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Attenuation of fluoride-induced nephrotoxicity in rats by leaf extract of punarnava (*Boerhaavia diffusa* L.)

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Abstract:

The present study investigated the toxic effects evaluated as histopathological changes of sodium fluoride in kidney of rats. Wistar albino rats were divided into eight groups. Group I received 1ml deionized water /kg b.w. / day orally daily by a gastric tube for 40 days. The control and experimental animals were sacrificed and the kidney tissue. Group II were orally administered with 300 ppm of NaF for 40 days were, pre and post-treated with 500ppm /kg b.w. /day + 300 ppm NaF /kg /b.w /day of leaf extract of Boerhaavia diffusa L. for 20 days respectively. Group III were orally administered with 600 ppm of NaF for 40 days were, pre and post-treated with 500 ppm /kg b.w. /day + 600 ppm NaF /kg /b.w /day of leaf extract of Boerhaavia diffusa L. for 20 days respectively Group IV positive control were administrated with 500 ppm /kg b.w. / day of leaf extract of Boerhaavia diffusa L. for 20 days. processed for histopathological examination. Fluoride caused degenerative changes in the renal glomeruli and tubules.There was renal tubular necrosis, dilation of proximal and distal tubules, vacuoles formation, sloughing of the tubular epithelial cells, and cloudy swellings, and the glomerular degeneration along with alterations in glomerular size, proliferation of mesangial cells,

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marked lobulation, hypertrophy, atrophy or shrinkage with expanded Bowman's capsule were visible. These changes were reduced in the recovery group but not returned to normal. The findings implied that leaf extract of Boerhaavia diffusa L. can be used as an effective protecting agent against sodium fluoride induced nephrotoxicity.

Key words: Albino rats, *Boerhaavia diffusa* L., Nephrotoxicity, pathological changes, Sodium fluoride.

INTRODUCTION

Fluoride is an essential trace element from the halogen group that has protective effects against bone mineral loss. In the halides group of the periodic table, fluoride has great importance due to its smallest size and most electronegativity. Although the mechanisms of fluoride in biological forms are remains unclear but it has the unique chemical and biochemical properties for the size and reactivity. It is ubiquitously present in soil, water, plants and air. In the animal body, fluoride makes its presence through water and food [1].If fluoride is consumed in high quantities, it can cause severe damage to most tissues. Fluoride renal excretion is one of the most important mechanisms for the regulation of fluoride levels in the body. Approximately, 50% of the daily absorbed fluoride is excreted by the kidney. Fluoride is freely filtered through the glomerulus and undergoes a variable degree of proximal tubular reabsorption, directly related to glomerular ultrafiltrate pH. It has been reported that, the proximal tubule is more susceptible to damage than the glomerulus or any other tubular structure in rat models of acute fluoride nephrotoxicity [2].

Quite sensitive in their histopathological and functional responses to toxic amounts of fluoride, the kidneys are the primary organs concerned with excretion and retention of fluoride after chronic fluoride intoxication. Numerous

structural and functional changes have been noted in kidneys of animals receiving increased amounts of fluoride under different conditions. Many studies have shown that elevated concentrations of fluoride can occur in the kidney as it has a major route in removal of fluoride from the body [3,4,5]. Fluoride nephrotoxicity causes pathological changes in the glomeruli and in the proximal, distal, and collecting tubules of experimental animals [6,7].

Boerhaavia diffusa L. is herbaceous plant of the family Nyctaginacea. It is claimed to be rejuvenative to the urinary system [8] and improves the function of impaired kidneys, and expel the excess fluid out of the body very effectively [9].The present study assessed the possible protective effect of *Boerhaavia diffusa* L. against sodium fluoride-induced nephrotoxicity in rats.

MATERIAL AND METHODS:

Animals: Young Wistar albino rats, weighing between 100-200gm were housed in polypropylene cages with stainless grill tops and fed with standard rat pellet diet (Hindustan lever limited, India) and water was given *ad libitum*. Animals were maintained at a constant room temperature of 20-22°C and 60% humidity. The experiment were performed under the approval of the animal ethical committee of Punjabi University, Patiala (Animal Maintenance and Registration No. 107/99/ CPCSEA /2014-23).

