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Cyperus esculentus suppresses hepato-renal oxidative stress, inflammation, and caspase-3 activation following chronic exposure to sodium fluoride in rats' model

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ABSTRACT

Background: Death arising from hepato-renal related diseases is on the increase. *Cyperus esculentus* (CE) possesses antioxidants potentials. This study aim at investigating the effect of *Cyperus esculentus* on sodium fluoride (NaF)-induced hepato-renal toxicity in rats.

Methods: Twenty-four male rats weighting (10–12 weeks old, 200± 20 g) randomized into group A (control) received 1 ml normal saline; group B administered 5 mg/kg bwt of NaF; group C received 500 mg/kg bwt CE; group D received 5 mg/kg bwt NaF and 500 mg/kg bwt CE through gastric gavage for 30 days. Liver and kidney histology, serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH), Creatinine (Cr), and Blood urea nitrogen (BUN), Hepatic and renal nitric oxide (NO), myeloperoxidase (MPO), tumor necrosis factor-alpha (TNF-α), interleukin-1 β (IL-1β), caspase-3, superoxide dismutase (SOD), catalase (CAT), glutathione (GSH), glutathione peroxidase (GPx) and malondialdehyde (MDA) were performed.

Results: The observed increases in AST, ALT, ALP, LHD, Cr, and BUN were alleviated in NaF+CE treated rats. The reduction of antioxidant activity was assuaged in rats treated with NaF+CE. In addition, NaF increases liver and kidney MDA, NO, MPO TNF-α, IL-1β, and caspase-3 activity, significantly decreases in rats treated with NaF+CE. Histological observation showed swelling glomeruli and renal tubules lesion while the liver sections showed an extensive histopathological change in NaF exposed rats. However, the intervention of CE alleviated the severity of histopathological lesions induced by NaF.

Conclusion: Therefore, CE ameliorate NaF-induced oxidative stress, inflammation, and caspase 3 activation in the liver and kidney of the rats.

1. Introduction

Sodium fluoride is used globally as an important element in the prevention of tooth decay, and it has proven its effectiveness in dental caries prevention when its low level in oral hygiene materials (Clarkson et al., 2011). Fluoridated water is a natural source of fluoride pollution, and is defined through the prevalence of fluoride-containing minerals in groundwater (Barbier et al., 2010). However, recent studies estimated that about 20% of pharmaceuticals and 30–40% of agrochemicals are organofluorines (Nabavi et al., 2012a). Fluoride-containing compounds are also commonly used in household products and offices (*e.g.*, dental products, refrigerants, and fire extinguishers) (WHO, 2002). Prolonged intake of fluoride or excessive consumption results in fluorosis, leading to progressive degenerative disease, often characterized by dental mottling and skeletal dysfunctions including crippling deformities, osteoporosis, and osteosclerosis (Nabavi et al., 2012a). The kidney has a

Abbreviation: NaF, Sodium fluoride; CE, Cyperus esculentus; AST, Aspartate aminotransferase; ALT, Alanine aminotransferase; ALP, Alkaline phosphatase; LDH, Lactate dehydrogenase; Cr, Creatinine; BUN, Blood urea nitrogen; NO, Hepatic and renal nitric oxide; MPO, myeloperoxidase; TNF-α, tumor necrosis factor-alpha; IL-1β, interleukin-1 β; caspase-3, SOD, superoxide dismutase; CAT, catalase; GSH, glutathione, GPx, glutathione peroxidase; MDA, malondialdehyde.

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prominent role in fluoride metabolism as 50–80% of fluoride is removed via urinal excretion (Xiong et al., 2017). Not surprisingly, the kidney is one of the major organs affected by fluoride intoxication, and numerous studies have established a close correlation between fluoride intake and renal injury (Nabavi et al., 2012b).

Drug-induced nephrotoxicity is a prominent cause of renal failure (Shashi and Kaur, 2017). The kidney is part of the target organs attacked by excessive fluoride accumulation (Couser et al., 2011). Fluoride plays a part in the cellular respiratory process like in free radical reactions. Fluoride reacts with polyunsaturated fatty acids and initiates lipid peroxidation leading to necrosis and apoptosis (Hamza et al., 2015; Samanta et al., 2016). As the primary organ concerned with the retention and excretion of fluoride, the kidney is quite sensitive to fluoride toxicity (Luo et al., 2017). Various studies have shown that exposure to high fluoride concentrations in drinking water elevated the renal and liver function enzyme levels in serum and cause severe changes in the histology of the liver and kidneys (Zhan et al., 2006; Anjum *et al.*, 2014). According to a study carried out by Zhan et al. (2006), pigs that were exposed to fluoride concentrations between 100 and 250 mg/Kg showed a significant increase in the serum creatinine and urea levels and also showed detrimental effects on kidney function and structure. The situation of serious imbalance between oxidant and antioxidant is referred to as oxidative damage. In many diseases, tissue damage is accompanied by an imbalance in the oxidant and antioxidant status (Shashi and Kaur, 2017). Exposure to fluoride results in the generation of anion superoxide increased oxygen concentration and its downstream consequences such as hydrogen peroxide, hydroxyl radicals, which are important in mediating the toxic effects of fluoride (Shashi and Kaur, 2017). Intake of high levels of fluoride is a known cause of altered enzyme activities, structural changes, and influences lipid metabolism. Acute poisoning can terminate in death due to blocking of cell metabolism since fluoride inhibits enzymatic processes, mainly metalloenzymes responsible for important vital processes (Shivarajashankara et al., 2012).

Medicinal plants and their formulations have continued to attract attention as a result of the strong speculations and belief that they are safe and very effective (Farnsworth and Soejarto, 1995). This strong speculation and assumption have led to its indiscriminate use especially in developing countries (Mbaka et al., 2010). Recently, some studies show that some of these medicinal plants alter the physiological status of some vital organs in the body while affecting their medicinal actions (Mbaka et al., 2010). Consequently, it has become imperative to ascertain the effects of those plants used as medicines on the physiological status of vital organs. *Cyperus esculentus* (CE) commonly called tigernut, earth almond or yellow nutsedge is a crop of the family, Cyperaceae that is widely spread across the globe (Sanchez-Zapata *et al.*, 2012). It is a tuber that has abundant energy contents and also contains starch, protein, fat, sugar, dietary minerals, among other things (Zhang et al., 2006). The Hausas, Yorubas, and Igbos in Nigeria call *Cyperus esculentus* Aya, Imumu, and Ofio, respectively (Omode et al., 1995). Nigerians eat this plant either fresh or after it has been dried, roasted, or turned into a beverage called Kunu (Oladele and Aina, 2007). *Cyperus esculentus* has long been used in herbal medicine to treat indigestion, diarrhea, and dysentery (Abano and Amoah, 2011). Vitamin C, Vitamin E, and Quercetin are among the antioxidants contained in *Cyperus esculentus* as well as minerals like phosphorus, potassium, and zinc (Belewu and Belewu, 2007; Allouh et al., 2015). Studies have also shown that tiger nuts have high energy nutrients such as glucose oleic acid, starch, fats, sugars, and proteins (Kim et al., 2007). *Cyperus esculentus* milk is suitable for diabetic individuals and also helps in weight control because of its high fiber content (Emple et al., 2009). *Cyperus esculentus* is beneficial in the treatment of flatulence, constipation, indigestion, and diarrhea due to the presence of digestive enzymes such as lipase, catalase, and amylase (Ubhenin et al., 2016). The high content of oleic acid has a positive effect on cholesterol, thereby preventing heart attacks, thrombosis, and activating the blood content of soluble glucose (Kim et al., 2007). Tiger nut reduces the risk of colon cancer. Tiger nut is high in vitamin B1, which helps to balance the central nervous system and improve the body's ability to respond to stress (Allouh et al., 2015). Ogbuagu and Airaodion (2020) recently observed that tiger nut milk boosts male fertility. In another study, Airaodion and Ogbuagu (2020) reported the hematopoietic potential of tiger nut. This study investigates the effect of *Cyperus esculentus* on sodium fluoride-induced renal toxicity in Sprague Dawley rats.

