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Expert Opinion Article

A Scanning Electron Microscopy Study of The Renal Glomeruli and Tubules in Adult Albino Rats Following Oral Administration of Sodium Fluoride, And the Possible Protective and Curative Role of Plant Leaf Extract *Boerhaavia Diffusa* L.

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ABSTRACT
This study used microdissection methods, such as scanning electron microscopy, to clarify the morphological alterations in the kidneys of albino rats treated with sodium fluoride. Eight groups of six rats each were randomly selected from a total of forty-eight animals. Rats in Control I were given deionized water orally. For 40 days, rats in the first and second groups received daily doses of 300 and 600 mg NaF/kg bw, respectively. For 20 days, 500 mg/kg b.w./day of *Boerhaavia diffusa* L. leaf extract was given orally to Control II rats. After receiving a 20-day pretreatment of 500 mg/kg bw/day of *Boerhaavia diffusa* L. leaf extract, groups third and fourth were subjected to 40 days of exposure to 300 and 600 mg NaF/kg bw/ /day. Groups fifth and sixth were exposed firstly to 300 and 600 mg NaF/ kg bw /day and then post-treated with leaf extract of *Boerhaavia diffusa* L. for 20 days. Scanning electron microscopy was used to examine the morphological characteristics of the rat kidneys treated with sodium fluoride. The features included the shortening of the podocyte processes, cytoplasmic blebs, hypertrophy, atrophy, knob-like projections at the end of the podocytes, shrinkage of the cell body, extreme deprivation of erythrocytes, and collagen fibers. It might be said that sodium fluoride negatively impacted the kidney's morphological structure. The renal damage changes may be lessened by administering *Boerhaavia diffusa* L. leaf extract both before and after treatment.

KEYWORDS: Albino rats, *Boerhaavia diffusa* L., Scanning electron microscopy, Sodium fluoride.

Introduction

Fluoride is found in many forms in nature, and too much of it can cause fluorosis, which is characterized by mottling of the teeth and skeletal symptoms such as osteoporosis, osteosclerosis, and deformities that are incapacitating (Wang *et al*., 2020 and Kaur ,2022). The kidneys are particularly vulnerable to the toxicity of fluoride because they are the main organ involved in the excretion and retention of fluoride (Alhusaini *et al*., 2018). Fluoride has been related to kidney structural damage and malfunction, which leads to nephrotoxicity (Tian *et al*., 2020).

Numerous structural and functional alterations have been observed in the kidneys of animals given higher fluoride dosages under various circumstances (Forbes and Thorburn, 2018). The synthesis of energy by the mitochondria is closely linked to kidney function, and both diabetic and chronic kidney disease are linked to mitochondrial malfunction. Strong mitochondrial toxicity is caused by fluoride (Tan *et al*., 2018).Numerous studies have demonstrated that consuming too much fluoride can harm the mitochondria, which releases cytochrome c and activates the caspase cascade, ultimately resulting in apoptosis (Babaei *et al*., 2017). Various investigations have demonstrated that because the kidneys play a crucial role in the body's clearance of fluoride, increased fluoride concentrations can occur there. Experimental animals with fluoride nephrotoxicity have pathological alterations in their proximal, distal, and collecting tubules as well as in their glomeruli (Pillai *et al*., 2017).

Boerhaavia diffusa L. is a common weed all throughout India. It is a perennial herb that spreads and creeps, with numerous upright branches and a strong root. In Ayurveda, the plant is popularly referred to as *Punarnava* because, according to the belief, its top dies during hot summers and regrows new branches following rains (Chaudhary and Dantu, 2011). *Boerhaavia diffusa* L. is thought to be therapeutically effective for the treatment of inflammatory renal illnesses as well as frequent clinical issues such as nephrotic syndrome, oedema, and ascites because of its combination of diuretic, antioxidant, and anti-inflammatory actions (Padmini and Kumar, 2013). *Boerhaavia diffusa* L. is so widely distributed, its application in reducing NaF toxicity

has become essential and might eventually be incorporated into routine human supplements.

MATERIAL AND METHODS

Young Wistar albino rats, weighing between 100-200g were housed in polypropylene cages with stainless grill tops and fed with commercial rat pellet diet (Hindustan Lever Limited, India) and water was provided *ad libitum*. Experimental protocols and procedures used in this study were approved by the Animal Ethical Committee of Punjabi University, Patiala (Animal Maintenance and Registration No. 107/99/ CPCSEA /2014-23).

EXPERIMENTAL STUDY

The animals were randomly divided into eight experimental groups with six animals in each group:

- Rats served as control 1 and were given 1 ml of deionized water per kilogram of body weight every day for 40 days.
- For 40 days, Group I and II received oral gavage treatments of 300 and 600 mg NaF/kg b.w./day.
- For 20 days, rats were fed 500 mg/kg b.w./day of *Boerhaavia diffusa* L. leaf extract (Control-II).
- Rats in Groups III and IV were given a single oral dosage of 500 mg/kg b.w./day of *Boerhaavia diffusa* L. leaf extract for 20 days, and then they were given 300 and 600 mg of NaF/kg b.w./day for 40 days.
- After 40 days of treatment with 300 and 600 mg NaF/kg b.w./day, rats in Groups V and VI received a 20-day post-treatment of 500 mg/kg b.w./day of *Boerhaavia diffusa* L. leaf extract. During the exposure period, animals were placed in metabolic cages, the rats were weighed, fasted overnight, and were excised under ether anesthesia. The kidney tissues were rapidly excised, weighed, and processed for histopathological examination.

PREPARATION OF *BOERHAAVIA DIFFUSA* L. PLANT EXTRACT

Fresh leaves of *Boerhaavia diffusa* L. were collected from Botanical Garden, Punjabi University, Patiala and got identified in Department of Botany, Punjabi University, Patiala. The plant leaf extract was prepared by the method of Narendhirakannan *et al*. (2006).The collected leaves were shade dried and ground to a coarse powder. The powder was extracted in 95% ethanol in a soxhlet apparatus at 60° C for 35 hours. After cooling and filtration, the filtrate was concentrated at 65° C in rota vapor to obtain dry powder.

PREPARATION FOR SCANNING ELECTRON MICROSCOPY:

For scanning electron microscopy viewing, the samples were fixed in 2.5% glutaraldehyde and 2 % paraformaldehyde, washed in 0.1 M sodium phosphate buffer (pH 7.3) for 12 hours at 4°C, postfixed in 1% osmium tetraoxide for 2 hours at 4°C. After few washes in 0.1 M phosphate buffer, the samples were dehydrated through graded acetone and dried by the critical point method (Hollenberg and Erickson, 1973). 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 15 kV. Images were digitally acquired by using a CCD camera attached to the microscope.

RESULTS AND DISCUSSION OF SCANNING ELECTRON MICROSCOPIC STUDY

The kidney of a control rat was examined under scanning electron microscopy, revealing renal convoluted tubules with many apical microvilli and an outer basement membrane. (Figure . 1).

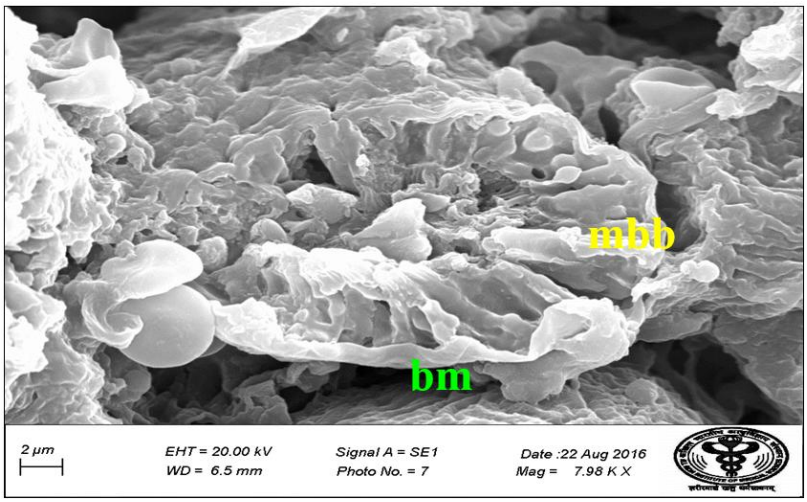

Figure.1 Scanning electron micrograph of renal tubule of the control rat showing tubular basement membrane (bm), and microvilli brush border (mbb). X 7980

The podocytes, possessing several cytoplasmic processes, protruded from the cell body in different directions. They produced a large number of primary processes, which produced secondary processes. (Figure .2).

