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Ameliorative Efficacy of *Moringa oleifera* Lam. against the Sodium Fluoride Induced Nephrotoxicity in Wistar Rat

Trishna Kalita[1] and Uma Dutta[2*]

Department of Zoology, Cotton University, Panbazar, Guwhati – 781001, Assam, India; uma.dutta@cottonuniversity.ac.in

Abstract

A study was conducted to evaluate the protective efficacy of a methanolic leaf extract of *Moringa oleifera* L. (MO) for the remediation of nephrotoxicity induced by exposure to a significant groundwater pollutant, fluoride. Wistar albino male rats weighing 150–200 g were divided into five groups, each containing five animals. The control group received normal drinking water, and Group I received sodium fluoride in drinking water (20 mg/l daily) for 90 days. Group II received *M. oleifera* extract (250 mg/l) for 90 days. Group III received sodium fluoride 20 mg/l along with a low dose of *M. oleifera* extract (250 mg/l) for 90 days, and Group IV received sodium fluoride 20 mg/l along with a high dose of *M. oleifera* extract (500 mg/l) for 90 days. After 90 days, the kidneys and blood samples were obtained to evaluate their histological characteristics and assess the levels of oxidative stress and various enzyme parameters. The current investigation showed that sodium fluoride has a notable impact on renal function by elevating levels of creatinine, Blood Urea Nitrogen (BUN), urea, as well as Serum Glutamic Oxaloacetic Transaminase (SGOT), Serum Glutamate Pyruvate Transaminase (SGPT), and Alkaline Phosphatase (ALP) indicators. Further exposure resulted in a decrease in Superoxide Dismutase (SOD) and Catalase (CAT) levels, whereas Lipid Peroxidation (LPO) activity increased in kidney tissue. The histopathological examination of the kidney revealed glomerular enlargement, degenerative changes in Bowman's capsule, and alterations in the tubular portion of the glomerulus. The study's findings indicate that the administration of *M. oleifera* extract had a notable impact on the renal function and antioxidant status of rats subjected to sodium fluoride treatment. The administration of *M. oleifera* extract demonstrated a significant ability to mitigate the nephrotoxic effects induced by sodium fluoride in rats, mainly when both substances were concurrently administered. *M. oleifera* extract may also provide hepatoprotective effects by modifying membrane permeability and blocking enzymes from entering the serum.

Keywords: Antioxidant, Histopathology, Nephrotoxicity, Plant Extract, Sodium Fluoride

1. Introduction

Fluoride is a highly electronegative[1] and reactive element that never occurs in a free state. Fluoride compounds are common and widely dispersed in diverse amounts throughout the environment. Fluoride exposure is primarily caused by drinking contaminated groundwater. Other sources of fluoride consumption include diet, toothpaste, mouthwash, and other anti-caries medications[2]. The prolonged absorption of fluoride causes fluorosis, an irreversible condition that is a significant public health issue in India and other nations[3]. Fluoride can pass through cell membranes by simple diffusion and then reach soft tissues and cause damage[4]. Research indicates that the

**Author for correspondence*

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presence of fluoride can cause an excessive generation of Reactive Oxygen Species (ROS), resulting in a disruption of the antioxidant defence system[5]. In addition, studies have shown that fluoride can negatively affect other vital organs, such as the liver and kidneys. It is widely known that a considerable amount of fluoride absorbed by the body is excreted through the urinary system, primarily through glomerular filtration. Previous research findings have indicated a potential correlation between exposure to fluoride in occupational or environmental settings and adverse renal health outcomes. It is established that exposure to fluoride, whether acute or chronic, can result in renal toxicity in both humans and animals[6,7]. Researchers are always vigilant in their search for biologically active compounds derived from plants, aiming to safeguard humans from the detrimental impacts of drugs and toxins.

