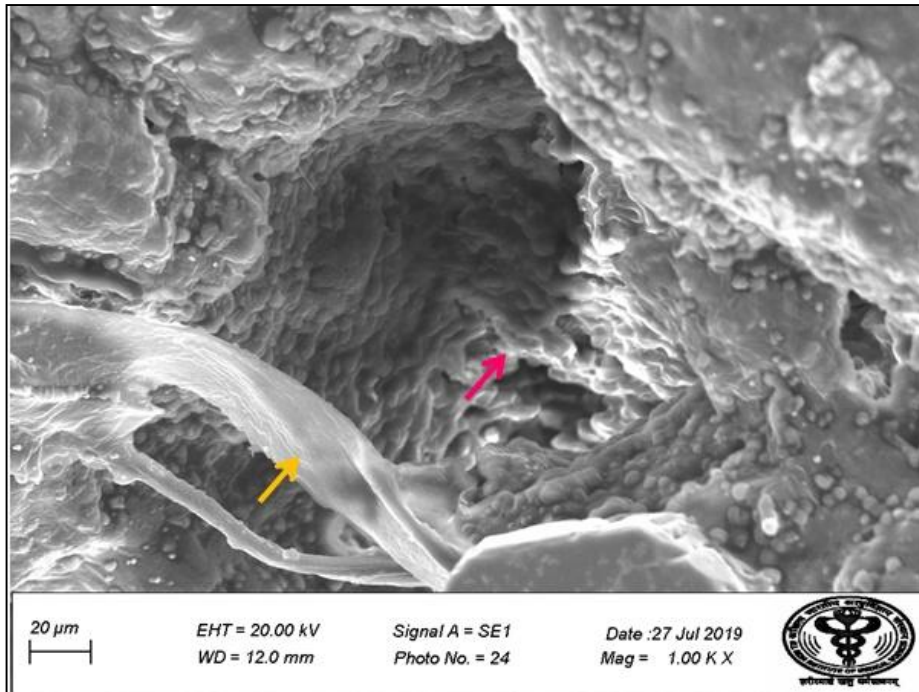


Fig. 11. Scanning electron micrograph of adrenal gland of rat treated with 600 mg/kg b.w./day of sodium fluoride for 40 days showed rough outline on the walls of cavity (↑) and fibrous structure (↑). X 1000

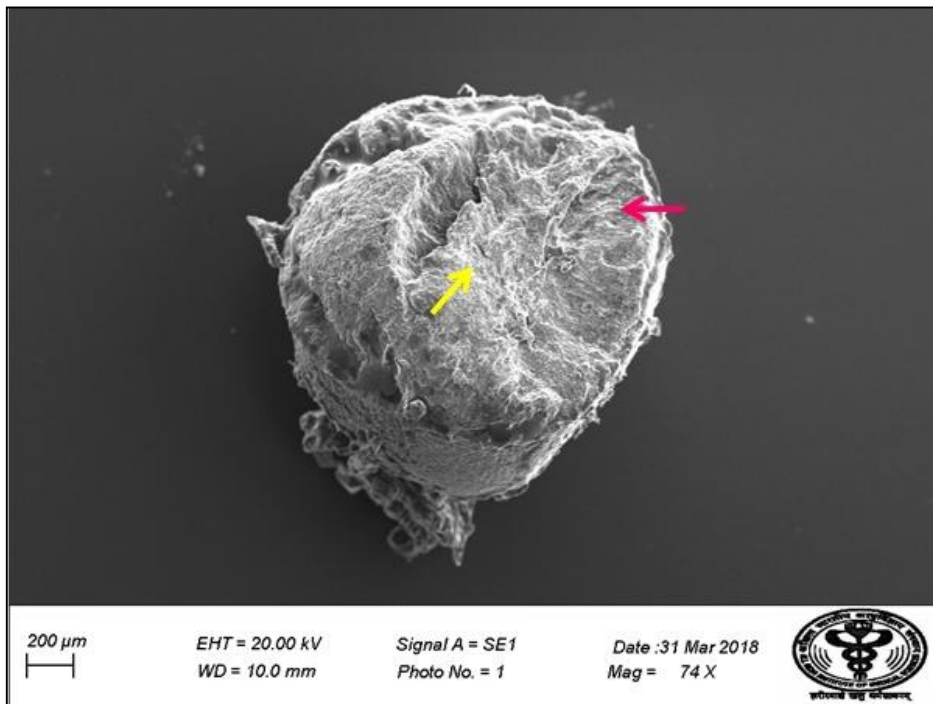


Fig. 12. Scanning electron micrograph of adrenal gland of rat treated with 200 mg/kg b.w./day of Curcumin showing adrenal cortex (↑) and medulla (↑). X 74