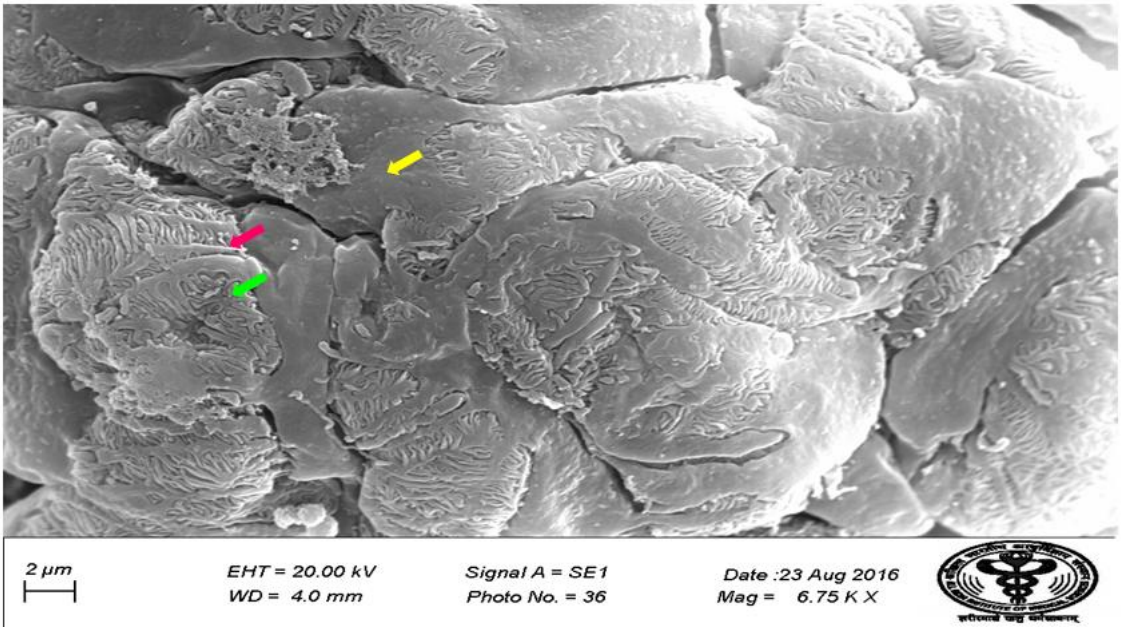

Figure 2: Scanning electron micrograph of glomeruli of control rat showing several cell bodies of the podocytes (↑) with their primary (↑), and secondary processes (↑). X 6750

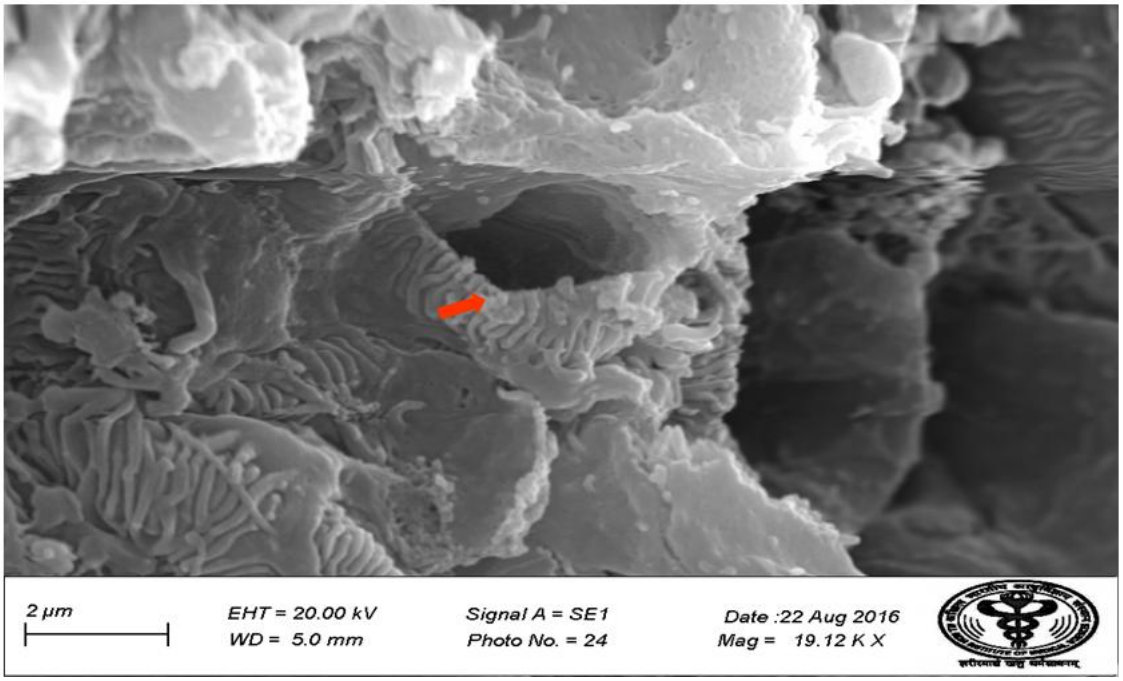

Figure 3: Scanning electron micrograph of a renal glomerular capillary of control rat showing interdigitating foot processes (↑) over outer capillary surfaces. X 19120

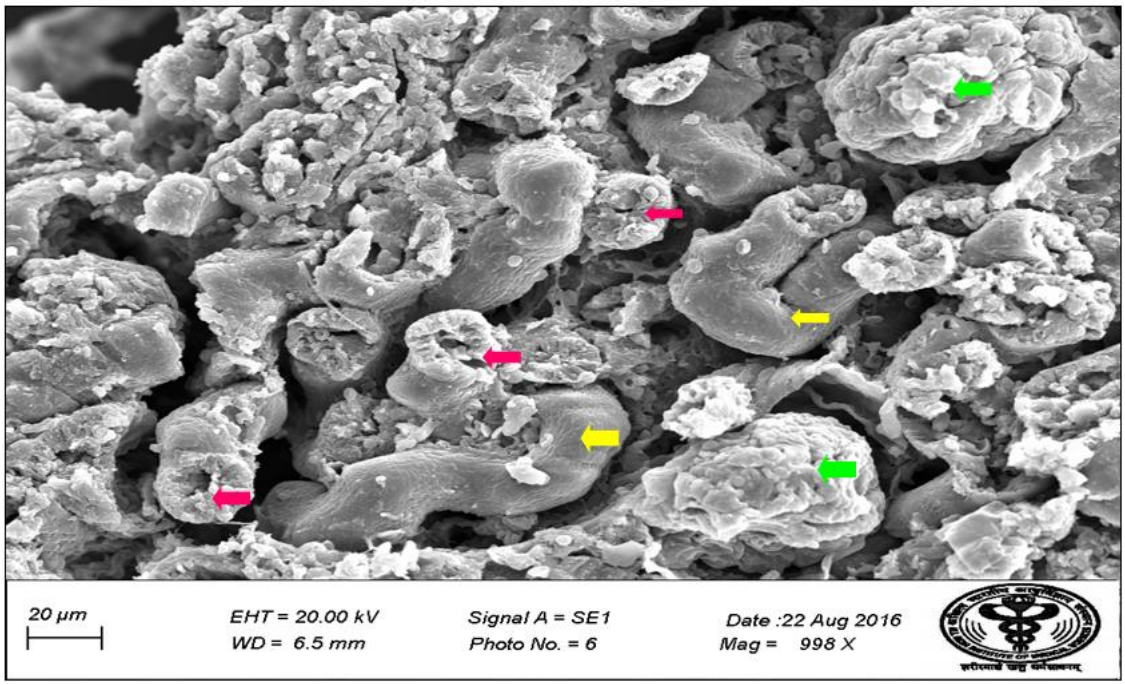

Figure 4: Scanning electron micrograph of the kidney of rat treated with 500 mg /kg b.w. /day leaf extract of *Boerhaavia diffusa* L. for 20 days showing normal architecture of renal tubule with brush border (↑), basal lamina (↑) and glomeruli (↑). X 998

The interdigitating foot processes covered the outer glomerular capillary surface (figure. 3)

The rats treated with 500 mg /kg b.w./day of leaf extract of *Boerhaavia diffusa* L. for 20 days, the renal convoluted tubule showed apical brush border, and outer basal lamina. The renal corpuscles were composed of glomerulus, Bowman's capsule, and Bowman's space (figure. 4).