Moringa oleifera, a member of the Moringaceae family, is a tree with edible components such as leaves, fruit, and flowers. The geographical distribution of this entity encompasses regions of Asia and Africa that fall within the tropical and subtropical zones. The extracts derived from *M. oleifera* have been found to exhibit a diverse range of nutritional and pharmacological properties. These include but are not limited to anti-inflammatory, antioxidant, anti-cancer, hepatoprotective, neuroprotective, hypoglycemic, and blood lipid-reducing functions. The beneficial characteristics of *M. oleifera* are intricately associated with its phytochemical constituents, particularly flavonoids[8-10]. Prior research has indicated that administering *M. oleifera* leaf extract for 28 consecutive days after treatment can protect against cadmium-induced hepatotoxicity in rats. This is achieved through the suppression of increased levels of ALP, Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), and LPO while concurrently augmenting the level of SOD[11]. Also, the administration of *M. oleifera* leaf extract for six weeks has been identified as a significant determinant in the reduction of diabetic complications. This is achieved through safeguarding against inflammation and renal damage in a streptozotocin-induced diabetes rat model[12]. The objective of the current investigation was to assess the potential influence of *M. oleifera* on renal dysfunction caused by Sodium Fluoride (NaF) in Wistar rats.

2. Materials and Methods

2.1 Chemicals

The kidney markers urea, BUN, and creatinine were purchased from Sigma-Aldrich. Sodium fluoride (NaF), SGOT, SGPT, and ALP markers were purchased from Himedia Laboratories Pvt. Ltd. Additional chemicals were obtained from the laboratory of the Zoology Department, Cotton University, Guwahati, Assam.

2.2 Collection, Identification and Extraction of Plant Sample

The leaves of *M. olerifera* were gathered from the campus of Cotton University, located in Guwahati, Assam. (Latitude: 26.1874°N, Longitude: 91.7467°E). The process of identification and authentication was conducted at the Department of Botany, Gauhati University. The *M. oleifera* leaves were washed, air-dried, and subsequently pulverized into a coarse powder using an electric blender. The leaf powder underwent a 72-hour immersion in methanol, followed by filtration. Thereafter, the filtrate was concentrated at 37°C using a rotary evaporator to give a residue methanol extract of MO.

2.3 Experimental Animals

Wistar albino male rats weighing 150-200 g were procured from the Animal House Facility of the Pharmacology Department, College of Veterinary Science, Guwahati, Assam. We obtained permission to conduct the experimental procedures from the Institutional Animal Ethics Committee (IEAC), Cotton University, with reference number 10/IAEC/CU/05/01/2021. All institutional and national guidelines of CPCSEA (Committee for Control and Supervision on Experiments of Animals, India) and NIH (National Institute of Health) for the maintenance, transportation, experimental procedures, and sacrifice of laboratory animals were strictly followed during the experimental procedures. The animals were allowed to acclimate in the laboratory for one week. They were kept in plastic cages, ensuring appropriate humidity levels, and exposed to a 12-hour cycle of light and darkness. The animals were provided with a commercial pellet diet and clean drinking water ad libitum throughout the experiment.

2.4 Experimental Design

After one week of acclimation, animals were divided into five groups, each group containing five animals. The control group received normal drinking water, and Group I received sodium fluoride (NaF) in drinking water (20 mg/l, daily) for 90 days. Group II received *M. oleifera* extract (250 mg/l) for 90 days. Group III received sodium

fluoride 20 mg/l along with a low dose of *M. oleifera* extract (250 mg/l) for 90 days, and Group IV received sodium fluoride 20 mg/l along with a high dose of *M. oleifera* extract (500 mg/l) for 90 days.

2.5 Collection and Preparation of Blood and Tissue

Blood is drawn by a cardiac puncture after deeply anaesthetizing the rat and collected in a centrifuge tube[13]. To acquire the clear serum, the blood samples underwent centrifugation at a speed of 5000 revolutions per minute for 15 minutes. The serum was subsequently aliquoted into individual tubes and stored at a temperature of -20°C to facilitate subsequent biochemical analysis. Rats were sacrificed by cervical dislocation, and their kidneys were removed, washed in normal saline, and then fixed in Carnoy's fixative for histopathological investigations. Another set of kidneys was homogenized (1:10 w/v) in 100 mM phosphate buffered saline with EDTA (1 mM, pH 7.4) and centrifuged for 30 min at 12,000. The resulting supernatant was separated and used for the antioxidant tests.