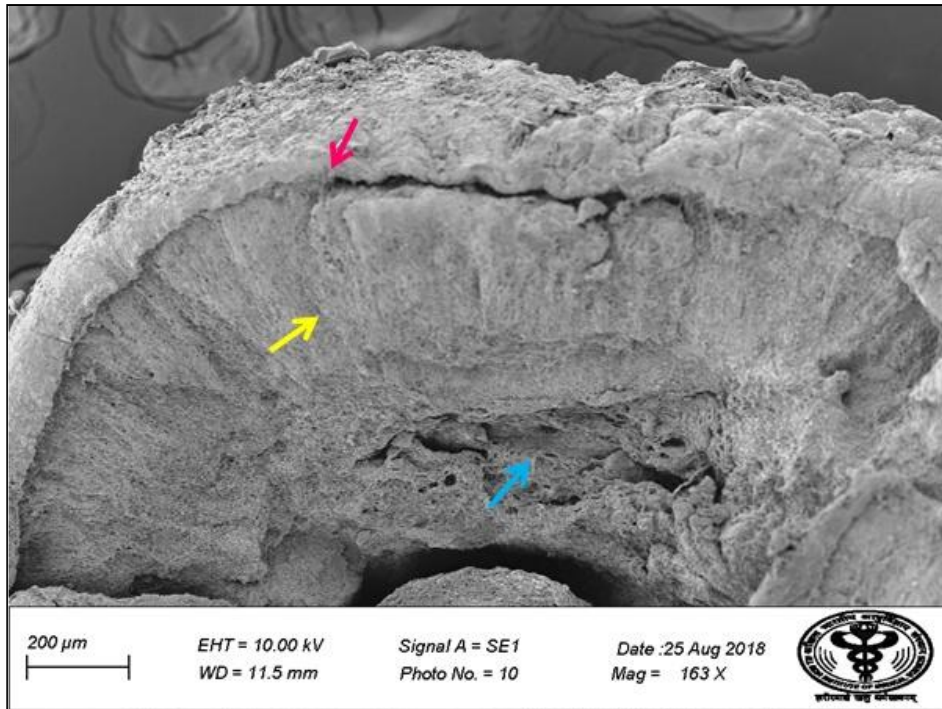


Fig. 13. Scanning electron micrograph of adrenal gland of rat treated with 300 mg/kg b.w./day of sodium fluoride for 40 days post-treated with 200 mg /kg b.w./day of Curcumin for 20 days showing capsule (↑), adrenal cortex (↑) and adrenal medulla (↑) similar to control. X163