Experimental Design

Rats were allowed a 2-week acclimatization period and then they were divided randomly into eight groups 6 rats each. Group I received 1 ml double distilled water/kg b.w./day orally daily by a gastric tube for 40 days. Group II were orally administered with 300 ppm of NaF for 40 days were, pre and post-treated with 500 ppm /kg b.w. /day + 300 ppm NaF /kg

/b.w /day of leaf extract of *Boerhaavia diffusa* L. for 20 days respectively. Group III were orally administered with 600 ppm of NaF for 40 days were, pre and post-treated with 500 ppm /kg b.w. /day + 600 ppm NaF /kg /b.w /day of leaf extract of *Boerhaavia diffusa* L. for 20 days respectively Group IV positive control were administrated with 500 ppm /kg b.w. / day of leaf extract of *Boerhaavia diffusa* L. for 20 days. The animals of all groups were sacrificed and the kidney tissue were processed for pathological examination. The leaf extract of *Boerhaavia diffusa* L. was prepared by the method of Narendrikhanan *et al*.,2006 [10].

HISTOPATHOLOGY

The kidney tissue was removed and fixed in Bouin's fluid for 24 hours, washed in 70% alcohol, dehydrated in graded series of alcohol, cleared in xylene and processed for paraffin embedding and 5-7µm thin sections were stained with haematoxylin and eosin. Histopathological changes were studied under light microscope at 400 and 1000 magnification.

OBSERVATION

Histology of normal kidney

Group 1 (control)

In the kidney of control rat, renal cortical tissue showed proximal and distal convoluted tubules and numerous renal corpuscles. The renal corpuscles contained tufts of capillaries the glomeruli which were surrounded by the Bowman's capsules. The urinary spaces were present between the glomeruli and Bowman's capsules. The parietal and visceral layers of each Bowman's capsule were distinct. The parietal layers were lined by simple squamous epithelium. The glomeruli were in intimate contact with the visceral layer of Bowman's capsules which was composed of podocytes. The proximal convoluted tubules were lined with thick large cubical

epithelium with acidophilic cytoplasm. The distal convoluted tubules exhibited lower cubical epithelium and larger regular distinct lumens with less acidophilic cytoplasm. A normal architecture of kidney was observed in the positive controls treated with leaf extract of *Boerhaavia diffusa* L. in the concentration of 500 mg/kg b.w./day for 20 days **(Fig. 1,2).**

Group 2 (300 NaF mg/kg b.w./day for 40 days)

In rats treated with 300 mg/kg b.w./day NaF for 40 days, there was significant morpho pathological damage to the renal cortex. The glomeruli exhibited different forms of degeneration. Some glomeruli appeared markedly lobulated whereas others appeared shrunken with a moderately congested capillary loops and an expanded Bowman's capsule. Some renal convoluted tubules were manifested either damaged with sloughing off their tubular epithelial cells and exhibited abnormal marked dilatation of their lumens.**(Fig.3)**.The oedematous and vacuolar tissue, vacuolar dystrophy, tubular atrophy, presence of protein casts in lumen were also visible.**(Fig. 4).**The glomeruli revealed hypertrophic changes as compared to control.**(Fig.5)**.Extensive interstitial hemorrhage was well demonstrated in the peritubular and perivascular areas along with degenerated glomeruli.(**Fig. 6).**

Group 3 (Pre-treated BD500 for 20 days+ 300NaF mg/kg b.w./day for 40 days)

In rats pre-treated with leaf extract of *Boerhaavia diffusa* L.(500 mg/kg b.w./day)for 20 days followed by 300 mg/kg b.w./day NaF for 40 days, revealed the normal renal corpuscles with glomeruli.Less degenerated tubules were seen. **(Fig.7)**.

Group 4 (Post-treated BD500 for 20 days + 300NaF mg/kg b.w./day for 40 days)

In rats post-treated with leaf extract of *Boerhaavia diffusa* L.500 mg/kg b.w./day for 20 days after 300 mg/kg b.w./day NaF

for 40 days, showed that the Bowman's capsule contained normal glomerulus and most of the renal tubules approximately returned to their normal appearance with mild atrophy.**(Fig. 8)**.