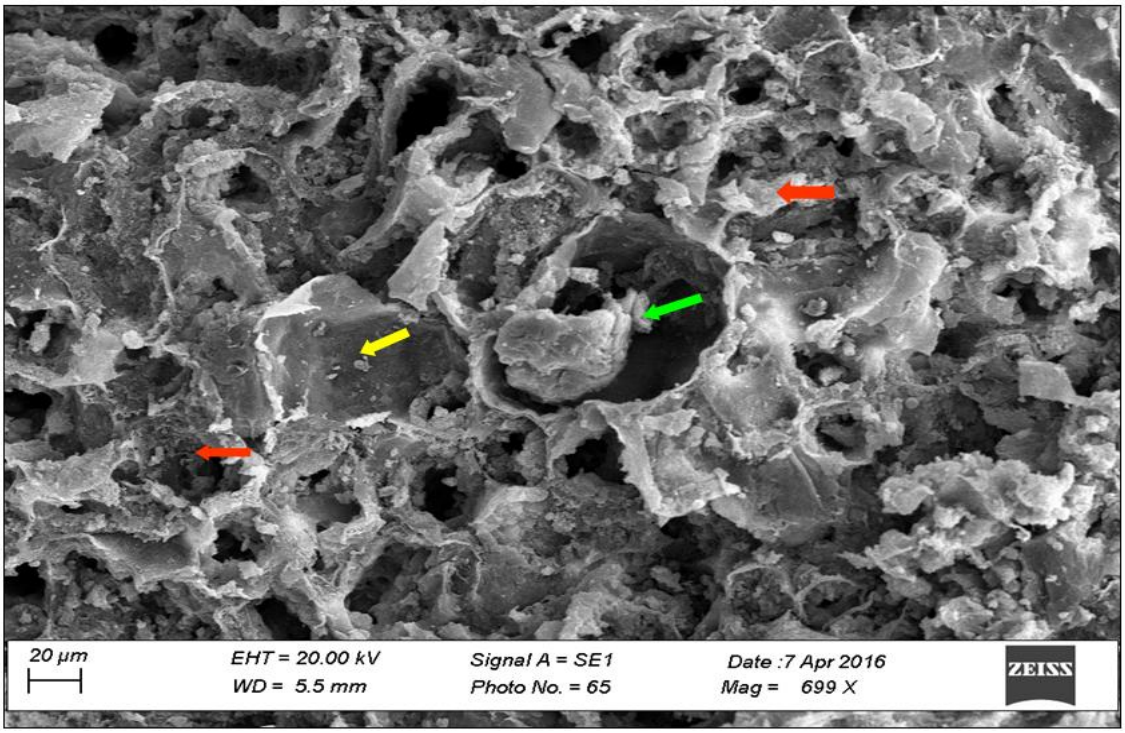

Figure 5: Scanning electron micrograph of the kidney of rat treated with 300 mg / kg b.w. /day of NaF for 40 days showing the renal tubules with disrupted architecture. The debris of the apical brush border (↑), and renal corpuscle with a degenerated (↑), and small atrophic glomerulus (↑) are visible. X 699

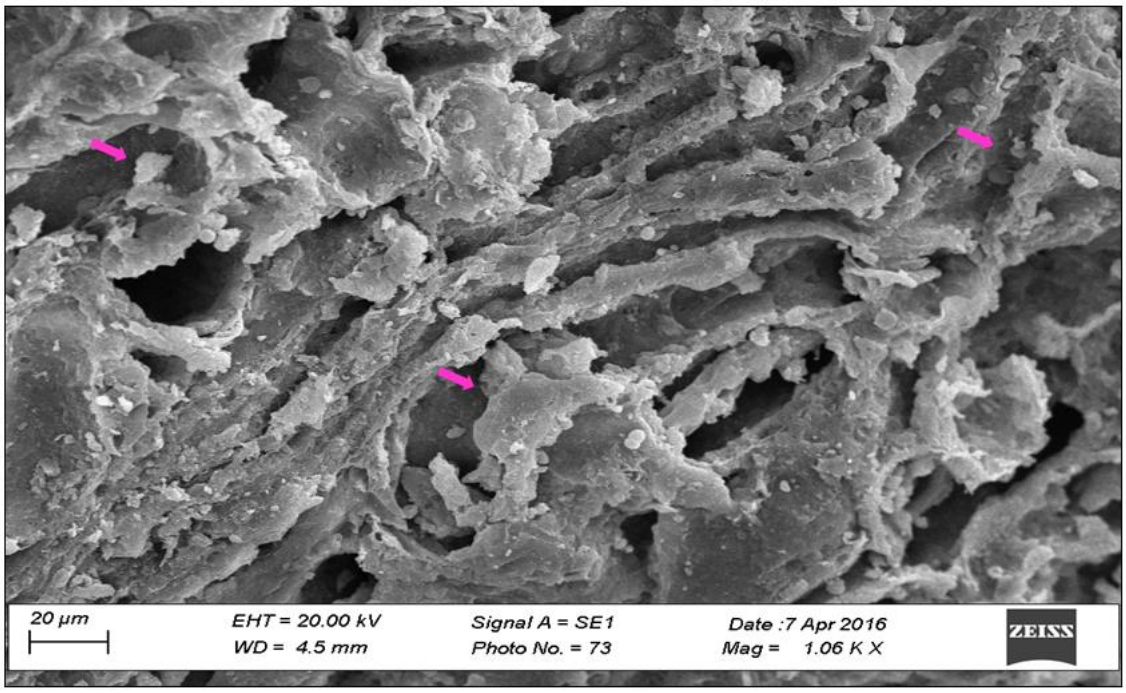

Figure 6: Scanning electron micrograph of the kidney of rat treated with 300 mg / kg b.w./day of NaF for 40 days showing the renal tubule with disrupted architecture and debris in the luminal cavity ( ). X 1060

The rats treated with 300 mg/ kg b.w. /day of NaF for 40 days showed the renal convoluted tubules with disrupted architectures.The renal corpuscle had atrophic glomerulus, their lumen contained debris and revealed considerable loss of brush border (figure. 5, 6).

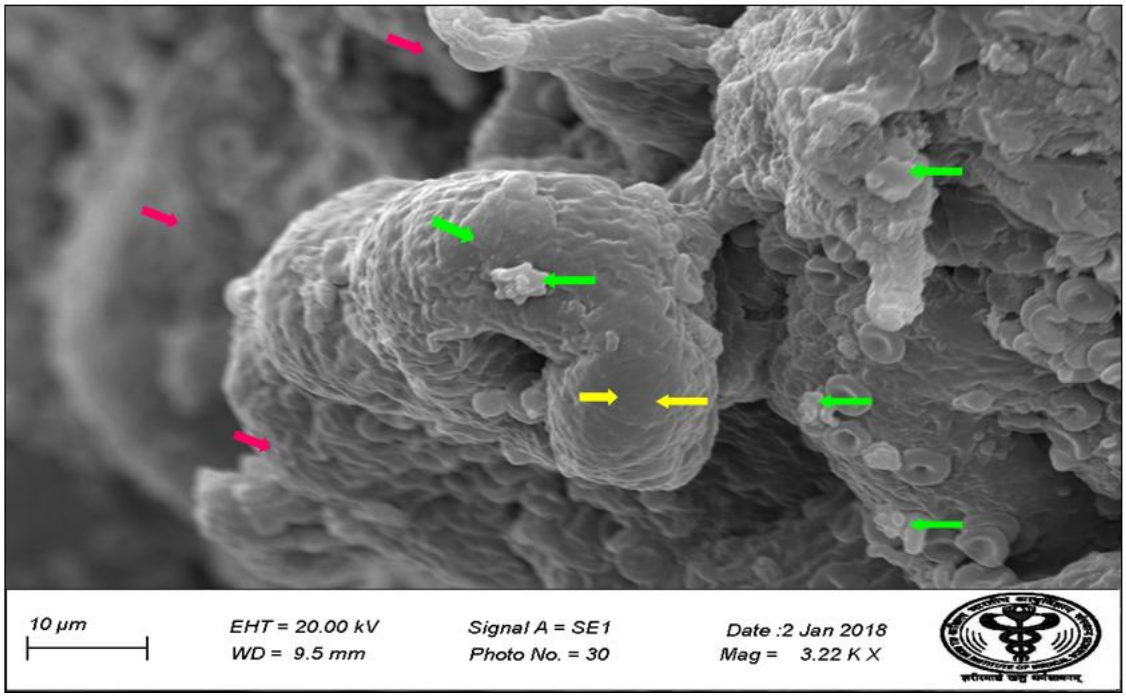

Figure 7: Scanning electron micrograph of the kidney of rat treated with 300 mg / kg b.w. / day of NaF for 40 days showing the glomerulus with distorted shape of erythrocytes ( ) and swelling or fused foot processes ( ). X 3220