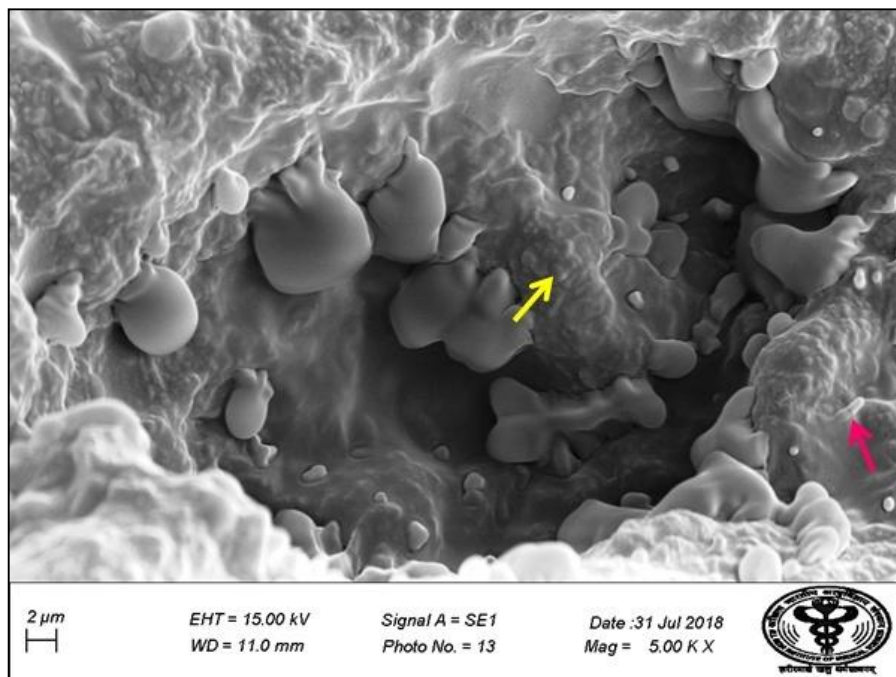


Fig. 14. Scanning electron micrograph of adrenal gland of rat treated with 300 mg/kg b.w./day of sodium fluoride for 40 days post-treated with 200 mg /kg b.w./day of Curcumin for 20 days showing restoration of cuboidal cells (↑) and less number of microvilli (↑). X2000

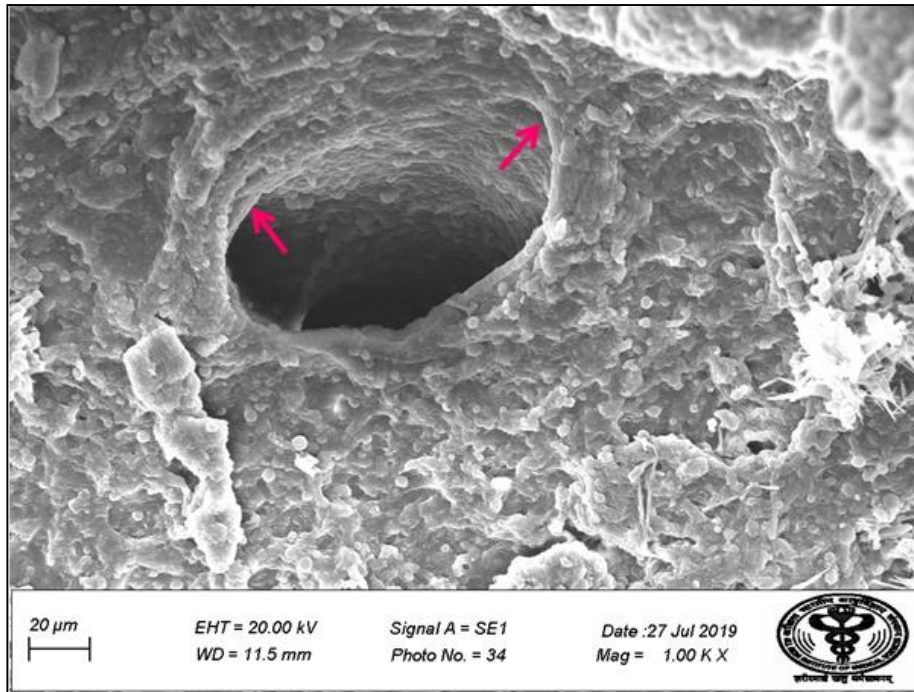


Fig. 15. Scanning electron micrograph of adrenal gland of rat treated with 600 mg/kg b.w./day of sodium fluoride for 40 days post-treated with 200 mg /kg b.w./day of Curcumin for 20 days showed walls of cavity having smooth outline (↑) and improved structure.X1000