2. Materials and methods

2.1. Chemicals

Fluoride in the form of sodium fluoride (NaF) was purchased from PASCAL Scientific (London, United Kingdom) (Product no.MW76613), thiobarbituric acid, and reduced glutathione were purchased from Sigma-Aldrich Corp. (St. Louis, MO USA). ELISA kits for the assessment of tumor necrosis factor-alpha (TNF-α), interleukin-1β (IL-1β), and caspase 3 (CASP3) activity were purchased from Elabscience Biotechnology Company, Beijing, China. All other chemicals used in the study were of analytical reagent grade.

2.2. Collection and extraction of plant material

Dried tubers of *Cyperus esculentus* were purchased from Isolo market and centre for research and development (CERAD), Federal University of Technology, Akure (FUTA), Ondo State, Nigeria for proper identification and authentication. The samples of the *Cyperus esculentus* nut were identified and authenticated by Mr. Omomoh Bernard and a sample of the plant voucher FUTA/0196 was deposited for reference purposes. The *Cyperus esculentus* tubers were washed and air-dried. The dried tuber was blended into a fine powder with the aids of an electronic blender (Kenwood 1.6 L, BL460 Prestons, Australia). The milled plant sample 450 g was later soaked in 1200 ml of phosphate-buffered saline (PBS) for 12 h at room temperature, and was later filtered through cheesecloth and then through Whatman #1 filter paper. The filtrate was then bottled in clean screw-cap bottles and stored in a refrigerator until use.

2.3. Experimental animal care

Twenty-four (24) mature healthy male Sprague-Dawley rats (10–12 weeks old, 200± 20 g) were obtained from the Central Laboratory of The Federal University of Technology Akure, Ondo State, Nigeria. The rats were kept in plastic cages located in a well-ventilated rat house, provided rat food and water *ad libitum*. The rats were subjected to natural photoperiod of 12-hr light: 12-hr dark and adequately cared for according to the conditions stated in the 'Guide for the Care and Use of Laboratory Animals published by the National Institute of Health (1985) and in line with Federal University of Technology Akure, Nigeria experimental animal Ethical guidelines.

2.4. Experimental design

The sexually mature male Sprague-Dawley rats were randomly divided into four groups of six rats each ($n = 6$). Group A served as control and was given 1 ml normal saline; group B administered 5 mg/kg bwt of sodium fluoride; group C received 500 mg/kg bwt *Cyperus esculentus*; group D received 5 mg/kg bwt sodium fluoride and 500 mg/kg bwt *Cyperus esculentus* through gastric gavage for 30 days. The doses of sodium fluoride and *Cyperus esculentus* used in the present investigation were chosen from our studies and previously published data (Adelakun et al., al.,2021).

2.5. Determination of body weight and absolute and relative weights of liver and kidney

Bodyweight was measured weekly using a sensitive electronic

Table 1
Effect of *Cyperus esculentus* nut extract on body weight, liver weights, and relative to body weight in experimental NaF-exposed rats.

Parameters	Treatment groups			
	Control	NaF	CE	NaF+CE
Initial body weight (g)	214.30±6.23	219.50±5.44	225.90±7.14	225.60±5.89
Final body weight (g)	253.80±8.15$^{\Psi}$	202.30±4.14*	263.20±3.14 $^{\Psi,\alpha}$	265.50±3.94 $^{\Psi,\alpha}$
Weight difference (g)	39.50±1.92	17.20±1.30*	37.30±4.00 $^{\alpha}$	39.90±1.95 $^{\alpha}$
Absolute Liver weight (g)	6.86±0.15	4.99±0.20*	6.81±0.15 $^{\alpha}$	6.33±0.14 $^{\alpha}$
Relative Liver weight (g)	2.66±0.12	2.49±0.07	2.57±0.08	2.37±0.07
Absolute Kidney weight (g)	0.66±0.01	0.48±0.02*	0.65±0.02 $^{\alpha}$	0.68±0.02 $^{\alpha}$
Relative kidney weight (g)	0.27±0.00	0.25±0.02	0.25±0.01	0.26±0.01

Values are expressed as Mean ± S.E.M, $n = 6$ in each group, *: represent a significant difference from control, α: represent a significant difference from NaF, Ψ: represent a significant difference from initial body weight at $p < 0.05$, One-Way ANOVA. Keys: CE, *Cyperus esculentus*; NaF, Sodium fluoride.

Table 2
Effect of *Cyperus esculentus* nut extract on liver oxidative and antioxidant status in experimental NaF-exposed rats.

Parameters	Treatment groups			
	Control	NaF	CE	NaF+CE
MDA Liver	0.74±0.13	2.00±0.18*	0.61±0.09 $^{\alpha}$	1.23±0.10$^{*,\ \alpha,\beta}$
Kidney	1.72±0.59	5.70±0.67*	1.82±0.34$^{\alpha}$	2.16±0.25$^{\alpha}$
SOD Liver	1.26±0.26	0.30±0.06*	1.31±0.26 $^{\alpha}$	1.02±0.26 $^{\alpha}$
Kidney	5.90±1.09	1.41±0.24*	6.15±1.03$^{\alpha}$	5.67±0.95$^{\alpha}$
CAT Liver	0.93±0.11	0.26±0.10*	0.83±0.10 $^{\alpha}$	0.70±0.11 $^{\alpha}$
Kidney	46.99±7.51	15.60±3.53*	47.94±7.56$^{\alpha}$	44.52±6.26$^{\alpha}$
GSH Liver	9.72±1.04	2.47±0.54*	9.53±1.16 $^{\alpha}$	8.12±1.19 $^{\alpha}$
Kidney	49.63±9.64	10.90±2.60*	52.10±9.33$^{\alpha}$	48.01±9.71$^{\alpha}$
GPx Liver	3.17±0.29	1.41±0.20*	2.97±0.27 $^{\alpha}$	2.73±0.28 $^{\alpha}$
Kidney	17.91±3.21	3.73±1.28*	19.16±3.22$^{\alpha}$	16.52±3.17$^{\alpha}$

Values are expressed as Mean ± S.E.M, $n = 6$ in each group, *: represent a significant difference from control, α: represent a significant difference from NaF, β: represent a significant difference from CE at $p < 0.05$, One-Way ANOVA. Keys: CE, *Cyperus esculentus*; NaF, Sodium fluoride.

weighing balance (Scout Pro, Ohaus Corporation, USA). Absolute weights of liver and kidney were measured using the same device during sacrifice while their relative weights were calculated using the relationship below: Relative weight of organ = (Absolute weight of organ / Final body weight) x 100%

2.6. Sample collection

Animals were anesthetized with a combination of ketamine/ xylazine (60/6 mg/kg, i.p.), after 24 h of last administration. Blood samples were collected from the jugular vein and centrifuged for 10 min at 3000 rpm for separating serum. Samples were then stored at −20 °C until analysis. Animals were sacrificed by decapitation and liver and kidney tissues were then isolated. Tissues were washed with normal saline afterward. For histological examination, one part of tissues was fixed in 10% phosphate-buffered formalin.

2.7. Liver and kidney homogenate preparation

The left kidney and part of the liver of each rat were homogenized separately in 50 mm Tris–HCl buffer (pH 7.4) containing 1.15% KCl to prepare a 20% (1/5 w/v) tissue homogenate using a Potter Elvehjem homogenizer. Thereafter, the homogenates were centrifuged at 10,000 g for 10 min in a cold centrifuge (4 °C). The supernatants were obtained and utilized for the determination of oxidants/antioxidants, pro-and anti-inflammatory cytokines, and apoptotic proteins.