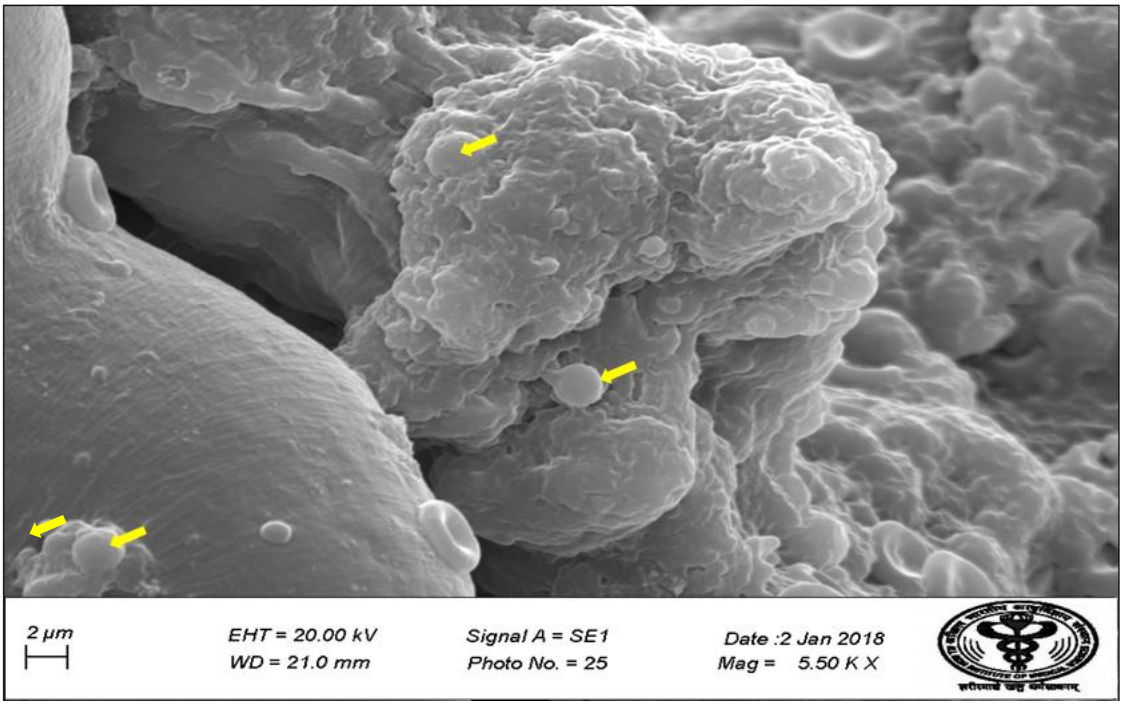

Figure 8: Scanning electron micrograph of the kidney of rat treated with 300 mg / kg b.w. /day of NaF for 40 days showing a glomerulus with cytoplasmic blebs ( ). X 5500

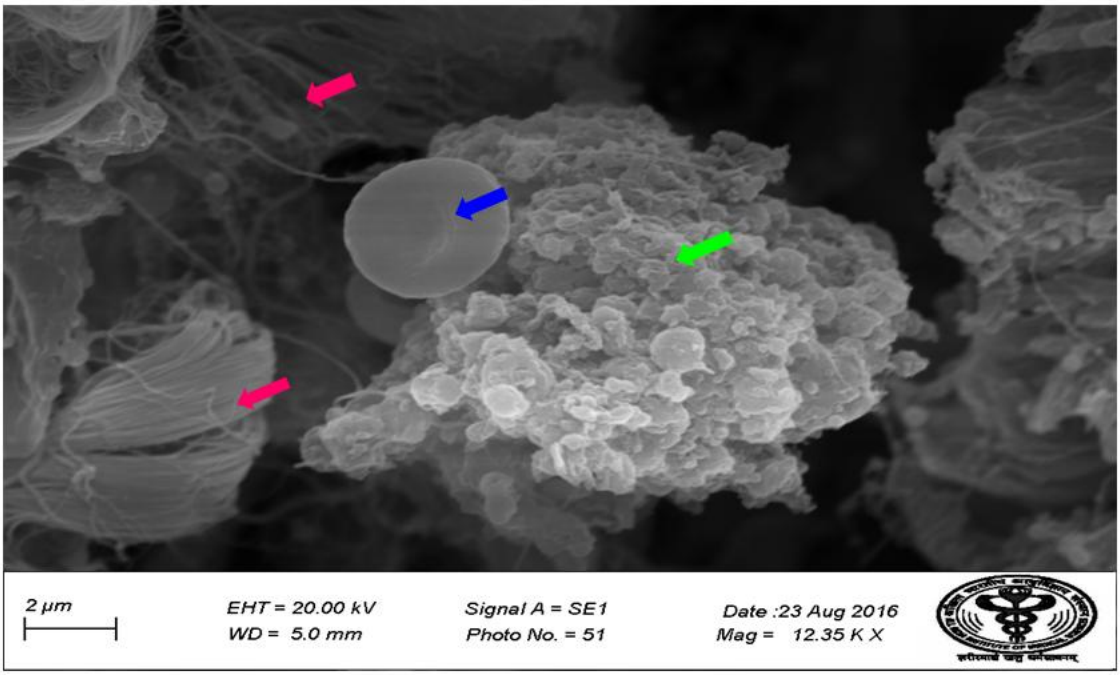

Figure 9: Scanning electron micrograph of the kidney of rat treated with 300 mg /kg b.w. /day of NaF for 40 days showing terminally sclerosed glomeruli ( ) with loss of foot processes, and abundant collagen fibres ( ). X 12350

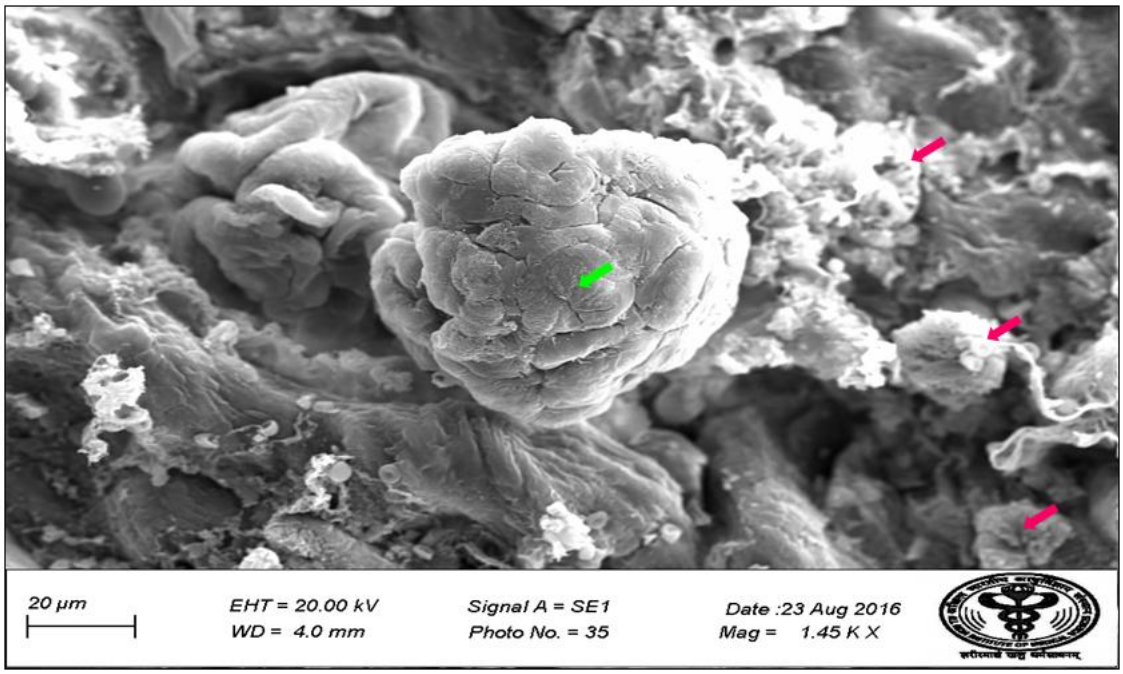

Figure 10: Scanning electron micrograph of the kidney of rat pre-treated with 500 mg / kg b.w./day leaf extract of *Boerhaavia diffusa* L. for 20 days before 300 mg / kg b.w./day of NaF for 40 days showing the improved structure of renal tubules with apical brush border (⬆) and preservation of glomerular tuft architecture (⬆) less capillary dilation. X 1450