Group 5 (600NaF mg/kg b.w./day for 40 days)

In rats treated with 600 mg/kg b.w./day NaF for 40 days there was dissolution of renal cortex. The tubular epithelia of proximal convoluted tubules showed vacuolar degeneration of their cytoplasm giving them cloudy swelling appearance and markedly dilated lumen.**(Fig.9)**.Some tubular epithelial cells exhibited cell swelling with lysis of their cytoplasm, vacuolation and organelles. The renal tubules were dilated and atrophied.**(Fig.10)**. The shape of proximal and distal convoluted tubules was distorted**.(Fig. 11).**

Group 6 (Pre-treated BD500 for 20 days + 600NaF mg/kg b.w./dayfor 40 days)

In rats pre-treated with leaf extract of *Boerhaavia diffusa* L.500 mg/kg b.w./day for 20 days followed by 600 mg/kg b.w./day NaF for 40 days, the degenerative changes in the glomeruli and tubules were improved as compared with group treated with 600 mg/kg b.w./day NaF for 40 days alone. The lumen in the most of proximal and distal convoluted tubules appeared normal.**(Fig. 12)**.

Group 8 (Post-treated BD500 for 20 days+ 600NaF mg/kg b.w./day for 40 days)

In rats post-treated with leaf extract of *Boerhaavia diffusa* L.500 mg/kg b.w./day for 20 days after 600 mg/kg b.w./day NaF for 40 days, the renal glomeruli and convoluted tubular cells both proximal and distal showed less degeneration and cytoplasmic vacuolation. The shape of collecting ducts and tubules was also normalized with intact epithelium.**(Fig. 13)**.

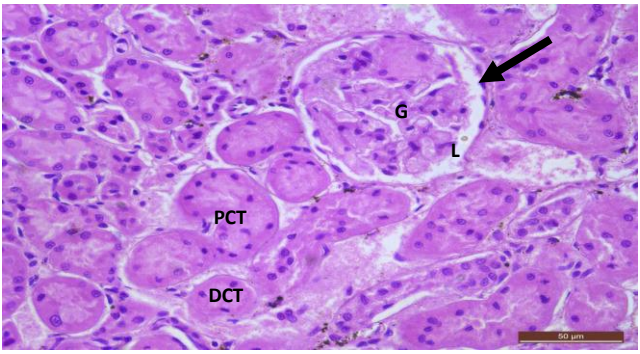

Fig 1. Photomicrograph showing the kidney of control rat with the proximal convoluted tubules (PCT) and distal convoluted tubules (DCT). The glomeruli (G) exhibit a tuft of glomerular capillaries surrounded by normal Bowman's capsule (↑). Bowman's capsule exhibits visceral and parietal layers with a lumen in between. H&E X 400.

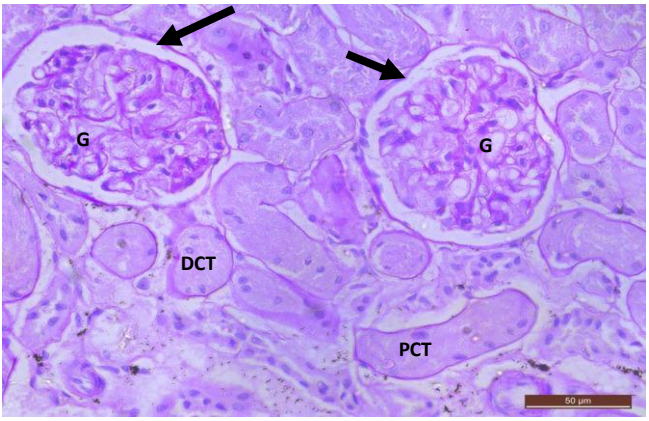

Fig 2. Photomicrograph showing the kidney of rat treated with 500 mg/kg b.w./day of leaf extract of *Boerhaavia diffusa* L.The contour of the proximal (PCT) and distal (DCT) tubules is intact and regular.The renal glomeruli (G) are seen with their tuft of capillaries and normal Bowman's capsule with their presence of urinary space (↑).H & E X400.