2.8. Assay of liver and kidney function indices

Analysis of serum activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and lactate dehydrogenase (LDH) as well as creatinine and urea levels were performed using available commercial kits from Randox Laboratories Limited (UK). Creatinine and Blood urea nitrogen (BUN) were assessed as markers of nephrotoxicity. Creatinine concentration was determined using the Jaffe reaction described by Toora and Rejagopal (2002). BUN was determined using a Randox Commercial Kit based on the methods of Fesus et al. (1983).

2.9. Assay of pro-inflammatory biomarkers and caspase 3 activity

Hepatic and renal nitric oxide (NO) level was assessed using Griess reagent according to established protocol (Green et al., 1982). Briefly, the reaction mixture consisting of an equal volume of sample and Griess reagent was incubated for 15 min before the absorbance was evaluated at 540 nm. The level of NO was extrapolated from the standard curve and then expressed as Units/mg protein. Moreover, myeloperoxidase (MPO) activity was evaluated according to the method described by Granell et al., 2003. Additionally, TNF-α and IL-1β concentrations as well as caspase-3 activity were evaluated using commercially available ELISA Kits (Elabscience Biotechnology Company, Beijing, China) with the aid of a Spectra Max plate reader (Molecular Devices, CA, USA) as stated in the manufacturer's manual.

2.10. Determination of superoxide dismutase (SOD) activity

The reaction mixtures contained sodium carbonate (1 mL, 50 mM), NBT (0.4 mL, 25 μm), and freshly prepared hydroxylamine hydrochloride (0.2 mL, 0.1 mM). After mixing the reaction mixtures by inversion, the supernatants of kidney homogenates were added (containing 5 μg proteins). The change in absorbance of the reaction mixture was recorded at 560 nm (Nabavi et al., 2012b).

2.10.1. Determination of catalase (CAT) activity

The catalase activity was measured by the method of Nabavi et al. (2012a). Proteins contained in liver and kidney tissue homogenates (5 μg) were mixed with hydrogen peroxide (2.1 mL, 7.5 mM), and a time scan was performed for 10 min at 240 nm at 25 °C. The disappearance of peroxide, which the level reflects catalase activity, was measured. One unit of catalase activity is defined as the amount of enzyme that reduces 1 μmol of hydrogen peroxide per minute.

2.10.2. Determination of glutathione (GSH) activity

Liver and kidney GSH level was determined according to the method of Ellman (1959). Briefly, TCA (5%) was added to diluted kidney tissue homogenates (containing 36 μg protein) to precipitate the proteins. After centrifugation (12,000 X g, 5 min), the supernatant was taken and 5,50- dithiobis(2-nitrobenzoic acid) solution (Ellman's reagent) was added. The absorbance of the reaction mixture was measured at 417 nm. Various concentrations of reduced GSH were also used to construct a calibration curve from which the levels of reduced GSH in homogenate samples were calculated.

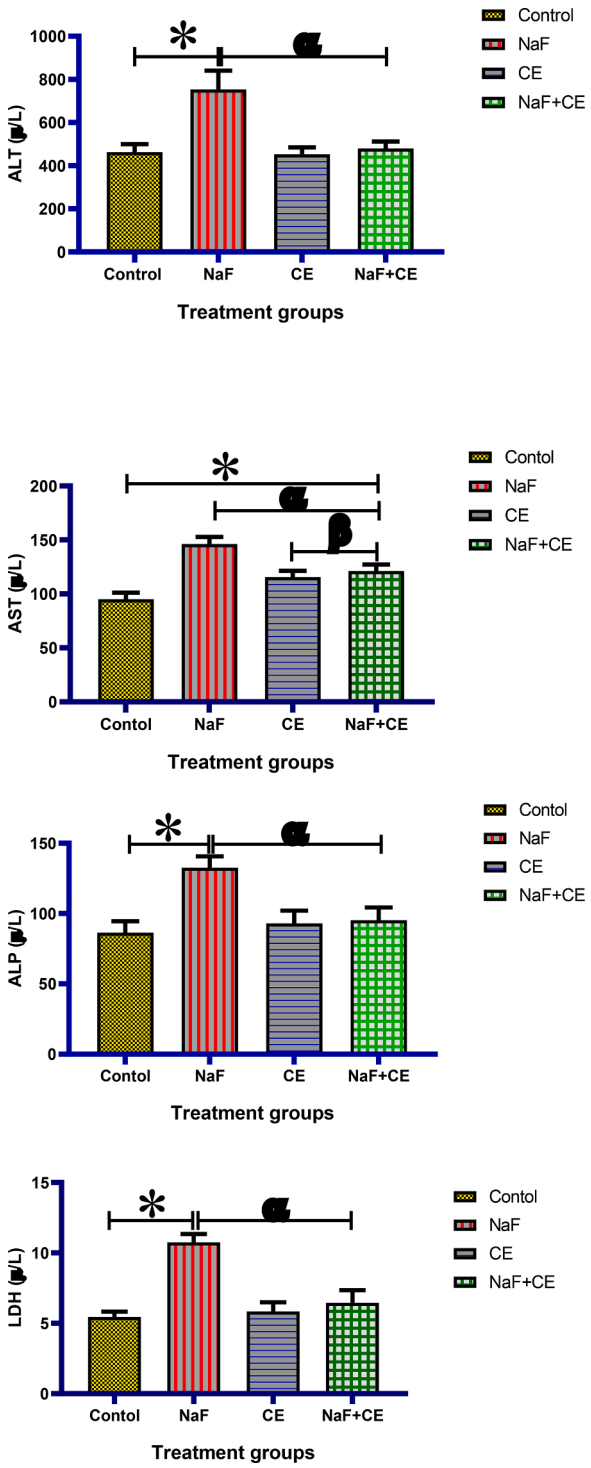

Fig. 1. Effect of *Cyperus esculentus* nut extract on liver function parameters in experimental NaF-exposed rats. Values are expressed as Mean ± S.E.M, $n = 6$ in each group, *: represent a significant difference from control, α: represent a significant difference from NaF, β: represent a significant difference from CE at $p < 0.05$, One-Way ANOVA. Keys: CE, *Cyperus esculentus*; NaF, Sodium fluoride.

2.10.3. Determination of glutathione peroxidase (GPx) activity

GPx activity was also measured according to Beutler *et al.* (1975). The reaction mixtures contain 0.5 mL of potassium phosphate buffer (pH, 7.4), 0.1 mL of Sodium azide, 0.2 mL of GSH solution, 0.1 mL of H_2O_2, 0.5 mL of sample and 0.6 mL of distilled water. The mixture was incubated in the water bath at 37 °C for 5 min and 0.5 mL of TCA was added and centrifuged at 4000 rpm for 5 min. A volume of 1 mL of the supernatant was taken and added 2 mL of K_2PHO_4 and 1 ml of Ellman's reagent. The absorbance was read at 412 nm using distilled water as blank.

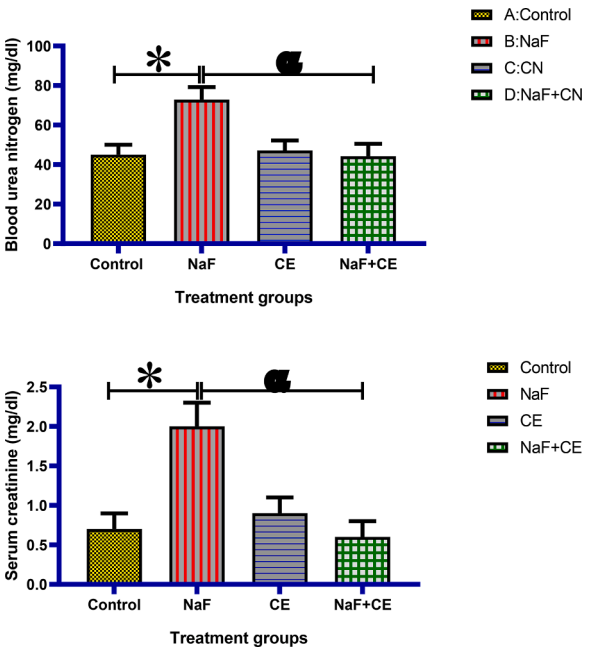

Fig. 2. Effect of *Cyperus esculentus* nut extract on serum creatinine (Cr) and blood urea nitrogen (BUN) in experimental NaF-exposed rats. Values are expressed as Mean ± S.E.M, $n = 6$ in each group, *: represent a significant difference from control, α: represent a significant difference from NaF at $p < 0.05$, One-Way ANOVA. Keys: CE, *Cyperus esculentus*; NaF, Sodium fluoride.