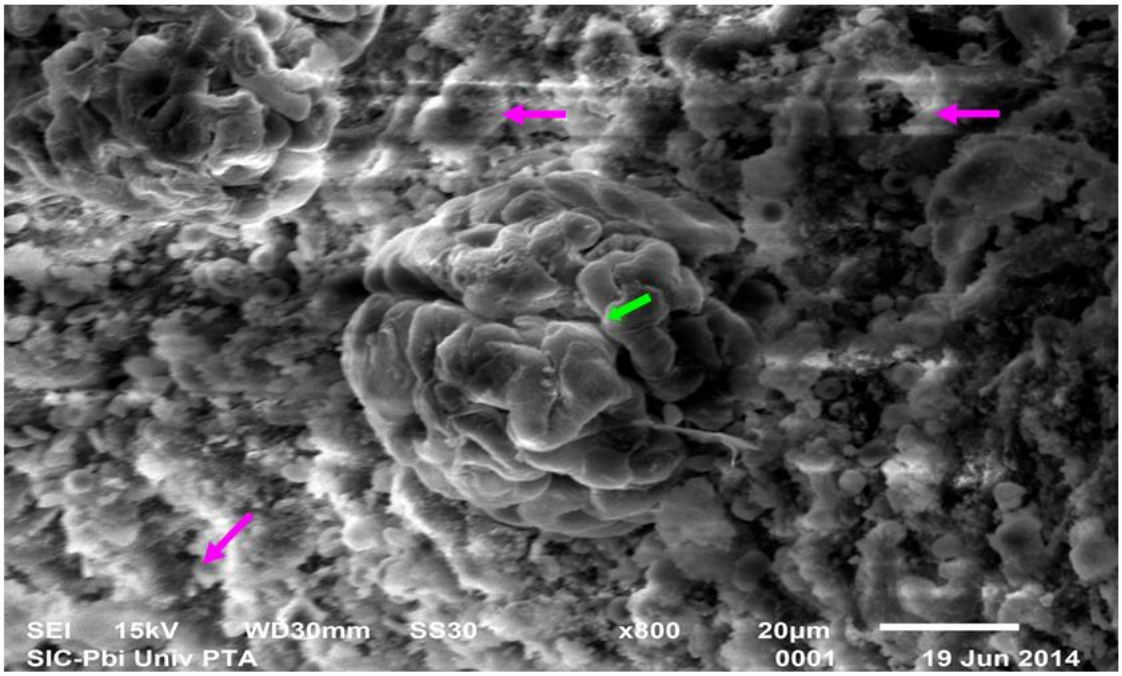

Figure 11: Scanning electron micrograph of the kidney of rat post-treated with 500 mg /kg b.w./day leaf extract of *Boerhaavia diffusa* L. for 20 days after 300 mg / kg b.w./day of NaF for 40 days showing preserved capillary loop of glomerulus (⬆) and some normal renal tubules (⬆). X 800

The majority of the podocyte cell bodies within the glomerulus experienced significant swelling, and the erythrocytes had a deformed shape that deviated from the typical biconcave discs under normal blood flow circumstances. Furthermore, a marked deficiency in erythrocyte counts was noted in the glomeruli (figure 7). There were cytoplasmic blebs in the glomerulus (figure. 8). Figure 9 displays collagen fibre deposition and a damaged capillary loop in the glomerular tuft.

The renal convoluted tubules in rats that were pre-treated with 500 mg/kg b.w. /day of *Boerhaavia*

diffusa L. leaf extract for 20 days showed retained normal appearance, followed by 40 days of 300 mg/kg b.w. /day NaF. The design of the glomerular tuft provides protection against capillary leakage, vasoconstriction, slightly increased capillary dilatation inside the glomerulus, and normal erythrocytes (figure. 10).

Following a 40-day exposure to 300 mg/kg b.w. /day NaF, the rats were given 500 mg/kg b.w. /day leaf extract of *Boerhaavia diffusa* L. for 20 days. This resulted in well-preserved glomerular capillaries loop with fewer surface lesions and preserved apical microvilli with normal renal tubule appearance (figure 11).

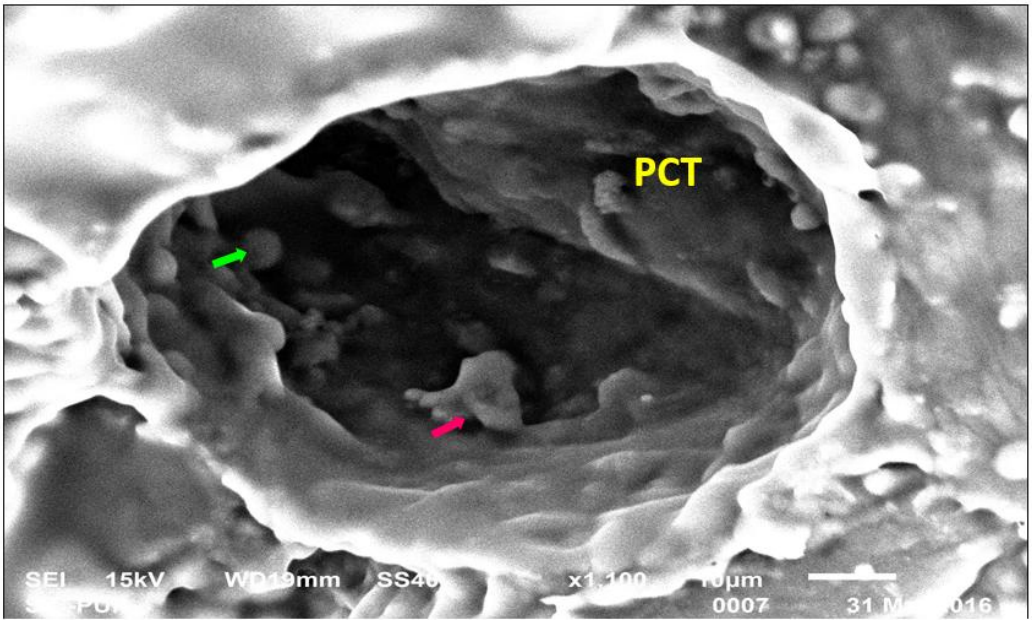

Figure 12: Scanning electron micrograph of the kidney of rat treated with 600 mg / kg b.w. / day of NaF for 40 days showing dilated proximal convoluted tubule (PCT) areas of hyperplasia (⬆) and cellular hypertrophy. The lumen contained distorted erythrocytes (⬆). X 1100

The renal tubules enlargement in rats given 600 mg/kg b.w./day of NaF showed signs of both cellular hypertrophy and hyperplasia (figure. 12). The renal tubule displayed luminal congestion, cytoplasmic bleb, vacuolization, sloughing off of the epithelium layer, and loss of the apical brush boundary (figure 13). The tubular blockage was symbolized by the formation of substantial crystal deposits, which were not seen in the control group (figure 14).