2.6 Doses Selection

A pilot study was conducted for a dose selection of sodium fluoride using an individual graded response method from Van den Heuvel *et al*[14]. When reviewing the literature, it was important to consider the preferred high doses and a very low dose to assess the dosage response of the necessary chemicals based on the No Observable Adverse Effect Level (NOAEL), Low Observable Adverse Effect Level (LOAEL), and intermediate impact level. Consequently, the appropriate dose levels were selected in a manner that prevents toxicity at modest doses. In addition, the doses should have a negligible harmful effect at intermediate levels and toxic effects at higher levels of exposure. However, the toxic impacts should not result in the mortality of more than 10% of the animals exposed to the chemical.

The methanolic leaf preparations of *M. oleifera* were subjected to an acute toxicity study by the Organization for Economic Co-operation and Development (OECD) Guidelines No. 423. The animals underwent an overnight fasting period and were given only water. Following this, the MO extract was orally administered at a dosage of 2000mg/kg. The animals were then observed for 7 days. If two out of three animals show mortality, the provided amount is classified as hazardous. If mortality is recorded in one-third of the animals, the same dose is administered again to validate the toxicity study. No deaths or noticeable physical abnormalities were found after administering a dose of 2000 mg/kg of body weight. Based on the acute toxicity research and preliminary analysis of the extract on experimental animals, oral doses of 250 mg/kg and 500 mg/kg body weight were chosen for the current investigation.

2.7 Assessment of Antioxidant Enzymes

2.7.1 Superoxide Dismutase and Catalase Activity

Superoxide dismutase activity in renal tissues was determined by the method of Misra and Fridovich[15]. The reaction shows absorption at 560 nm and is expressed in units per mg of protein. The catalase activity in renal tissues was determined by the method of Bonaventura *et al.*[16] and expressed in mol/min/mg protein.

2.7.2 Lipid Peroxidation

The quantification of oxidative stress levels can be assessed through the measurement of Malondialdehyde (MDA), a compound that is formed as a result of LPO. The reaction involving the compound MDA and the chemical thiobarbituric acid results in the formation of a distinct crimson-coloured end product known as a Thiobarbituric Acid Reactive Substance (TBARS). The estimation was conducted using the Buege and Aust approach[17].

2.8 Serum Biochemical Markers

The markers of nephrotoxicity were evaluated through the assessment of BUN, creatinine, and serum urea concentration. The parameters were determined via spectrophotometry using commercially available kits and adhering to the manufacturer's protocols based on serum samples. SGOT, SGPT, and ALP activities were assayed using the kits from Himedia.

2.9 Histopathological Study

The kidney was excised and underwent fixation using Carnoy's solution for 24 hours, after which the fixative was removed by continuous washing with tap water. The tissues were dehydrated using a sequence of alcohols with increasing concentration, then cleared in xylene, and finally embedded in paraffin. The sections were cut using a microtome with a thickness of 4µm. The tissue

slices were prepared for staining using the hematoxylin and eosin procedure developed by Luna[18].

2.10 Statistical Analysis

All data were expressed as mean ± SEM (Standard Error of Mean). Statistical analyses were performed using the Statistical Package for Social Sciences (SPSS) version 21. The significance of differences between groups was ascertained by a one-way ANOVA followed by Tukey's post hoc test.

3. Results

3.1 Antioxidant Activity

3.1.1 Superoxide Dismutase Activity and Catalase Activity

The administration of sodium fluoride had a noticeable impact on the activity of SOD and CAT. The enzymatic activities observed in the NaF-treated group (Group I) exhibited a notable decrease in comparison to the control group (Figure 1). The experimental group III, which received a low dose of MO, did not exhibit a statistically significant alteration in the activity levels of SOD and CAT when compared to the Group I. Nevertheless, it is worth noting that the administration of a high dose of MO in Group IV resulted in markedly elevated SOD and CAT activities when compared to Group I. Upon further analysis, it was observed that the administration of MO alone in Group II resulted in a notable improvement in the levels of these enzymes when compared to the control group. This observation suggests that MO may have a beneficial impact in reducing oxidative stress induced by NaF.