2.10.4. Determination of lipid peroxidation

The Malondialdehyde (MDA) content as an index of lipid peroxidation was quantified in the PMFs of the tissues according to the method Varshney and Kale (1990). 400 μL of Tris KCl, 125 μL of 30% TCA, 100 μL of sample and 125 μL of 0.75% TBA in 0.2 M HCl was immediately added. The reaction mixture was incubated in the water bath at 80 °C for 45 min, cooled on ice, and centrifuged at 3, 000 rpm for 15 min. 200 μL of supernatant was taken and the absorbance was measured against a blank of distilled water at 532 nm. Lipid peroxidation in units/mg protein was calculated with a molar extinction coefficient of 1.56 × 105/M/cm.

2.10.5. Histopathological examination

For the histological examination, the liver and kidneys were removed immediately and fixed in a 10% formalin solution. Then, dehydrated in graded alcohol concentrations and, embedded in paraffin. Sections of 5 μm were prepared, stained with hematoxylin and eosin (H&E), and evaluated with a light microscope (Olympus, Tokyo, Japan) connected to a camera (Digital Microscope BMZ-04-DZ). Six microscopy slides per animal were examined for assessment of histological changes such as congestion of RBCs, infiltration of inflammatory cells, and damage of proximal tubule cells (brush border loss, cell swelling, and nuclear pyknosis). For assessment of proximal tubule damages, the average percentage of damaged tubules was determined by dividing the number of tubules with histological criteria in a randomly microscopic field by the total number of tubules in the same field and the result was multiplied by 100. Infiltration of inflammatory cells and congestion of RBCs were graded into 4 categories: normal (0), weak (1), moderate (2), or intense (3), and the averages were considered. The diameter of the glomerulus was assessed by using Motic Images plus 2.0 image analysis software. For each slide, the mean of 6 fields was calculated. Slides were read in a 'blind' fashion

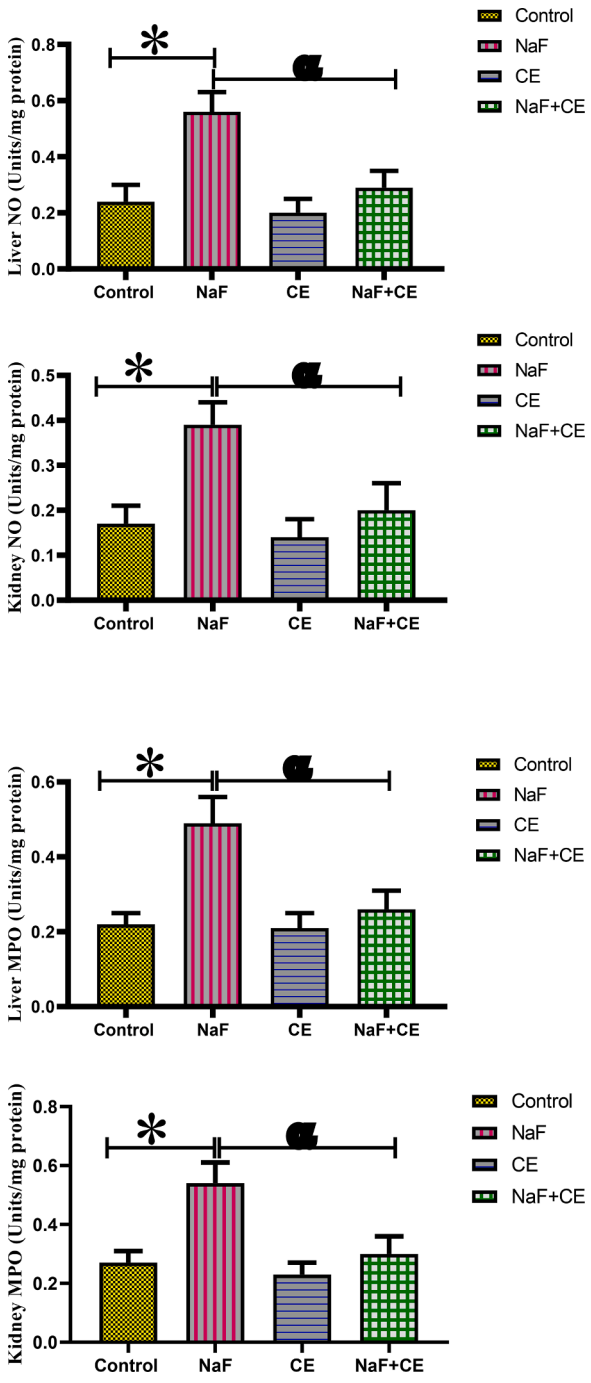

Fig. 3. Effect of *Cyperus esculentus* nut extract on NO and MPO activity in the liver and kidney in experimental NaF-exposed rats. Values are expressed as Mean ± S.E.M, $n = 6$ in each group, *: represent a significant difference from control, α: represent a significant difference from NaF at $p< 0.05$, One-Way ANOVA. Keys: CE, *Cyperus esculentus*; NaF, Sodium fluoride.

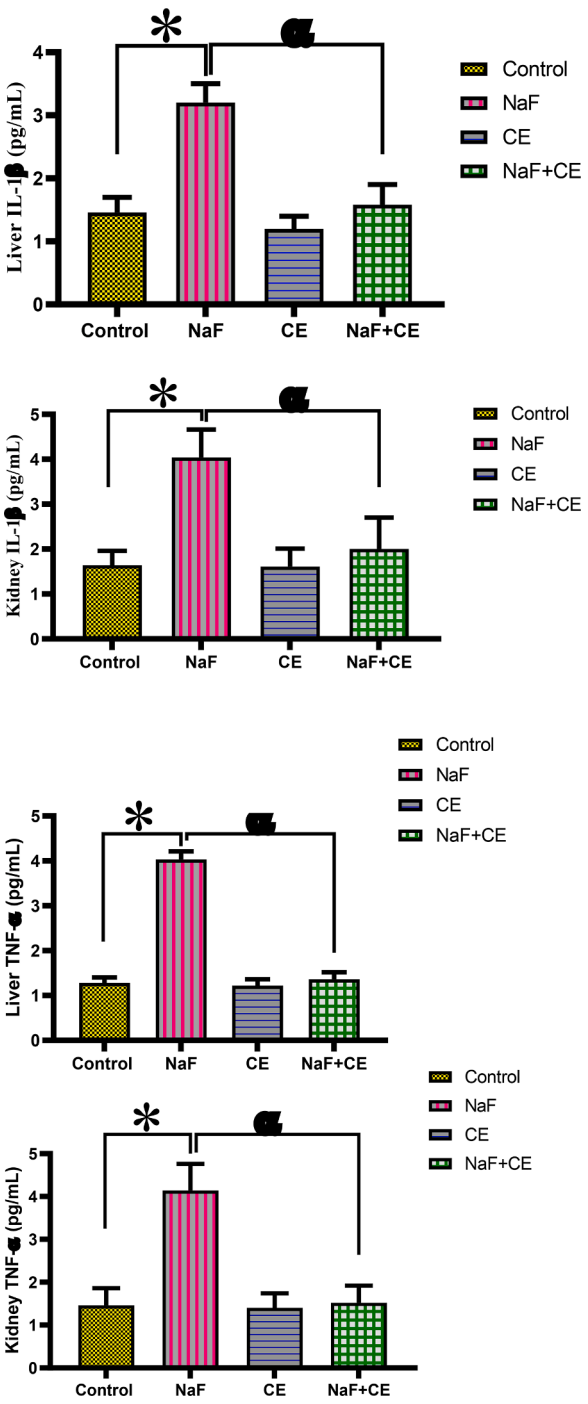

Fig. 4. Effect of *Cyperus esculentus* nut extract on the level of IL-1β and TNF-α in the liver and kidney in experimental NaF-exposed rats. Values are expressed as Mean ± S.E.M, $n = 6$ in each group, *: represent a significant difference from control, α: represent a significant difference from NaF at $p< 0.05$, One-Way ANOVA. Keys: CE, *Cyperus esculentus*; NaF, Sodium fluoride.