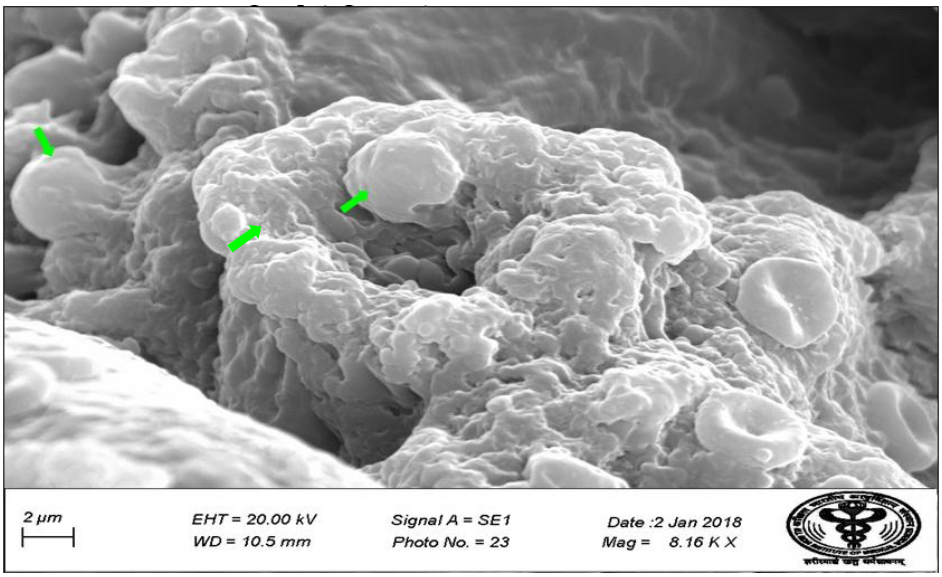

Figure 13: Scanning electron micrograph of the kidney of rat treated with 600 mg / kg b.w. /day of NaF for 40 days showing the renal tubules with rough surface areas and cytoplasmic blebs (⬆). X 8160

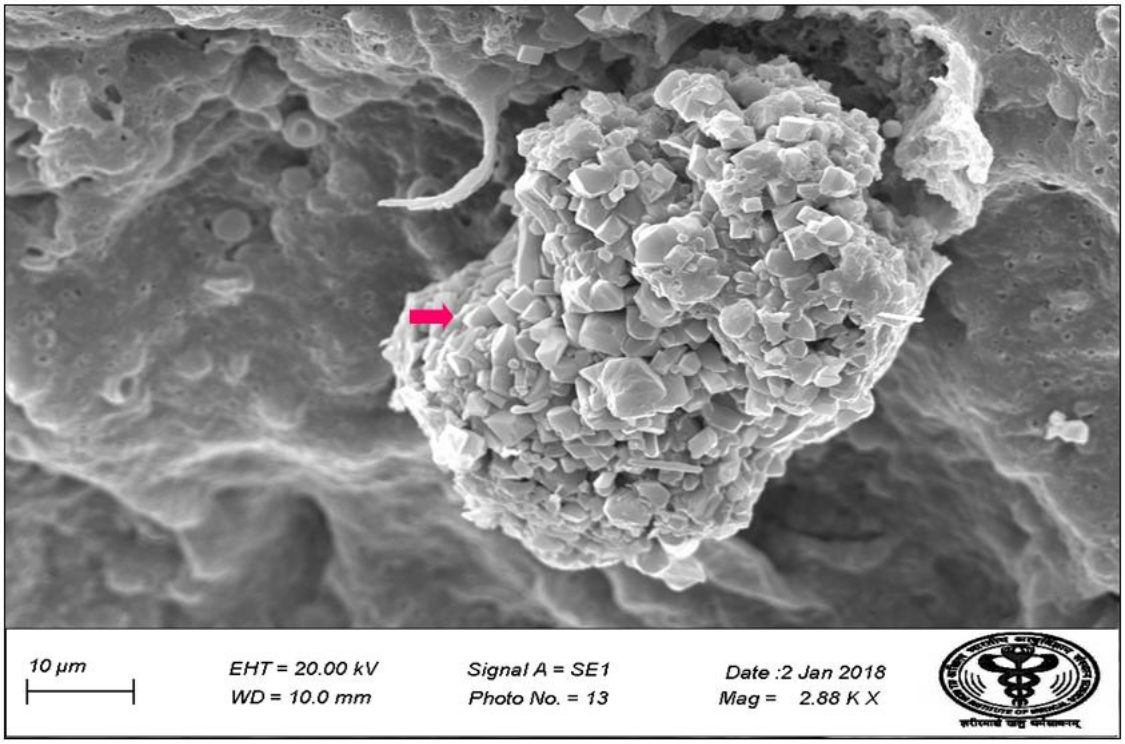

Figure 14: Scanning electron micrograph of the kidney of rat treated with 600 mg / kg b.w. /day of NaF for 40 days showing the large deposition of calcium oxalate crystals (⬆). X 2880

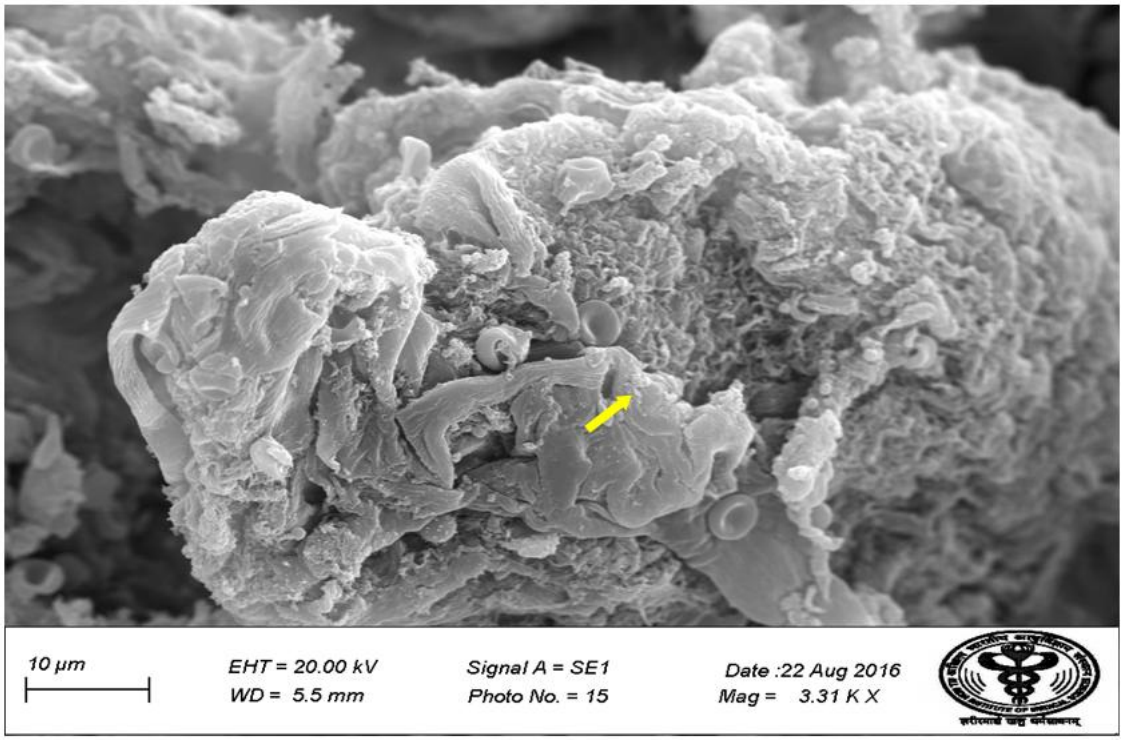

Figure 15: Scanning electron micrograph of the kidney of rat treated with 600 mg / kg b.w. /day of NaF for 40 days showing the segmental necrosis of glomerular tuft (⬆). X 3310

The glomerular basement membrane abruptly changed from its normal state to that of a necrotic lobule, where fibrin fibrils and the dissolving glomerular basement membrane were mixed together (figure. 15).