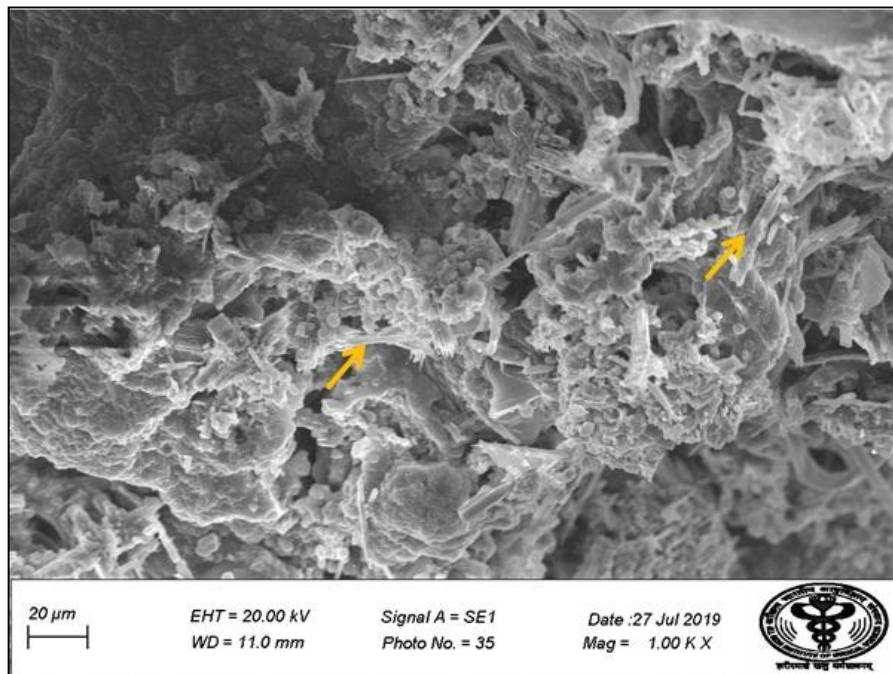


Fig. 16. Scanning electron micrograph of adrenal gland of rat treated with 600 mg/kg b.w./day of sodium fluoride for 40 days post-treated with 200 mg /kg b.w./day of Curcumin for 20 days showing less filopodia (↑).X1000

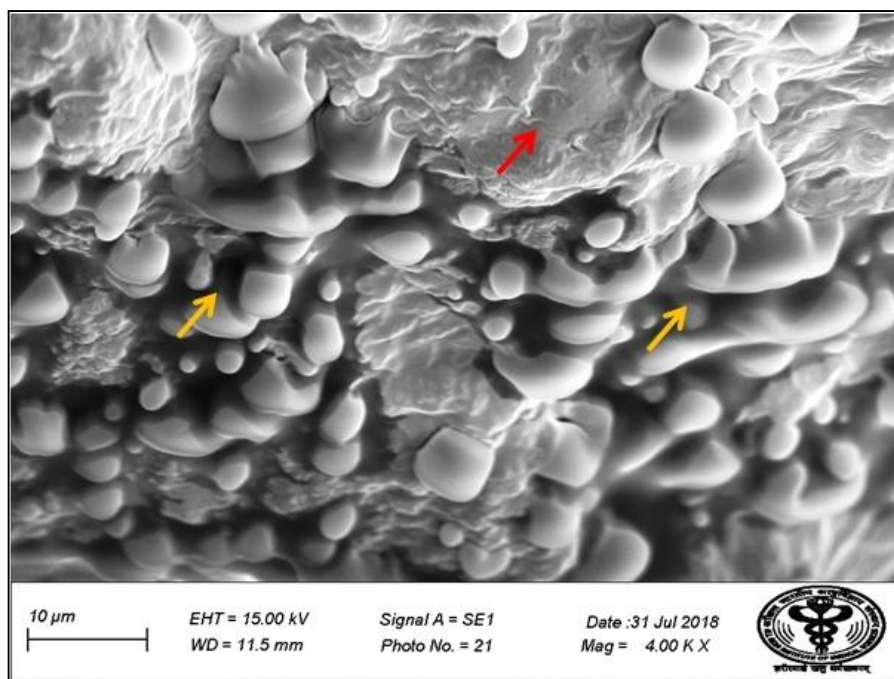


Fig. 17. Scanning electron micrograph of adrenal gland of rat treated with 600 mg/kg b.w./day of sodium fluoride for 40 days post-treated with 200 mg /kg b.w./day of Curcumin for 20 days showing reappearance of cuboidal cells (↑) and improved surface epithelium (↑). X1000

The clumping of the surface epithelium and distorted cuboidal cells were observed in the adrenal gland of fluoridated rats. Similar findings were reported by Kumar and Kumari [20] who evaluated abrasion and clumping of surface epithelium, absence of microvilli, blebs and distorted shape of cuboidal epithelium in NaF treated rats. These alterations may have been accumulated due to cells damage caused by induced toxicity.