2.10.6. Statistical analysis

Data were subjected to analysis using Analysis of Variance (ANOVA) with the aid of graph pad prism. Data from each parameter was expressed as mean value ± standard deviation. Data were considered to be significantly different at a 95% confidence level ($P<0.05$).

3. Results

3.1. Body, liver, kidney, and relative organ weight

There was no significant difference between the initial and final body in animals treated with NaF alone ($p>0.05$). However, the animals in control, CE, and NaF+CE showed a statistically significant increase in final body weight as compared with initial body weight ($p<0.05$). The final body weight, weight of liver and kidney of the animals in NaF alone

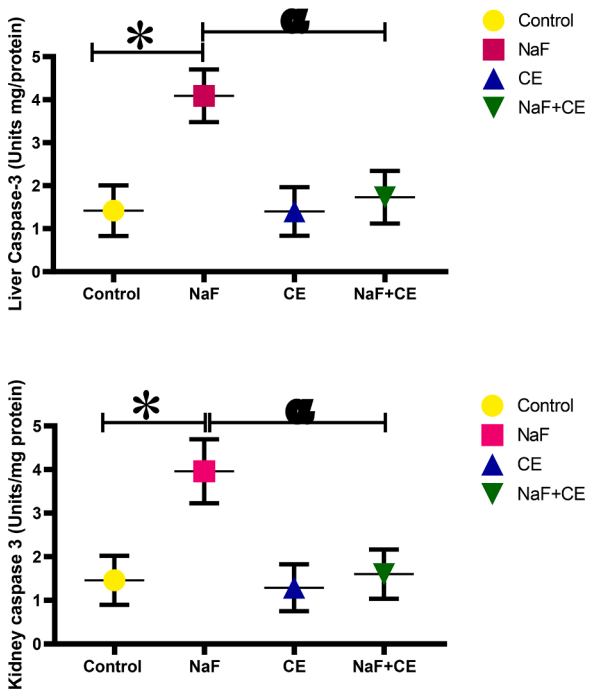

Fig. 5. Effect of *Cyperus esculentus* nut extract on caspase 3 activity in the liver and kidney in experimental NaF-exposed rats. Values are expressed as Mean ± S.E.M, $n = 6$ in each group, *: represent a significant difference from control, α: represent a significant difference from NaF at $p < 0.05$, One-Way ANOVA. Keys: CE, *Cyperus esculentus*; NaF, Sodium fluoride.

exposed group significantly decrease when compared to that of the control group. However, co-administration of NaF and CE significantly increase the final body weight and liver weight as compared to NaF administered only ($p<0.05$). Although, the final body weight, liver and kidney weight in CE alone, and NaF+CE showed no significant difference when compared to that of the control group. Also, the ratio of liver and kidney weight to body weight was almost the same in all groups [Table 1].

3.2. Hepatic and renal oxidative and antioxidant concentration

Chronic exposure to NaF alone significantly increases the concentration of liver and kidney MDA compared to control ($p<0.05$). However, the intervention of CE reduced the concentration of MDA in the liver and kidney significantly when compared to NaF alone treated animals ($p<0.05$). Also, CE administration significantly reduced liver and kidney MDA concentration as compared to that of NaF alone exposed group and NaF+CE treated animals ($p<0.05$). Although, the mean value of liver and kidney MDA in CE alone treated animals is insignificantly lower than that of a control animal ($p>0.05$) [Table 2].

Rats treated with NaF alone present a significant decrease in liver and kidney SOD, CAT, GPx, and GSH when compared with the control. However, the decrease in liver and kidney antioxidant enzymes concentration was elevated in rats co-treated with NaF and CE when compared with NaF alone ($p<0.05$). Furthermore, the group treated with CE alone exhibit a significant increase in liver and kidney SOD, CAT, GPx, and GSH activities compared to NaF alone exposed group ($p<0.05$). No significant difference in the liver and kidney SOD, CAT, GPx, and GSH activities in CE alone, NAF+CE, and control group ($p>0.05$) [Table 2].

3.3. Biomarkers of liver function

Administration of NaF significantly increase the serum activities of AST, ALT, ALP, and LDH compared to control ($p < 0.05$). Conversely, simultaneous administration of NaF and CE decreases the activities of AST, ALT, ALP, and LDH when compared to the group treated with NaF. Alone ($p<0.05$). The decrease in activities of ALT, ALP, and LDH in the group treated with NaF+CE was not significant compared to CE treated group. Whereas, decrease in activities of AST in NaF+CE treated group is significant when compared with CE alone treated rats. Treatment with CE alone showed a significant reduction in serum activities of AST, ALT, ALP, and LDH compared to NaF alone exposed rats ($p<0.05$) [Fig. 1].

3.4. Serum creatinine (Cr) and blood urea nitrogen (BUN)

Sodium fluoride administration significantly increase serum creatinine and blood urea nitrogen as compared with the control group ($p<0.05$). Administration of CE significantly decrease the elevated level of Cr and BUN levels induced by NaF as compared with NaF treated group ($p<0.05$). No significant difference in serum Cr and BUN levels between the CE-only treated rats and the control group [Fig. 2].

3.5. Pro-inflammatory biomarkers and caspase 3 activation

Administration of NaF significantly increased the hepatic and renal MPO activity as well as the levels of NO, IL-1β, and TNF-α in the treated rats when compared to control. Also, there was an increase in activity of hepatic and renal caspase-3 significantly in the treated NaF alone treated rats when compared with the control. However, the increase in MPO activity, as well as the levels of NO, IL-1β, and TNF-α decrease significantly in the liver and kidney of rats in the group treated with NaF+CE compared with NaF alone, treated rats. No observed significance is different in hepatic and kidney inflammatory and apoptotic makers between the CE, NaF+CE, and the control groups [Figs. 3-5].

3.6. Histological observation of liver and kidney

The histological section of the liver of the control group revealed normal histo-architecture of the liver tissue as well as a normal and organized monolayer of hepatocytes.

Section of liver of NaF exposed rats showed extensive histopathological changes including interface hepatitis around portal space (lymphocytic infiltration and necrosis of hepatocytes), necrosis of acinar hepatocytes, congestion, and tissue degeneration, sinusoidal dilation, vacuolar degenerations, severe hemorrhage and congestion, hydropic degeneration and disruption of epithelium lining of the central vein.

Section of the liver of CE treated rats: showed normal hepatic tissue with central vein surrounded by hepatocyte with polyhedral shape and view nuclei of eosinophil.

Section of the liver of NaF+CE treated rats: showed a normal and organized layer of hepatic tissue with evidence acinar hepatocytes and vascular regeneration and organization of epithelium lining of the central vein with view nuclei of eosinophil [Fig. 6].

The microscopic structure of the kidney section of the control group showed complete regular glomeruli, clear capillary network, the renal tubules had a clear outline, the surface of the brush was clear, and the epithelial cells were arranged regularly.