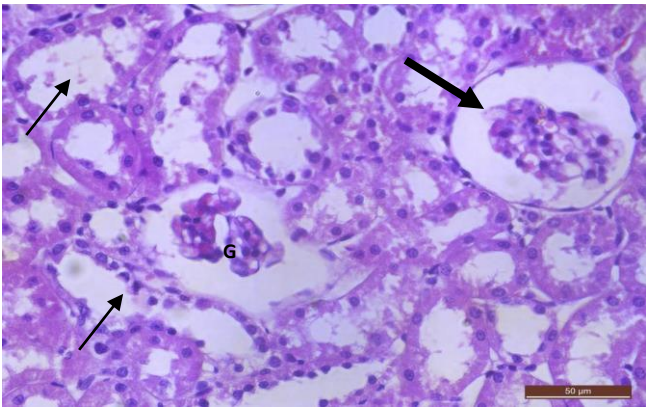

Fig 3. Photomicrograph of the kidney of rat treated with 300 mg/kg b.w./day of NaF showing renal tubules with marked dilated lumen (↑), denuded epithelium lining (↑), shrunken glomeruli (G) and expanded Bowman's capsule (↑), H & E X400.

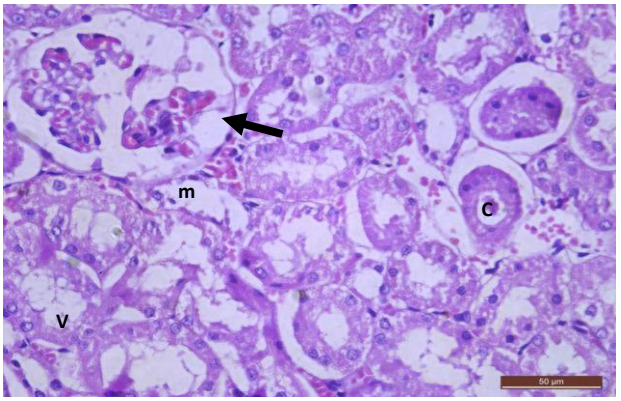

Fig 4. Photomicrograph of the kidney of rat treated with 300 mg/kg b.w./day of NaF with oedematous, vacuolated areas (↑), desquamated epithelial cells in the lumina of the tubules (↑) and intratubular casts (C).The nuclei with chromatin margination (m) are present. H & E X 400.

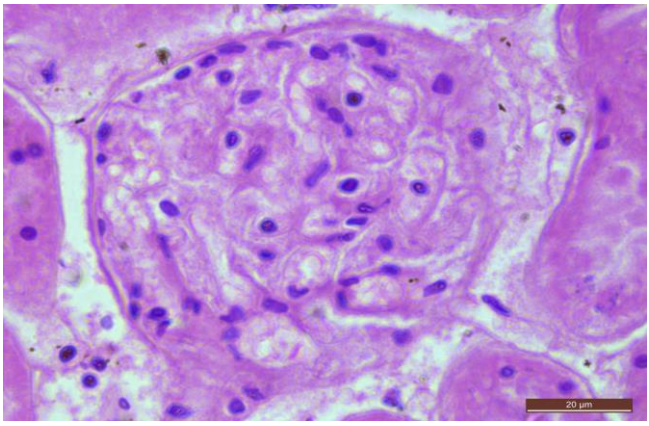

Fig 5. Photomicrograph of the kidney of rat treated with 300 mg/kg b.w./day of NaF showing a hypertrophied glomerulus. H & E X1000 photomicrograph under oil immersion lenses.

