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.

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 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...
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 sympa-tho-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 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 tetraoxide 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. 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
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. Adrenocorticotropic 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 wanderings of intermediate filaments to lipid droplets, is the source of steroid substrate cholesterol. The lipid droplets normally store the excess amount of 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 Almahboobi [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 fibrillar 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.

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 interacellular 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.

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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 miceration 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).

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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