NaF kidney section showed swelling glomeruli, cracked capillary network, and renal tubules were extensively lesion, the epithelial cells became necrotic, a large number of cells accumulated in the lumen and were positive for eosin, renal interstitial congestion was more serious, and more inflammatory cells infiltrated into the interstitium. The kidney section of CE treated group revealed complete renal structure, regular glomeruli, and clear capillary network, clear renal tubules outline and brush surface, and regular arranged epithelial cells. The kidney section of NaF+CE treated rats had varied areas of normal dilated tubules and glomeruli at the periphery of the cortex and degenerated cortical tubules, with mild cellular infiltration of the renal parenchyma [Fig. 7].

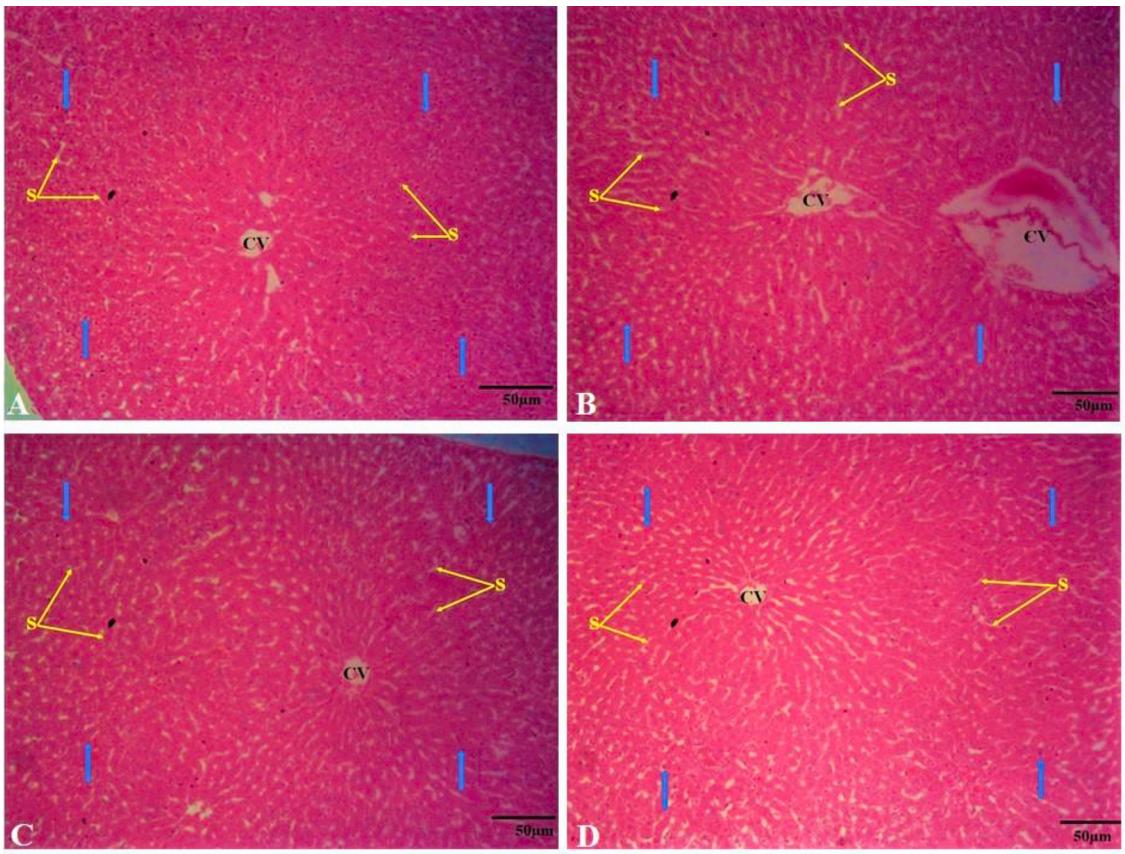

Fig. 6. Photomicrographs of the Liver of NaF treated rats showed marked degeneration, focal area of necrosis, and the presence of inflammatory cells. CE-treated rats appear somewhat similar to control. Rats co-exposed to NaF and CE showed the presence of few inflammatory cells.
CV: Central vein; G: Sinusoids; Arrows: Hepatocytes
Stains: H&E. Mg: X100, scale bar=50 μm.

3.7. Liver and kidney histopathological changes

There was observed a significant increase in pyknosis cell, fat deposition, infiltration of inflammatory cells, and congestion of RBCs of the liver tissue in NaF-treated rats as compared to the control group ($p<0.05$). however, co-administration of NaF and CE reduced fat deposition tissue and cell pyknosis, infiltration of inflammatory cells, and congestion of RBCs in liver tissue significantly compared to NaF exposed rats ($p<0.05$). No statistically significant difference in hepatic histopathological parameter between rats treated with CE and rats in the control group ($p>0.05$). Group treated with NaF+CE showed a significantly higher value in congestion of RBCs than the control group [Fig. 8].

Rats in the group treated with NaF showed a significant reduction in glomerulus diameter, and elevation in proximal tubule damage, infiltration of inflammatory cells, and congestion of RBCs of the renal tissue compared to the control group ($p<0.05$). Simultaneous administration of NaF and CE significantly increase glomerulus diameter and reduced proximal tubule damage, infiltration of inflammatory cells, and congestion of RBCs. No significant changes in renal abnormalities or histological changes between the CE and control group ($p>0.05$) but there was statistical significant between NaF+CE treated groups and the control group ($p<0.05$) [Fig. 9].

4. Discussion

The kidney and Liver are important organs involved in the detoxification, synthesis, and excretion of xenobiotics and related metabolites in the body (Airaodion et al., 2020a). They are especially vulnerable to damage by xenobiotics (Brzóska et al., 2003). Drug-induced nephrotoxicity is an important cause of renal failure (Shashi and Kaur, 2017). As the primary organ concerned with excretion and retention of fluoride, the kidney is quite sensitive to the toxicity of fluoride (Luo et al., 2017). Not surprisingly, the kidney is one of the major organs affected by fluoride intoxication, and numerous studies have established a close correlation between fluoride intake and renal injury (Nabavi et al., 2012a). Hence, rats chronically intoxicated with sodium fluoride (NaF) have been shown to display histological renal changes, interstitial edema, tubular destruction, and glomerular and medullary hyperemia (Nabavi et al., 2012a). Hand in hand with the typical kidney pathology, fluoride-intoxicated rats showed an increased rate of reactive oxygen species (ROS) generation and lipid peroxidation (Kobayashi et al., 2009). *Cyperus esculentus* is appeared to have more prospective usage as nourishment and industrial materials (Achoribo and Ong, 2017). Minerals such as potassium, phosphorus, magnesium, calcium, and iron are abundant in *Cyperus esculentus*. It's also rich in vitamins E and C, as well as a significant amount of vitamin B1 (Maduka and Ire, 2018).