The fluorotic rats showed bulging in the surface, numerous microvilli, many filopodia and network of collagen fibres, crystals deposition and wandering cell. Feinmesser *et al.* [21] also demonstrated that adrenal gland of rats with tumors showed a large number of microvilli pointing in different directions. The microvilli were thin and branched. Similar observation was recorded earlier in human normal and neoplastic adrenocortical cell. Adrenocorticotrophic hormone (ACTH) stimulated cells showed many microvilli on the surface [22]. Previously another study has also investigated that human cells stimulated with ACTH for 8 hours caused rounded stellate morphology and increase in microvilli [23]. The ACTH stimulated the rapid development of the microvilli and suggested to be a specific hormone mediated response [24].

Previously, it has been documented that adrenocortical cells observed under SEM showed three dimensional images outlining bulging surface along with numerous long microvilli on the cell membrane [25]. The prominence of microvilli resulted in the increase of cell surface area which caused the absorption of hormone precursors and elevation in the release of glucocorticoid hormone. This might be explained as why hyperplastic adrenocortical cells showed an increased response to ACTH as compared with normal cells. Tiny pits present on the cell membrane proposed active endocytosis of steroid hormone precursor [26]. Clusters of presumptive fenestrae with large transendothelial openings were observed on the luminal surface of adrenocortical endothelial cells [27].

Matsuo and Tsuchiyama [28] demonstrated that in cushing's adenoma, the collagen fibrils and fibrous substances were entangled with the parenchymal cells. The sinusoidal walls contained numerous fenestral pores and vesicles like processes. Similar findings were noticed by Nozaki *et al.* [29] who observed that numerous microvilli were entangled with collagen fibrils present on the parenchymal surface of the cells

in adrenal cortex of monkey. The wandering cells having pseudopodia like processes on the surface appeared monstrous in intracellular spaces. The wandering cells structure interpretation was coincide with that of macrophages in various organs [30]. Although, adrenal cortex have few number of macrophages, Motta *et al.* [31] in their SEM study observed cytoplasmic processes of the macrophages in the pores of endothelial lining in the pig. The SEM images of the phagocytosis of the cells were similar to kupffer cells [32].

Pudney *et al.* [33] reported that cells from ACTH treated animals showed extensive branched filopodia. The surface of the cells was covered by many filopodia. ACTH stimulated the intact perfused gland and expanded both the capillary and intercellular spaces within the cells of all zones which correlate with steroid secretion. The filopodia were developed by all cell types and occupied the intercellular and subendothelial space. Similar findings were reported by Surleef and Papadimitriou [34] who investigated that zymosan treatment showed variation in size and shape of the cells adhere to the sinus wall. Numerous folds, cytoplasmic lamellipodia, filopodial processes and cytoplasmic projections were observed prominently. Above mentioned studies indicate that when an external stimulus e.g. ACTH is administered in the animals, corresponding cell showed behavioural changes including altered morphology and impaired functioning.

The cell membrane has the capacity to form filopodia. The development of filopodia in Leydig cells of rat has been noticed to be associated with the degree of stimulation of cells [35]. The development of complex system of filopodia may clearly be a part of number of different processes. Aggregations of the crystals were reported in human suffering from the chronic pancreatic diseases [36]. Similar phenomenon of crystals aggregation in the present study under NaF toxicity was observed in the adrenal gland of fluorotic rats. A network of filaments with lipid droplets, collagen fibres, numerous spheroidal bodies, decellularized tissue and many secretory granules were observed. These findings coincided with a study by Almahbobi [37] who demonstrated that Field emission SEM revealed high resolution 3-D image of close contact of intermediate filaments with lipid droplets. The image cleared that intermediate filaments were interconnected with each other and showed a close association with lipid droplets. The binding

of intermediate filaments to lipid droplets, is the source of steroid substrate cholesterol. The lipid droplets normally store the excess amount of cellular cholesterol in case of basal steroidogenesis.