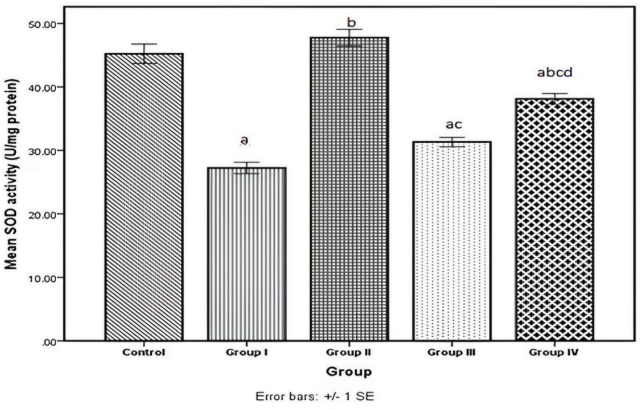


Figure 1. "a" indicates the significant difference from the Control group, "b" indicates the significant difference from Group I (NaF treated group), "c" indicates the significant difference from Group II (MO pretreated group), "d" indicates the significant difference from Group III (Low dose of MO) ($p<0.01$).

3.1.2 Lipid Peroxidation

In the group treated with sodium fluoride (Group I), the level of MDA is significantly higher compared to

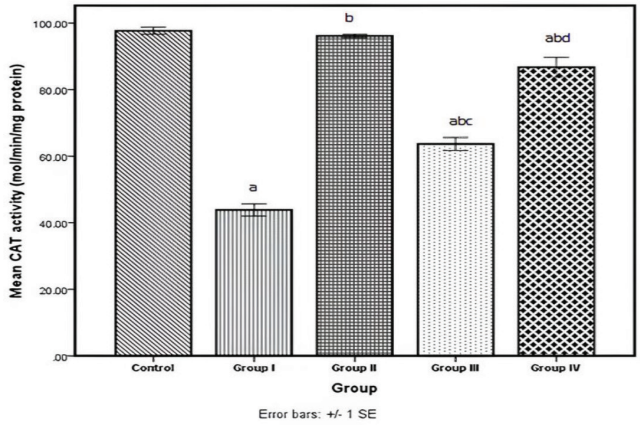


Figure 2. "a" indicates the significant difference from the Control group, "b" indicates the significant difference from Group I (NaF treated group), "c" indicates the significant difference from Group II (MO pretreated group), "d" indicates the significant difference from Group III (Low dose of MO) ($p<0.01$).

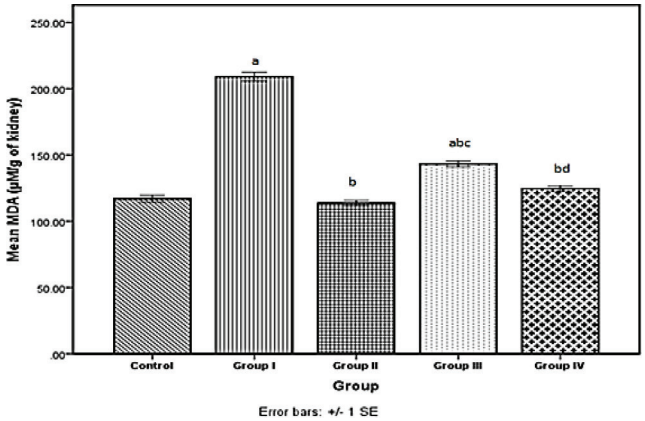


Figure 3. "a" indicates the significant difference from the Control group, "b" indicates the significant difference from Group I (NaF treated group), "c" indicates the significant difference from Group II (MO pretreated group), "d" indicates the significant difference from Group III (Low dose of MO) ($p<0.01$).

the control group. Administration of sodium fluoride in combination with high-dose MO in Group IV led to a notable reduction in MDA levels when compared to Group I. The levels of MDA in the control group, Group II, and Group IV are similar. This study demonstrates that MO has the potential to mitigate the adverse impacts of sodium fluoride-induced toxicity.