According to the results of this study, there are was a significant decrease in the body weight and the relative kidney and liver weights of the animals administered with NaF only when compared with the control. The evaluation of relative organ weight changes has been used to detect the effects of chemically stimulated changes to an organ either by oral or inhalation routes in the evaluation of toxicological studies (Sellers et al., 2007). Further, it can be used to assess whether an organ or tissue was subjected to any damages or not (Chanda et al., 2015). However, the administration of CE extracts only, as well as its co-administration with NaF significantly increased the body and organ weights when compared with the NaF only group. *Cyperus esculentus* has

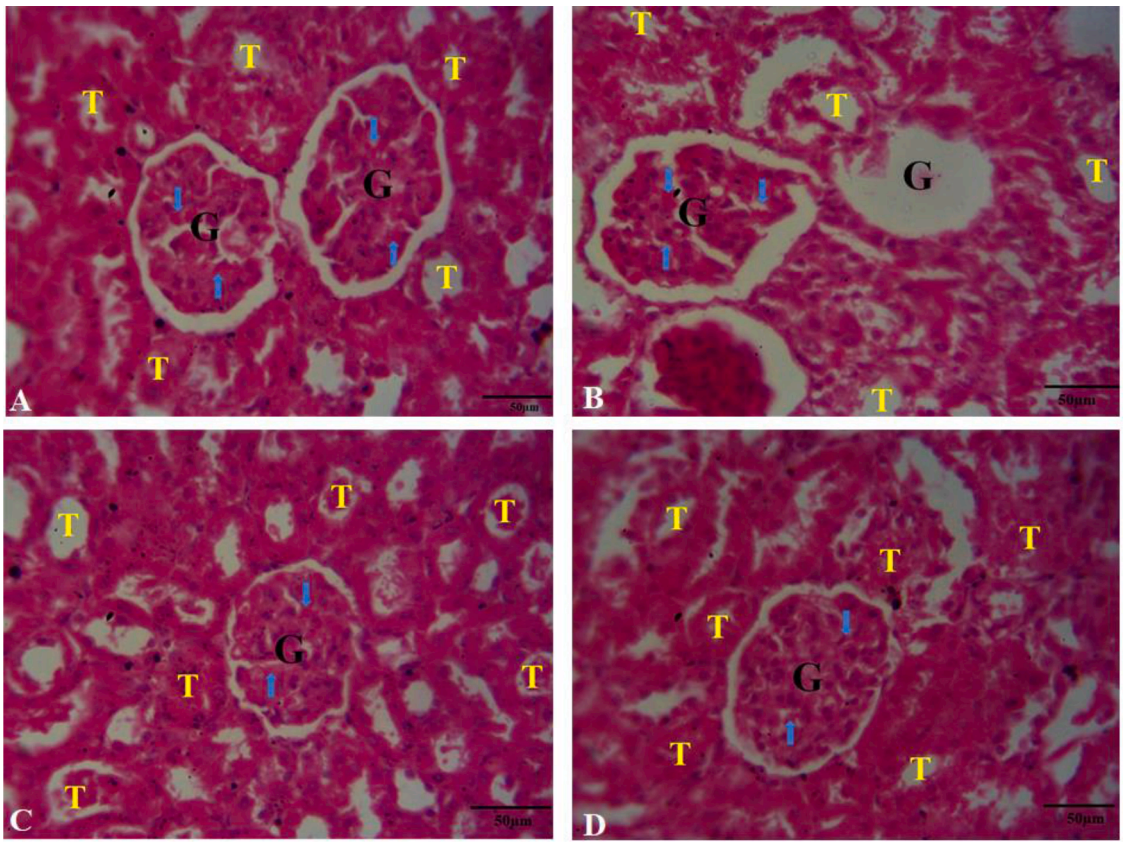

Fig. 7. Kidney of rats treated with NaF alone showing marked degeneration, focal area of inflammation, and disseminated congestion of vessels whereas CE alone showed kidney morphology. The kidney of rats co-exposed to NaF and CE showed mild disseminated congestion of vessels with few inflammatory cells.
G: Glomerulus; T: Renal tubules; Arrows: Renal capillaries
Stains: H&E. Mg: X100, scale bar=50 µm.

been shown to contain unsaturated fatty acids attributed to the positive role in the preservation of glomerular filtration rate and effective renal plasma flow (Siqun et al., 2013; Ezeh et al., 2014) which is an indication of its nontoxic nature which is consistent with the findings of (Hanaa and Hassan, 2007) and nutritional profiles have made tiger nut as unique food (Ekeanyanwu and Ononogbu, 2010).

Lipid peroxidation from oxidative stress disturbs the integrity of cellular membranes leading to the leakage of cytoplasmic enzymes (Salam and Agha, 2007). Free radicals and oxidative stress have been implicated in the pathogenesis of several xenobiotic toxicities, compromise in antioxidant defense, and increase in lipid peroxidation products in experimental fluorosis (Pandey et al., 2017). From this study, the kidney and liver tissues of NaF-treated animals showed significant elevation in the MDA levels in comparison with the control group. A significant reduction in the content of GSH and the activities of GPx, SOD, and CAT in kidney tissues in NaF-treated animals were also observed when compared to the control group. Experimental evidence has indicated that exposure to fluoride results in oxidative stress in both *vitro* and *in vivo* in soft tissues (Guan et al., 2000). Oxidative stress is induced by the increasing production of reactive oxygen species, such as superoxide anion, hydrogen peroxide, and hydroxyl radicals (Barbier et al., 2010). Reactive oxygen species can induce lipid peroxidation, inactive cellular enzymes, depolymerize polysaccharides, and induce deoxyribonucleic acid breaks and chromosome breakage (Salam and Agha, 2007). However, the administration of CE extracts only, as well as its co-administration with sodium fluoride significantly inhibited the elevation of MDA while also increasing the level of GSH, GPx, SOD, and CAT in the kidney and liver tissues when compared with the sodium fluoride only group. The ability of CE extract to positively impact these hepato-renal oxidative stress markers and antioxidant concentration levels could be due to the presence of antioxidants like quercetin, vitamins C and E, and trace element such as zinc (Belewu and Belewu, 2007; Allouh et al., 2015). These phytochemicals have powerful antioxidant properties and have been reported to be associated with improvement of oxidative stress and free radical scavenging (Dissanayake et al., 2009; Mohammadirad et al., 2013).

Serum urea and creatinine levels are an indication of kidney function both in men and in rodents (Airaodion et al., 2020b). Although serum creatinine and blood urea levels are both considered established markers for kidney function, serum creatinine is a more sensitive indicator since numerous other renal conditions such as dehydration can affect urea levels (Salhen and Mahmoud, 2016). The integrity of the kidneys was evaluated in this study via serum creatinine and urea levels. NaF administration significantly increased serum creatinine and blood urea nitrogen (BUN) as compared with the control group. This is following a previous study that revealed an augmented serum level of creatine and BUN following the administration of NaF, suggesting glomerular damage by fluoride intoxication (Nabavi et al., 2012b). The increase in serum BUN and creatinine levels are an indication of renal damage (Huang et al., 2018; Purena et al., 2018). The increase in BUN and creatinine might also be associated with hypertension-induced renal damage (Abdel-Zaher et al., 2018; Nunes et al., 2018). However, the administration of CE extracts only, as well as its co-administration with NaF significantly decreased the elevated creatine and BUN levels induced by sodium fluoride when compared with the NaF only treated group. This result is in agreement with the findings of Hassan (2007) who reported a decrease in the concentrations of creatinine and urea when animals were treated with *Cyperus esculentus* oil. The effect of administration of CE extract in the serum creatinine and urea levels observed in this study might be an indication that the integrity of the