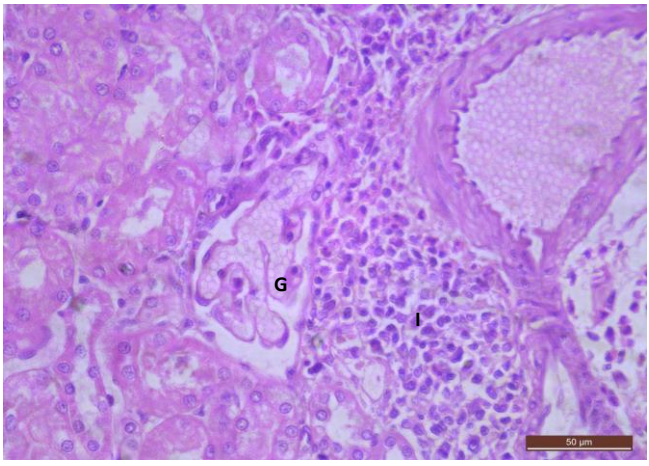

Fig 6. Photomicrograph of the kidney of rat treated with 300 mg/kg b.w./day of NaF showing inflammatory cells (I) infiltrated in the perivascular area, and showing degenerated glomeruli (G). H & E X400.

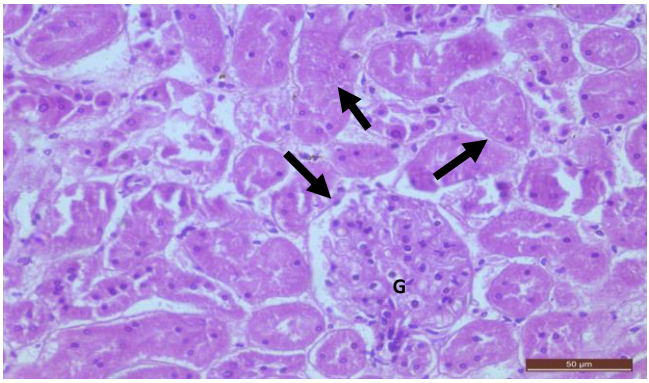

Fig 7. Photomicrograph of the kidney of rat pre-treated with leaf extract of *Boerhaavia diffusa* L.500mg/kg b.w./day for 20 days followed by 300 mg/kg b.w./day of NaF showing with normal glomeruli (G) somewhat intact capsular wall (↑), and reduced oedema and tubular atrophy. H&E X400.

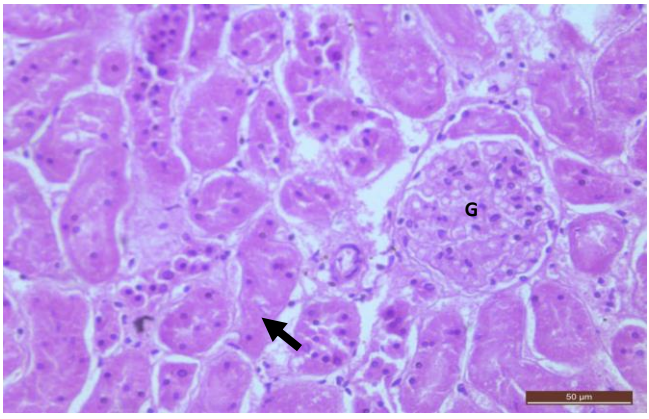

Photo 8. Photomicrograph of the kidney of rat post-treated with leaf extract of *Boerhaavia diffusa* L.500mg/kg b.w./day for 20 days after 300 mg/kg b.w./day of NaF showing normal renal glomeruli (G), and almost less degenerated proximal and distal convoluted tubules (↑).H&E X400.

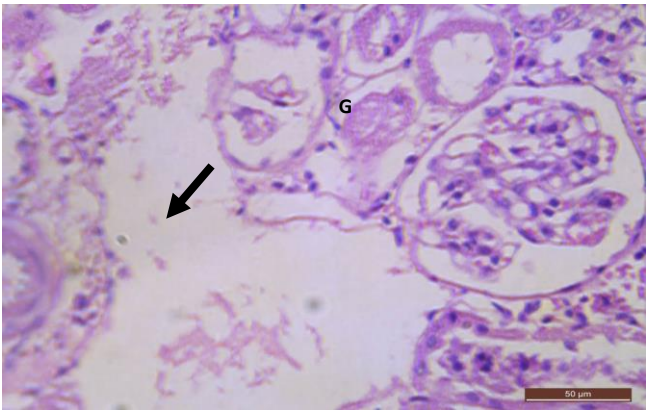

Fig 9. Photomicrograph of the kidney of rat treated with 600 mg/kg b.w./day of NaF showing with shrunken glomeruli (g), and vacuolar glomerular dissolution in cortex (↑) H&E X400.