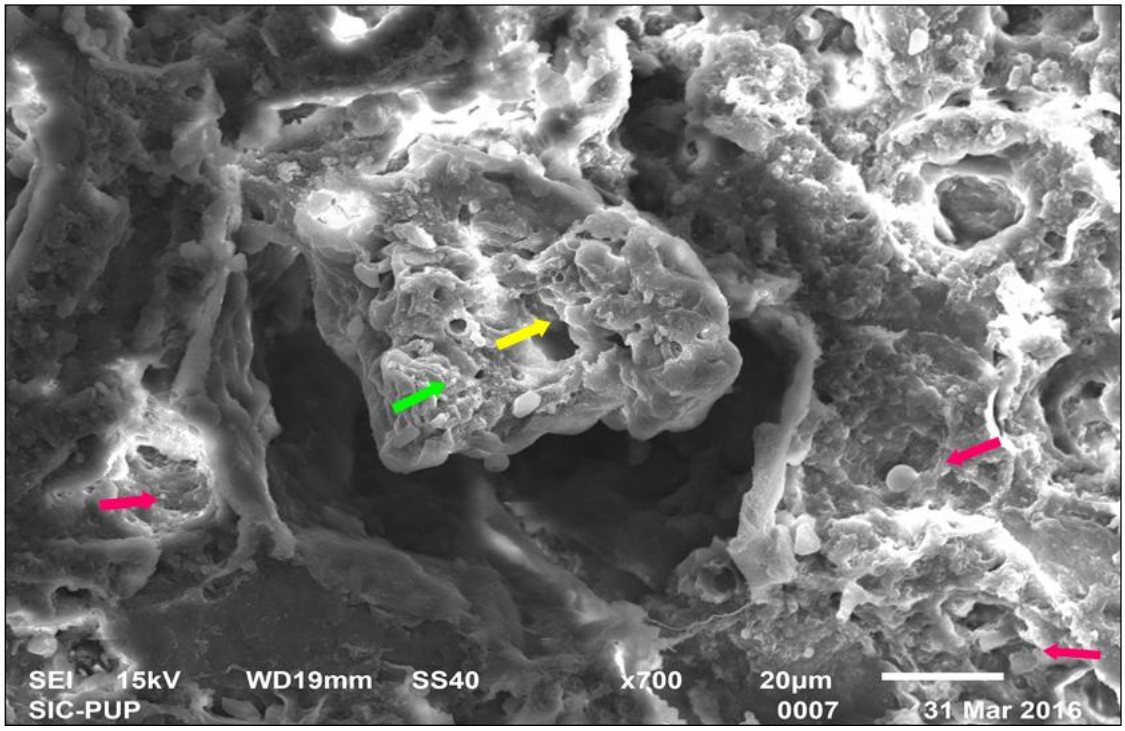

Figure 16: Scanning electron micrograph of the kidney of rat treated with 600 mg / kg b.w. / day of NaF for 40 days showing necrotic renal tubule (↑), and atrophic glomerulus (↑) with damage capillaries (↑). X 700

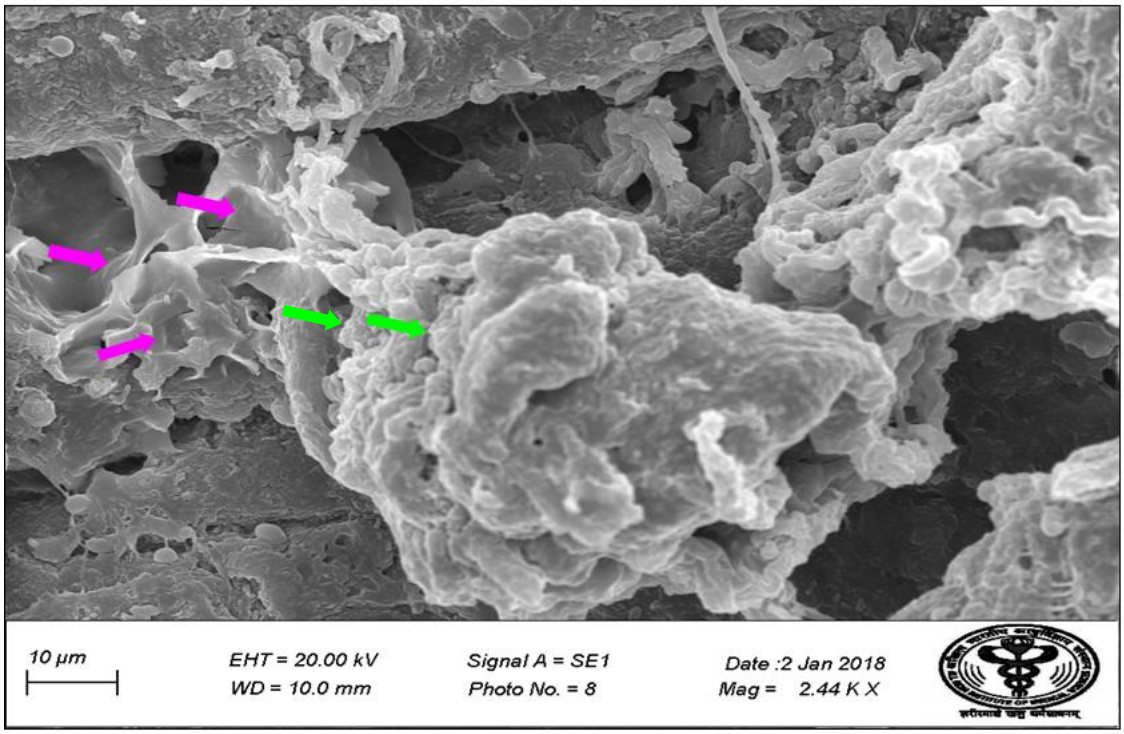

Figure 17: Scanning electron micrograph of the kidney of rat treated with 600 mg / kg b.w. / day of NaF for 40 days showing damaged glomerular tuft (↑) containing fibrocellular crescents (↑). X 2440

There were several noticeable features, including lesions in the glomerular podocytes, a shrinking glomerular tuft, structural cohesion loss, and tubular epithelial cell vacuolar degeneration (figure 16). Figure 17 shows the creation of fibrocellular crescents and vast masses of collagen fibrils with few cell lacunae.

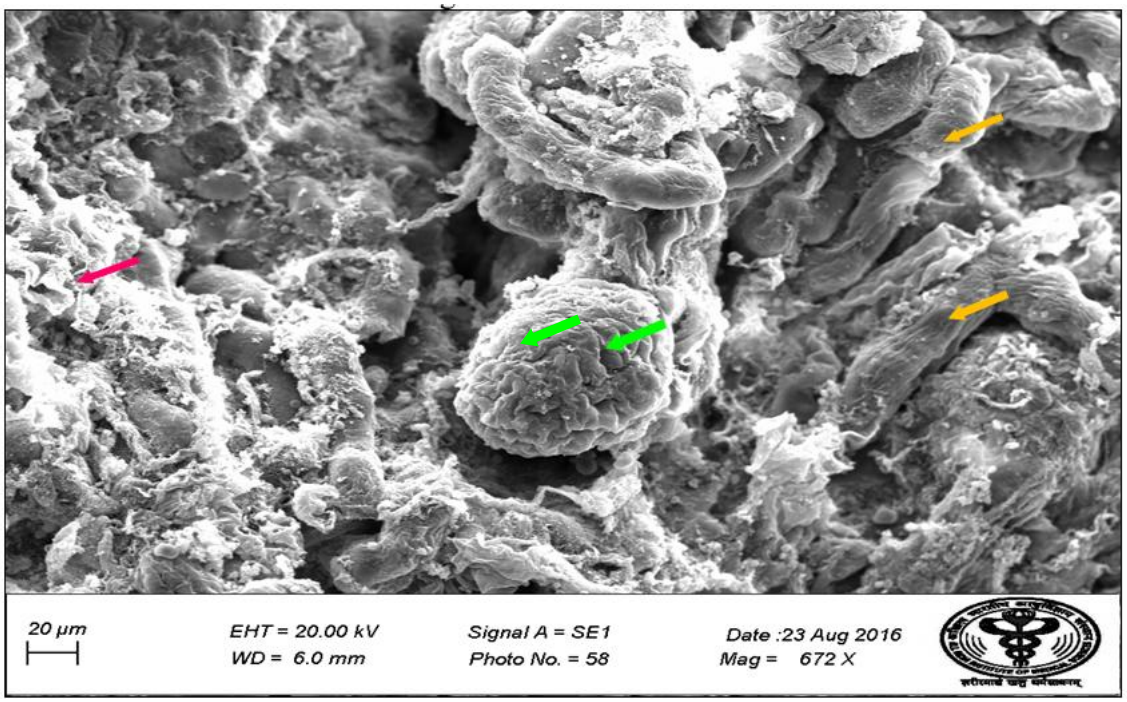

Figure.18: Scanning electron micrograph of kidney of rat pre-treated with 500 mg / kg b.w./ day leaf extract of *Boerhaavia diffusa* L. for 20 days before 600 mg / kg b.w./day of NaF for 40 days showing slight fibrin material (⬆), improved architecture of glomerulus tuft (⬆), and preservation of renal convoluted tubules with recovery of brush border and basal lamina without pores (⬆). X 672

The renal tubules with the apical brush border of the rats pre-treated with 500 mg/kg b.w. /day of Boerhaavia diffusa L. leaf extract for 20 days showed improvement, and the glomeruli recovered against swollen podocytes, dense fibrin material deposition, atrophy, and hypertrophy (figure. 18).