3.2 Toxicity Assay

The serum creatinine, urea, and BUN levels significantly increased in the sodium fluoride-treated group (Group I) compared to the control group. On the other hand, the MO significantly lowered the elevated creatinine, urea, and BUN levels compared with the sodium-fluoride-treated group. The improvement is more pronounced in the co-treated group with a high dose of MO. The SGOT, SGPT, and ALP activities in serum significantly increased in the sodium-fluoride-treated group compared to the control group (Table 1). Co-administration of sodium fluoride with MO (both high and low doses) in Groups III and IV resulted in a significant decrease in these enzyme activities compared to the sodium fluoride-treated group (Group I). The SGPT and ALP activities remained almost on par with the control group in the high dose of MO co-administered group.

3.3 Histopathological Results

Histology is commonly acknowledged as a valuable method for identifying and characterizing pathological lesions caused by toxic substances in different tissues. The intricate structure of kidneys comprises an assemblage of fundamental components known as nephrons. The nephron is composed of the glomerulus and the tubules. The transverse section of the kidney in the control rat exhibited the presence of normal proximal and convoluted tubules, characterized by their polygonal shape and distinct nuclei located centrally (Figure 4(A)). In the experiment, the kidney's transverse section from a rat exposed to fluoride for 90 days (Group I) revealed hypertrophy in the glomerulus, degenerative modifications in Bowman's capsule, and variations in the tubular region of the glomerulus (Figure 4(B)). Similarly, rats treated with MO alone did not exhibit histological lesions (Figure 4(C)). Furthermore, the renal corpuscle and normal glomerulus improved with co-administration of NaF with MO low dose (Group III) and NaF with MO high dose (Group IV), with a few proximal convoluted tubules displaying disrupted epithelial lining.

G - Glomerulus, BC - Bowman's capsule, WS - Wider Bowman's space, RBS - Restoration of Bowman's space

4. Discussion

Several studies have elucidated the significance of conducting investigations on the efficacy of plant-derived pharmaceuticals in mitigating the harmful effects of fluoride toxicity. The current study aimed to evaluate the effectiveness of *M. oleifera* extract in a rat model of fluorosis. This was achieved by analyzing serum biochemical parameters and antioxidant parameters and conducting a histopathological examination of the kidney. Oxidative stress is a well-established factor in the onset of fluoride-induced nephrotoxicity, with the production of ROS being crucial in the progression of this illness[19]. The investigation of the effects of NaF revealed a notable elevation in LPO levels within the kidney tissues. Additionally, a concurrent reduction in the activities of SOD and CAT was observed. The observed alterations in the experimental subjects' physiology indicate an excessive formation of oxygen-free radicals in the rats subjected to NaF treatment[20]. Oxidative stress, as defined

Table 1. Serum creatinine, urea, BUN, SGOT, SGPT and ALP levels in all the experimental groups

Parameters	Control	Group I	Group II	Group III	Group IV
Serum creatinine (µmol/l)	93.94±0.66	114.58±1.05^{a}	92.02±0.66^{b}	107.8±1.12^{abc}	98.88±1.56^{bc}
Serum urea (mg/dl)	52.19±1.16	29.32±0.74^{a}	52.63±1.16^{b}	44.17±1.92^{abc}	49.38±1.16^{b}
BUN (mg/dl)	25.37±0.38	13.73±0.5^{a}	21.08±1.41^{b}	17.3±0.84^{a}	20.36±0.44^{ab}
SGOT(U/L)	93.94±0.66	114.58±1.05^{a}	92.02±0.66^{b}	107.8±1.12^{abc}	98.88±1.56^{bcd}
SGPT(U/L)	52.08±1	84.06±1.37^{a}	52.3±2.07^{b}	62.12±1.47^{abc}	51.88±1.82^{bd}
ALP(U/L)	94.44±0.25	126.3±0.63^{a}	95.3±0.66^{b}	105.81±1.32^{abc}	96.53±0.25^{bd}

Values are presented in mean ± SEM. Superscripts indicate significant differences from each other. "a" is significantly different from the Control group, "b" is significantly different from Group I, "c" significantly different from Group II, and "d" is significantly different from Group III.