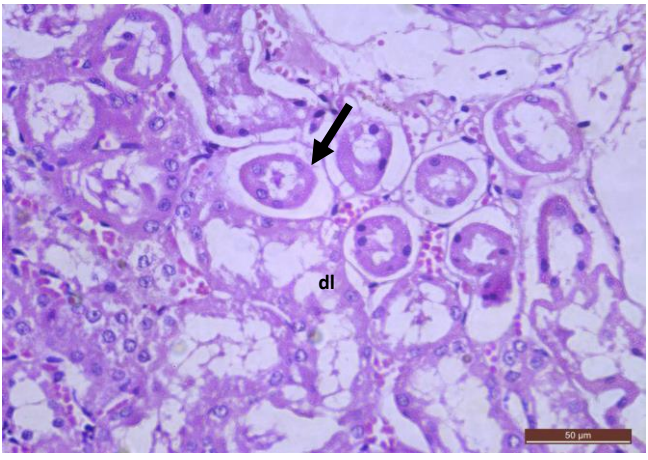

Fig 10. Photomicrograph of the kidney of rat treated with 600 mg/kg b.w./day of NaF with multifocal vacuolation of cytoplasm in a segment of proximal convoluted tubule and atrophy of some renal tubules (↑) H & E X400.

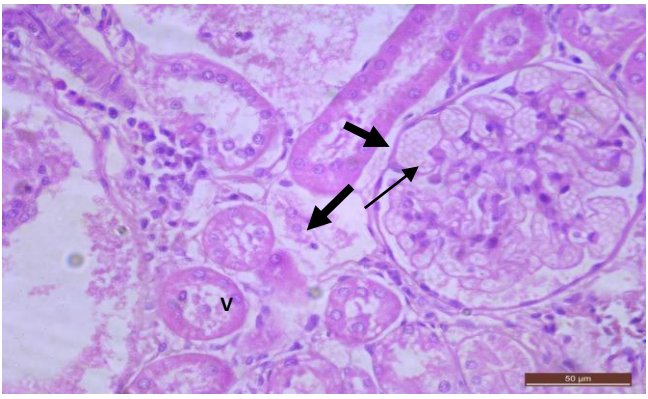

Fig 11. Photomicrograph of the kidney of rat treated with 600 mg/kg b.w./day of NaF with mesangial and endothelial capillary hypercellularity (↑) and thick basement membrane (↑),degenerated tubules (↑) and vacuolation of cytoplasm in tubules (V). H & E X400.

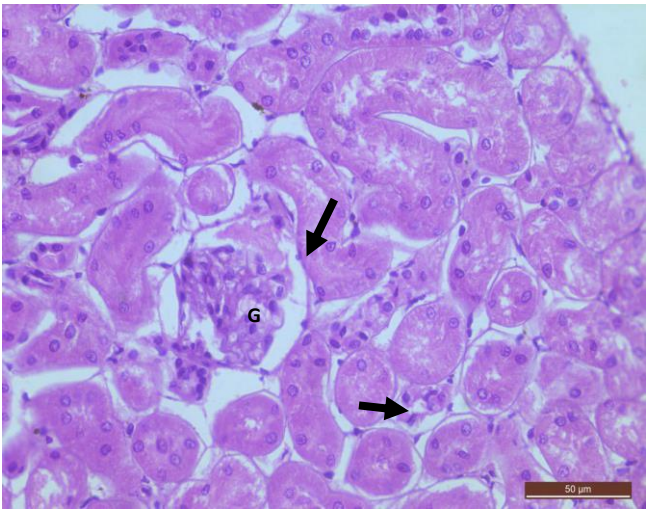

Fig 12. A photomicrograph of the kidney of rat pre-treated with leaf extract of *Boerhaavia diffusa* L.500mg/kg b.w./day for 20 days after 600 mg/kg b.w./day of NaF with deformed glomeruli (G),degenerated Bowmans capsule (↑) with few degenerated collecting tubules (↑). H&E X400.