When ACTH stimulates the adrenal cells the steroid production is increased by using cholesterol reserve stored in lipid droplets. It has been investigated that intermediate filaments are involved in steroid hormone synthesis [38,39]; therefore, association between filaments and droplets might be involved in steroidogenesis. Kikuta *et al.* [40] demonstrated the three dimensional organization of the collagen fibrillar network using an alkali water method and scanning electron microscopy. The collagen fibril plexuses extending the adrenal capsule, cortex and medulla were observed. The collagen fibres were increased in skeletal muscle after fluoride induced toxicity [41]. Similar collagen fiber network formation was observed in the present study.

Motta *et al.* [31] evaluated the three dimensional organization of mammalian adrenal cortex. The concave facets of the cells showed long microvilli and few invaginations. The small spheroidal bodies of unknown nature were also observed. The rat medulla treated with osmium microradiation method and observed by high resolution SEM showed that the cells exhibited plentiful secretory granules [42].

Recently on study, Yeung *et al.* [43] performed a bibliometric analysis and observed that United State, China, India, Japan and South Korea contribute mainly to the scientific area on the bioactive effect of Curcumin, with more focus on the potential of anticancer, inflammatory and antioxidative properties as already reported by Xu *et al.* [44]. Administration of Curcumin after fluoride treatment showed normal structure of adrenal gland similar to control. Preservation of cuboidal cells and less number of microvilli were noticed. Similar observations were reported by recent study who observed restoration in ovarian surface epithelium, shape of cuboidal and squamous cells epithelium after Curcumin administration [45].

5. CONCLUSION

It was concluded that NaF induce alterations in the ultrastructure of adrenal gland. The scanning electron microscopic examination of adrenal gland treated with NaF showed clumping of the

surface epithelium, rough edges with surface covered with numerous pores, decellularized adrenal tissue and network of collagen fibres. The distorted cuboidal cells, numerous microvilli, numerous filopodia and deposition of the crystals on the surface were observed. However, post-treatment with Curcumin showed amelioration against fluoride induced toxicity in adrenal gland of rat and hence advocated Curcumin's curative ability.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

The experiments were performed under the approval of Institutional Animal Ethics Committee of Punjabi University, Patiala (Animal maintenance and Registration No.107/GO/ReBi/S/99/CPCSEA 2017-19).

ACKNOWLEDGEMENT

The financial assistance from CSIR-UGC joint test JRF fellowship under University Grant Commission fellowship schemes, Govt. of India is greatly acknowledged. The authors are thankful to all faculties and staff associated with SAIF facility at All India Institute of Medical Sciences, New Delhi, India where Scanning Electron Microscopy was performed.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Shashi A, Kumar M, Bhardwaj M. Incidence of skeletal deformities in endemic fluorosis. Trop Doct. 2008; 38(4):231-233.
2. Zuo H, Chen L., Kong M, Qui L, Lu P, Wu P, Yang Y, Chen K. Toxic effects of fluoride on organisms. Life Sciences. 2018;198: 18-24.
3. Shashi A, Bhardwaj M. Study on blood biochemical diagnostic indices for hepatic function biomarkers in endemic skeletal fluorosis. Biol Trace Elem Res. 2011; 143(2):803-14.
4. Shashi A, Kumar J. Neurotoxicity induced by fluoride in rat cerebral cortex. Int. J. Curr. Microbiol. app. Sci. 2016;5(10):938-951.
5. Shashi A, Kaur J. Protective and therapeutic effect of *Boerhaavia diffusa* L. on fluoride-induced ultrastructural changes in kidney of rats. International Journal of Current Medical and Applied Sciences. 2017;13(3):159-167.
6. A Shashi, N Sharma, M Bhardwaj. Pathological evaluation of pancreatic exocrine glands in experimental fluorosis. Asian Pacific Journal of Tropical Medicine. 2010;3(1):36-40.
7. Shashi A, Singla S. Parathyroid Function in Osteofluorosis. World Journal of Medical Sciences. 2013;8(1):67-73.
8. Shashi A, Kumar P. Ultrastructural changes in rat thyroid gland exposed to fluoride. Int. J. Curr. Microbiol. App. Sci. 2016;5(5):521-531.
9. Shashi, A. and Kaushal, P. 2020. Curative effects of Curcumin on gonadotropin and steroid hormones in female rats exposed to fluoride toxicity. British Journal of Medical and Health Sciences. 2020; 2(8):420-427.
10. Mitani F. Functional zonation of the rat adrenal cortex: the development and maintenance by Proc. Jpn. Acad., Ser. B 90 The Japan Academy. 2014;163-183.
11. Wornska D, Kania BF, Balchuta M. Direct effect of hypothalamic neuropeptides on the release of catecholamines by adrenal medulla in sheep - study ex vivo. Pol J Vet Sci. 2017;20(2):339-346.
12. Ranabir S, Reetu K. Stress and hormones. Indian J Endocr Metab. 2011;15(1):18-22 .
13. Joe B, Kumar, MV, Lokesh, BR. Biological properties of curcumin-cellular and molecular mechanisms of action. Crit. Rev. Food Sci. Nutr. 2004;44(2):97-111.
14. Waghmare P, Patingrao D, Kadu P. Extraction, isolation, purification and identification of curcumin: A review article. Eur. J. Biomed. Pharm. Sci. 2015;2(3):108-123.