Along with a decrease in knob-like projection, there was an improvement in swelling podocytes and fused foot processes. Rats that were given 500 mg/kg b.w. /day of Boerhaavia diffusa L. leaf extract for 20 days after receiving 600 mg/kg b.w. /day of NaF for 40 days showed reduced disruption in the architecture of the renal tubule and a well-preserved glomerulus tuft (figure 19).

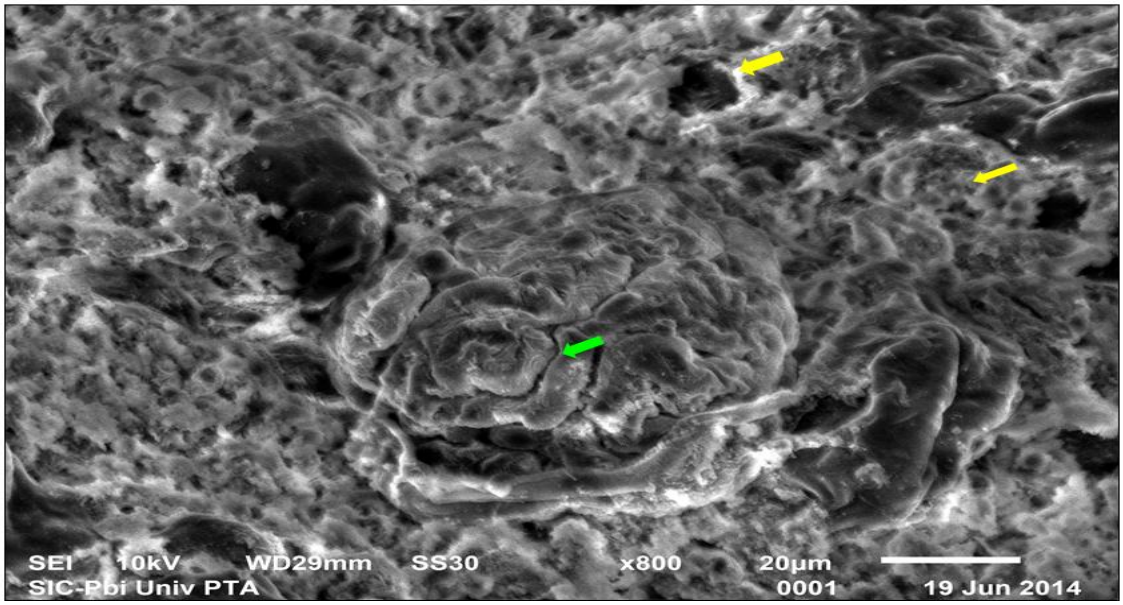

Figure.19: Scanning electron micrograph of kidney of rat post-treated with 500 mg / kg b.w./ day of leaf extract of *Boerhaavia diffusa* L. for 20 days after 600 mg/kg b.w./day of NaF for 40 days showing reduced swelling of glomerular tuft (⬆) and lumen of renal tubules lack debris (⬆). X 800

According to Davies and Azeez (2018), kidney damage, both acute and chronic, has been linked to fluoride exposure, commonly known as fluorosis, in both humans and experimental animals.

In the present study, scanning electron microscopy examination of the kidney of rats treated with sodium fluoride exhibited major renal tubular changes. The results are in accord with other investigators such as hyperplasia (Bernstein *et al*., 1987), number of pores (Blattmann *et al*., 2008), tubular injury and degeneration (Zaghloul, 2009), lumen contains cast and debris (Hafez, 2011), loss of apical brush border (Gupta *et al*., 2010), deposition of crystals (Mohamaden *et al*., 2013), cytoplasmic blebs, vacuolization, lumen congestion (Boa *et al*., 2015). Mohamed and Saleh, (2010) postulated that loss of microvilli reduced reabsorpitive surface due to damaged integrity of brush border membrane, might contribute to the reabsorpitive and secretory defects in these toxic states. These findings were in agreement with other investigator Khattab (1994) who postulated that damage to the kidney is both tubular and glomerular in nature. Also, it was found that the renal cortex was more affected than other parts of the kidney in organophosphorous toxicity as the cortex receives most of the total nutrient blood flow to the organ. Thus, when blood-born toxicants was delivered to the kidney, a high percentage of the material will reach the cortex.

Our study revealed cellular vacuolation in the renal convoluted tubules. Some investigators explained this finding as being most probably a cellular defence mechanism against injurious substances. These substances were segregated in the vacuoles and were thus preventing the interference with cellular metabolism (Mohamed and Saleh, 2010).

Mohamed and Saleh (2010) formulated that cells lining proximal convoluted tubules appeared to be the tissues in kidney most highly sensitive to toxic substances. It was noticed that proximal tubules are the first coming in contact with the toxic agent after it is filtered by the glomeruli.

Mohamaden *et al*. (2013) repeated that crystal deposition produce proximal tubular cell necrosis. The present fluorosis study, led to crystal deposition in tubular lumens. These crystals migrated to inter- and intracellular locations and eventually into the interstitium. Their movement into the interstitium was associated with inflammatory cell accumulation including lymphocytes and macrophages (Scheper *et al*., 2005). Interstitial inflammatory cell infiltrates around crystals may play an important role in renal tissue damage through the production of proteolytic enzymes, cytokines, and chemokines (Khan *et al*., 2002). Crystal retention and deposition was surrounded by tissue debris and fibrin. Crystal deposition and retention are not due to the lodgment of large crystals in tubular lumens, but rather as a result of debris adhesion to injured cells which stimulates additional crystallization (Menon and Resnic, 2002). This mechanism explains the relatively high expression of crystal binding proteins such as osteopontin, and hyaluronic acid (Asselman *et al*., 2003).

The present study of scanning electron microscopic study, revealed that exposure of rats to fluoride resulted glomerular alterations including cytoplasmic bleb (Hostetter *et al*., 2001), collagen fibrils, fibrocellular crescent (Makino and Ota, 1989), swelling in podocyte (Wagner *et al*., 2008), decrease in number of podocyte processes (Mohamed and Saleh, 2010), atrophy, enlargement of some glomeruli (Zaghloul *et al*., 2009; Hafez, 2011, Boa *et al*., 2015), tremendous deprivation of erythrocytes (Gupta *et al*., 2010), absence of podocyte covering (Hafez, 2011), foot processes replaced by broad cellular extensions (effacement) covering glomerular capillaries (Xu *et al*., 2015), and knob or bleb like microprojections (Tsuji *et al*., 2017).

The occurrence of fibrin/fibrinogen material is a result of filtration of various types of plasma proteins over the glomerular capillary wall, and precipitation of the fibrinogen. It is known that increased permeability to plasma proteins occurs after renal ischaemia (Racusen *et al*., 1982). Such leakage of proteins takes place despite a reduced glomerular filtration rate (Oken, I983; Enestorm *et al*., 1988).

Scanning electron microscopy revealed several patterns of disruptions which have been so difficult to envision by traditional morphological studies. The mildest form of injury appeared as segmental infrequent discrete perforations of the glomerular basement membrane lesions were similar to those observed by (Kondo *et al*. 1986; Castelino *et al*.2025).

CONCLUSION

In the present study, scanning electron microscopy was employed to evaluate the protective role of *Boerhaavia diffusa* L. leaf extract against fluoride-induced renal damage over a 20-day period. The ultrastructural analysis provided three-dimensional views, revealing marked glomerular and tubular injuries in fluoride-exposed rats. Interestingly, significant preservation of renal structures was

observed in the plant extract-treated group, highlighting the potential of this traditional medicinal plant for safeguarding kidney health in populations chronically exposed to fluoride contamination.

ACKNOWLEDGMENT

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