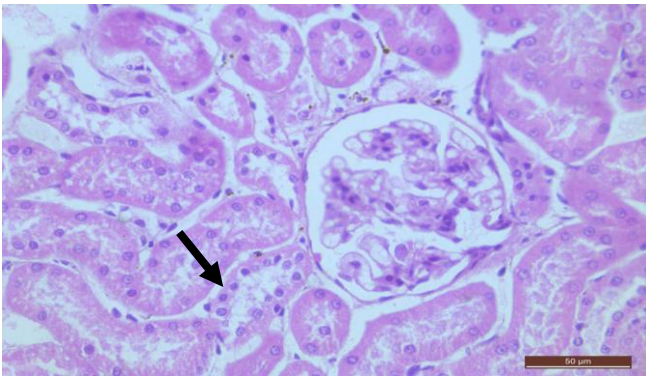

Fig 13. Photomicrograph of the kidney of rat pre-treated with leaf extract of *Boerhaavia diffusa* L. of 500mg/kg b.w./day for 20 days followed by 600 mg/kg b.w./day of NaF for 40 days showing with some normal renal tubules (↑).H&E X400.

DISCUSSION

The present study was an attempt to evaluate the nephrotoxicity of sodium fluoride and possible curative role of *Boerhaavia diffusa* L. leaf extract. The kidney is a site for potential fluoride toxicity, since it can be exposed to relatively high concentration of fluoride, and acute renal failure would contribute to the accumulation of fluoride [11].A relationship between the dose of fluoride and renal tissue injury has been reported [12].

Our findings revealed that sodium fluoride treatment to rats caused histopathological alterations in kidney. The dilated peritubular capillaries and inter tubular hemorrhages were found in some sections. Fluoride increased the permeability by affecting the communicating units between endothelia of the venules and capillaries, as a result oedema, hemorrhage, and necrosis occurred. The present study demonstrates desquamated epithelial cells in renal tubules. The result was compatible with the previous fluorosis study [13,6,14].In mice fed with 10 and 500 ppm sodium fluoride for 3 months shrunken kidneys, degeneration of tubular cells, and dilatation in the convoluted tubules were reported. [15].

In present work, the main changes observed in renal glomerulus were hypertrophy, atrophy with wide urinary spaces, and necrosis. Urinary spaces dilation may be the result of the high pressure across glomerular capillaries [16].Glomerular hypertrophy may be responsible for the podocytes abnormalities [17].

The renal tubules showed degenerated epithelial cells, presence of tubular casts in the lumen, oedema, dilation of tubular lumen, vacuolation, and lysis of cytoplasm. These changes are in agreement with the results of previous reports [3,6,18,4,2,14,19,20].The presence of necrosis may be related to the depletion of ATP, which finally leads to the death of the cells [21]. Renal medullary necrosis occurs as a primary manifestation of renal disease. The mechanism of which is poorly understood, but it seems to involve a vascular change. Also, prostaglandin synthetase is found in the kidney, primarily in the medulla, and inhibition of this enzyme resulted in decreased production of prostaglandin E2 (PGE2) and loss of its vasodilatory effect on juxtamedullary arterioles.[22,23].

In the present investigation, many renal tubules of the rat kidneys showed marked degenerative lesions under the effect of sodium fluoride. This is justifiable since the renal tubules are particularly sensitive to toxic influences, in part because they have high oxygen consumption and vulnerable enzyme systems, and in part because they have complicated transport mechanisms that may be used for transport of toxins and may be damaged by such toxins. Such degenerative changes were markedly pronounced in the proximal convoluted tubules. These findings reinforce previous study of fluorosis by some authors [19,20], found that many chemicals had a direct nephrotoxic action and exerted their effects principally on the proximal convoluted tubules.

The present study demonstrates that pre-treatment and post-treatment with *Boerhaavia diffusa* L.leaf extract protected

against degenerative changes of renal cortical architecture in the experimental. The herb is a diuretic that acts on the glomeruli of the kidney and also protects the kidney from being damaged.

CONCLUSION

Pre and post-treatment with leaf extract of *Boerhaavia diffusa* L.(500 mg/kg bw/day) for 20 days exhibited antinephrotic effects (curative). Histopathological findings indicate rejuvenation of necrotic cell in kidney.

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