15. Kulkarni SK, Dhir A, Akula KK. Potentials of curcumin as an antidepressant. *The Sci. world J.* 2009;9:1233-1241
16. Aggarwal R, Goel S, Behari J. Detoxification and antioxidant effects of curcumin in rats experimentally exposed to mercury. *J Appl Toxicol.* 2010;30(5):457-68.
17. Ezumi MFW, Amrah SS, Farid, CG, Mohsin SSJ. Morphological characteristics of the adrenal gland of *Rattus norvegicus* a revisit by scanning electron microscopy. *Annals of microscopy.* 2007;7:62-69.
18. Santos AC, Viana DC, Bertassoli BM, Vasconcelos BG, Oliveira DM, Rici REG, Oliveira MF, Miglino MA, Assis-Neto AC. 2015. Adrenal gland of Spix's yellow toothed cavy (*Galea Spixi's*, Wagner, 1831): morphological and morphometric aspects. *Braz. J. Biol.* 2016;76(3):645-655.
19. Kemoklidze KG., Alexandrov YK, Tiumina NA, Pukhov DE. Scanning electron microscopy of the rat adrenal gland after surgical laser exposure. *Bulletin of Siberian Medicine.* 2016;15(2):46-50.
20. Kumar A, Kumari S. Effect of fluoride toxicity on the ultrastructural morphology of ovary of mice. *International Journal of Applied Research and Studies.* 2015;4(8): 1-8.
21. Feinmesser M, Asa SL, Kovacs K, Low MJ. Fine structure of adrenal cortex in rats harbouring a medullary thyroid carcinoma transfected with a corticotrophin releasing hormone cDNA expression vector. *Journal of Endocrinology.* 1992;135:271-277.
22. Matsuo K, Tsuchiyama H. Human normal and neoplastic adrenocortical cells in tissue culture observed by scanning electron microscopy. *Acta Pathol Jpn.* 1987;37(1):65-76.
23. Rainey WE, Peter J, Hornsby PJ, Shay JW. Morphological correlates of adrenocorticotropin stimulated steroidogenesis in cultured adrenocortical cells: differences between bovine and human adrenal cells. 1983;113(1):48-54.
24. Cuprak LT, Lamini CJ, Bayer RC. Scanning electron microscopy of induced cells: rounding of mouse adrenal cortex tumour cells in culture. *Tissue and cell.* 1977; 9(9):667-680.
25. Li KH, Asa SL, Kovacs K, Murray D, Singer W. The adrenal cortex in ectopic adrenocorticotrophic hormone syndrome: a morphological study with histology, transmission and Scanning electron microscopy, flow cytometry and image analysis. *Endocrinology.* 1990;1(3):183-191.
26. Kolanowski, T., Crabbe, J. Characteristics of the response of human adrenocortical cells to ACTH. *Mol Cell Endocrinol.* 1976; 5:255-267.
27. Apkarian RP. The fine structure of fenestrated adrenocortical capillaries revealed by in lens field-emission scanning electron microscopy and scanning transmission electron microscopy. *Scanning.* 1997;19:361-367.
28. Matsuo, K., Tsuchiya, H. Adrenocortical adenoma with Cushing's syndrome: scanning electron microscopic observations. *Acta Pathol. Jpn.* 1986; 36(4):543-549.
29. Nozaki F, Miyoshi M. Perisinusoidal cells in a three dimensional organization of adrenal cortex in the monkey. *Arch. Histol, Jap.* 1984;47(4):345-357.
30. Miyoshi M, Fujita T. Stereofine structure of the splenic red pulp. A combined scanning and transmission electron microscope study on dog and rat spleen. *Arch. Histol. Jap.* 1971;33:225-246.
31. Motta P, Muto M., Fujita T. Three dimensional organization of mammalian adrenal cortex. *Cell Tissue Res.* 1979; 196:23-38.
32. Mutho-Kaas AC, Kaplan G, Seljelid R. On the mechanism of internalization of opsonized particles by rat Kuffer cells in vitro. *Exp. Cell Res.* 1976;103:201-212.
33. Pudney J, Sweet PR, Vinson GP, Whitehouse BJ. Morphological correlates of hormone secretion in the rat adrenal cortex and role of filopodia. *The anatomical record.* 1981;201:537-551.
34. Surleef SV, Papadimitriou JM. The mononuclear phagocytes of the rat adrenal. *Am J. pathol.* 1981;104:258-271.
35. Aoki A, Massa EM. Early response of testicular cells to stimulation by interstitial-cell stimulating hormone. *Am. J. Anat.* 1972;134:239-262.
36. Pramanik S, Ghos S, Roy A, Mukherjee AK. Biomineralization in human pancreas: A combined infrared-spectroscopy, scanning electron microscopy, X-ray rietveld analysis and thermogravimetric study. *J. Mater Res.* 2016;31(3):328-336.
37. Alamhobi G. Adhesion of intermediate filaments and lipid droplets in adrenal cells studied by field emission scanning electron