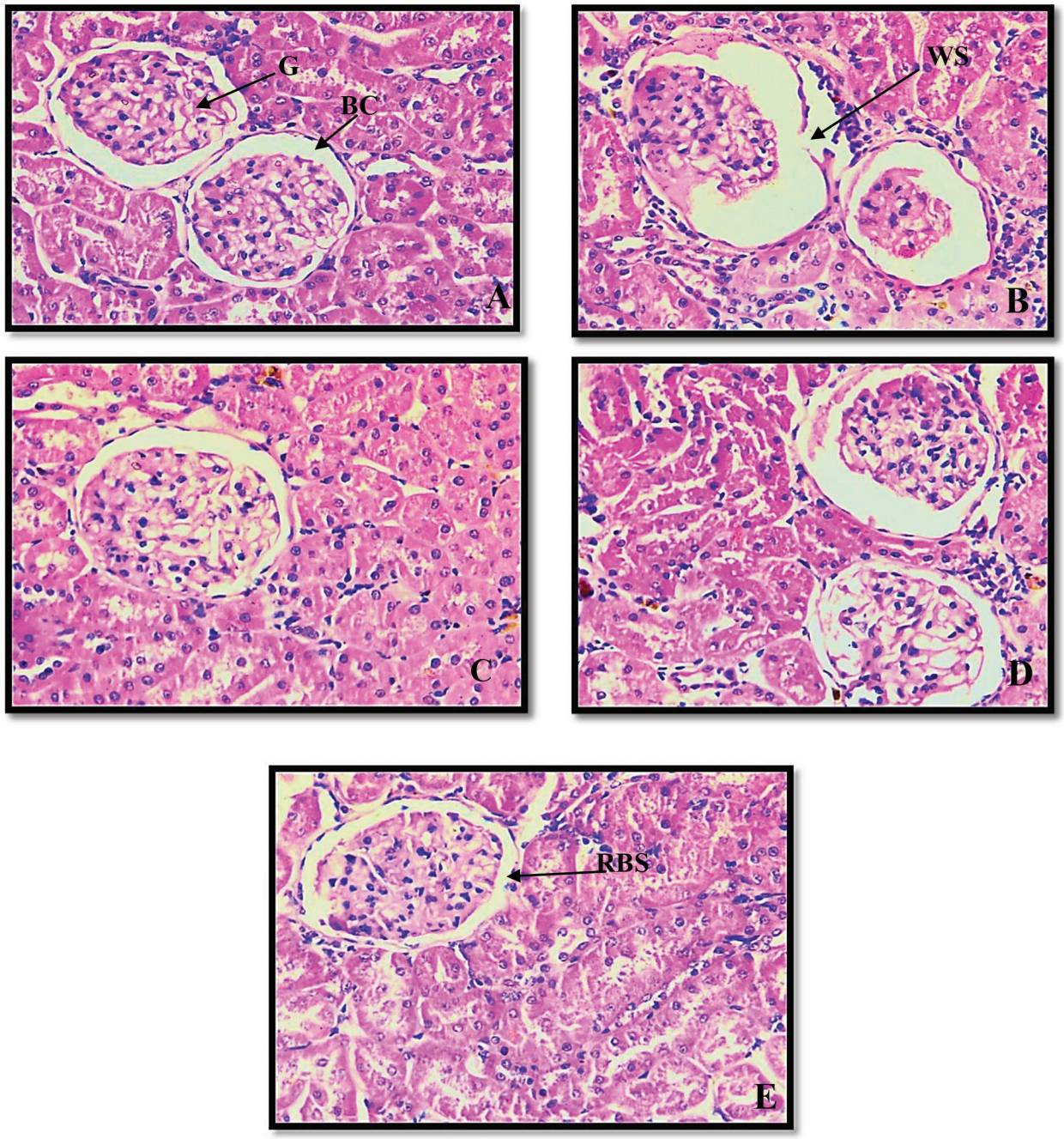


Figure 4. Photomicrographs of histoarchitecture of kidney from different groups. (A) Control, (B) Group I, (C) Group II, (D) Group III, (E) Group IV. Magnification 20X, Scale bar 50µm.