- microscopy. Cells and Tissue Research. 1995;281:387-390.
38. Sarria AJ, Panini S, Evans RM. A functional role for vimentin intermediate filaments in the metabolism of lipoprotein derived cholesterol in human SW-13 cells. J. Biol. Chem. 1992;267:19455-19463.
 39. Shiver, TM, Sackett DL, Knipling L, Wolff J. Intermediate filaments and steroidogenesis in adrenal Y-1 cells; Acrylamide stimulation of steroid production. Endocrinology. 1992;131:201-207.
 40. Kikuta A, Obtani O, Murakami T. Three dimensional organization of the collagen fibrillar framework in the rat adrenal gland. Arch. Histol. Cytol. 1991;54(2):133-144.
 41. Shashi A, Rana N. Scanning electron microscopic examination of surface lesions in fluorosed muscle of rat. Int. J. Curr. Med. Sci. 2016;6(9):81-86.
 42. Crivellato E, Solinas P, Isola R, Ribatti D, Riva A. Suggestive evidence of vesicle mediated mode of cell granulation in chromaffin cells. A high resolution scanning electron microscopy investigation. J. Anat. 2010;216:518-524.
 43. Yeung AWK, Horbanczuk M, Tzvetkov NT, Mocan A, Carradori S, Maggi F, Marchewka J, Sut S, Dall'Acqua S, Gan RY, Tancheva LP, Polgar T, Berindan Neagoe I, Pirgozliev V, Smejkal K, Atanasov AG. Curcumin: Total-Scale Analysis of the Scientific Literature. Molecules. 2019;24:1393.
 44. Xu XY, Meng X, Li S, Gan RY, Li Y, Li, HB. Bioactivity, health benefits, and related molecular mechanisms of curcumin: Current progress, challenges, and perspectives. Nutrients. 2018;10:1553.
 45. Shashi A, Kaushal P. Ultrastructural evaluation of oocytes during fluorosis in rat ovarian follicles. Manga Scientia Advanced Biology and Pharmacy. 2020;01(01):1-10.

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