by Halliwell, is the imbalance between oxidants and antioxidants[21]. Prior research has elucidated a correlation between fluoride exposure and oxidative stress in renal tissue[22]. When co-administered with NaF intoxication, the MO extract has been observed to prevent any alterations in the antioxidant system. This preventive measure helps maintain the overall health of these organs. In the present study, our findings revealed that the levels of SOD, CAT, and Malondialdehyde (MDA) were observed to be within normal limits. These findings imply that the animals stated above did not experience appreciable oxidative stress due to the fluoride and MO extract administrations. Similar results were observed when the methanol extract of *Azadirachta indica* (AI) showed comparable outcomes

in terms of mitigating sodium fluoride (NaF)-induced hypertension and genotoxicity in rats[23].

The findings of the current investigation indicate a notable increase in the concentrations of creatinine, urea, and uric acid in rats subjected to fluoride treatment compared to the control group, suggesting extensive glomerular damage[19]. The administration of MO (*M. oleifera*) treatment demonstrated an improvement in mitigating the adverse effects caused by fluoride-induced toxicity. This was achieved through significant alterations observed in various kidney parameters. A previous study has documented comparable and noteworthy improvements in markers associated with the restoration of renal function following the administration of argon oil (Saber *et al.*, 2020)[24]. The administration of Sodium Fluoride (NaF) has been observed to induce oxidative stress and adversely affect hepatic function. This conclusion is substantiated by the notable elevation of SGOT, SGPT, and ALP levels[25,26]. According to Trivedi *et al.* (2008), mice given oral NaF (6 and 12 mg/kg body weight/day) for 30 days showed a significant dose-dependent increase in blood SGOT and SGPT levels[27]. Our research also showed that all treatment groups experienced a substantial increase in SGPT and SGOT activity after 90 days of exposure to a NaF dosage. Elevated SGOT, SGPT, and ALP levels could be attributed to liver damage caused by sodium fluoride. After administering sodium fluoride together with MO extract, these concentrations were reversed. MO extract may benefit hepatoprotective by altering membrane permeability and preventing enzymes from seeping into the serum. The findings were consistent with earlier research[28].

Histopathological observations of the kidney revealed that sodium fluoride caused Bowman's capsule degenerative alterations, the glomerulus's enlargement, and alterations in the tubular area of the glomerulus. A similar observation was reported by Kour and Singh where a reduction in the size of the kidney structure, accompanied by the degeneration of glomeruli, as well as the deterioration of tubular cells and the expansion of convoluted tubules in mice, occurred due to the administration of sodium fluoride[29]. Ramseyer *et al.* found that rats exposed to 1.5 or 10 ppm of NaF in their drinking water had hypertrophy and hyperplasia in their tubules after 500 days[30]. The histological abnormalities observed in adult rats were shown to be substantially linked with variations in malondialdehyde components in the kidney. When NaF and MO were administered simultaneously, all of the histological alterations caused by NaF in rat kidney tissues were partially alleviated. This impact could be attributed to the antioxidant properties of MO.

5. Conclusions

The present study investigates the potential mitigating effects of co-administering *M. oleifera* extract with sodium fluoride over 90 days on nephrotoxicity and oxidative stress in rat renal tissues. The findings of this study indicate that *M. oleifera* exhibits potential as a viable ingredient in food supplements, particularly in the form of herbal formulations. These formulations can serve as nephroprotective agents, explicitly targeting the adverse effects of sodium fluoride toxicity. To obtain a comprehensive understanding, an additional examination is required to ascertain and thoroughly describe the active component(s) existing within the extract, and this process is presently underway.

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