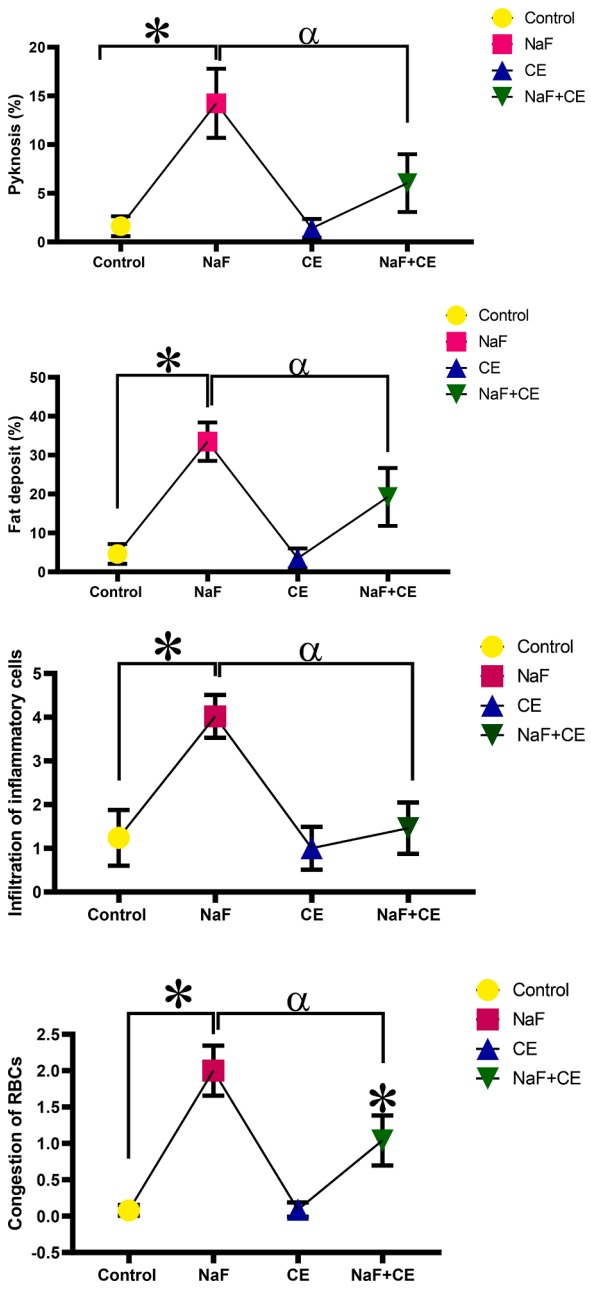

Fig. 8. Effect of *Cyperus esculentus* nut extract on liver histopathological changes in experimental NaF-exposed rats. Values are expressed as Mean ± S.E. M, $n = 6$ in each group, *: represent a significant difference from control, α: represent a significant difference from NaF at $p < 0.05$, One-Way ANOVA. Keys: CE, *Cyperus esculentus*; NaF, Sodium fluoride.

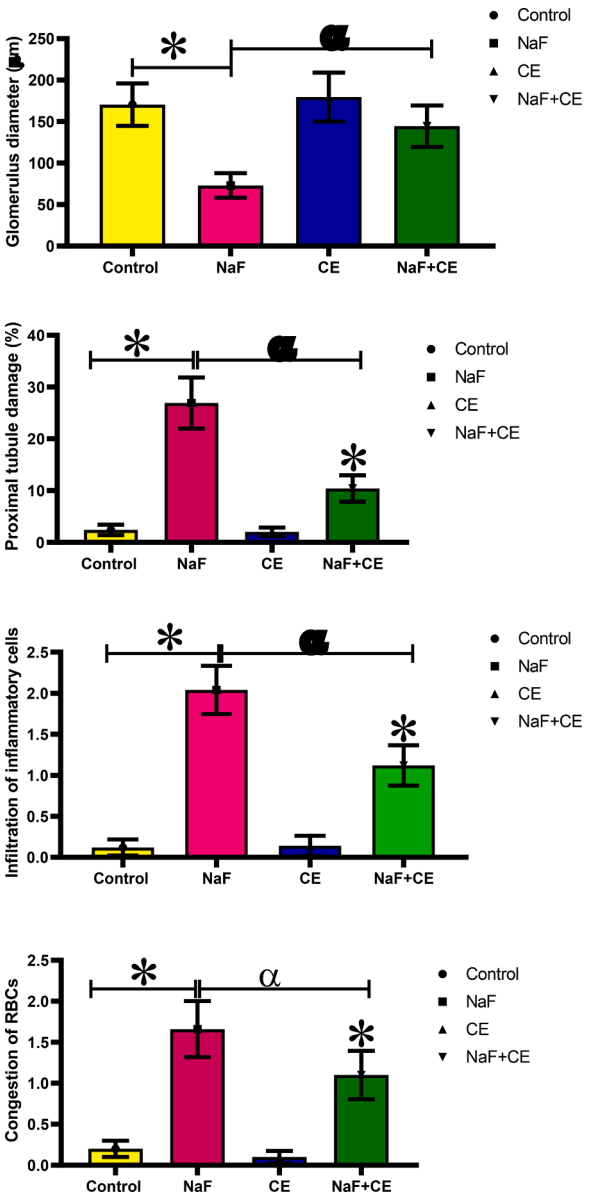

Fig. 9. Effect of *Cyperus esculentus* nut extract on Kidney histopathological changes in experimental NaF-exposed rats. Values are expressed as Mean ± S.E. M, $n = 6$ in each group, *: represent a significant difference from control, α: represent a significant difference from NaF at $p < 0.05$, One-Way ANOVA. Keys: CE, *Cyperus esculentus*; NaF, Sodium fluoride.

kidney was not compromised. *Cyperus esculentus* might have exerted this effect due to its phytochemical composition reported by Oguwike et al. (2017).

In this study, NaF treatment revealed a significant increase in MPO activity, the levels of NO, and pro-inflammatory cytokines (IL-1β and TNF-α) in the liver and kidney of rats. The MPO is often released by neutrophils and its activity is linked to the induction of oxidative stress and inflammation (Kato et al., 2016). TNF-α is a "master regulator" of inflammatory response because it recruits immune cells at the sites of injured tissues (Parameswaran et al., 2010). Therefore, an increase in the hepatic and renal concentrations of IL-1β and TNF-α in NaF-treated rats resulted in the induction of inflammation in the treated rats. Moreover, elevated TNF-α level has been shown to activate inducible nitric oxide synthase to produce NO which further combines with superoxide anion to form peroxynitrite, a noxious nitrogen species (Pacher et al., 2007). Thus, the increase in the hepatic and renal levels of NO and MPO in rats treated with NaF alone may contribute to hepatorenal damage by inducing inflammatory and nitrosative stress responses in the treated rats. Thus, NaF induced inflammation via mechanisms involving an augmentation in MPO activity with a concomitant increase in NO, IL-1β, and TNF-α levels in the liver and kidney in the treated rats. Conversely, a decrease in the MPO activity, levels of NO, IL-1β and TNF-α in rats treated with NaF+CE demonstrated the antagonistic impacts of CE on NaF mediated inflammatory response in liver and kidney of the treated rats and the impact of CE could be attributed to its strong antioxidant capacity. Caspase-3 is an aspartate-specific cysteine protease well reported to regulate the apoptosis cascade (Zhuang et al., 2000; Adedara et al., 2017). The marked increase in the hepatic and renal caspase-3 activity in CPF

alone-exposed rats connotes activation of apoptotic cell death. However, the reduction in the caspase-3 activity in rats treated with NaF+CE signifies the suppression of apoptotic cell death possibly due to the antagonistic and antioxidant role of CE on NaF in the treated rats.

The liver, being a very active organ, is involved in the metabolism and removal of toxins from the body. Its histology and biochemical characteristics are critical for detecting chemical toxicity. Section of liver of NaF exposed rats showed extensive histopathological changes including interface hepatitis around portal space, necrosis of acinar hepatocytes, congestion, and tissue degeneration, sinusoidal dilation, vacuolar degenerations, severe hemorrhage and congestion, hydropic degeneration, and disruption of epithelium lining of the central vein when compared the control that showed normal histoarchitecture characterized by a normal and organized monolayer of hepatocytes. This is following a previous study by Sewelam (2017), that also reported histological alterations in the liver of rats treated with fluoride characterized by shrinkage and nuclear dissolution of hepatocytes as well as other histopathological changes in the hepatic cells. The kidneys perform a lot of different functions, such as the excretion and removal of metabolic waste and foreign substances through urine (Pizzorno, 2015). The kidney is organized into many lobes, organized in a pyramidal structure, where the outer portion is made up of cortex, and the inner portion is made up of the medulla (Jayasumana et al., 2015). From this study, the microscopic structure of the kidney section of the control group showed complete regular glomeruli, clear capillary network, the surface of the brush was clear, the renal tubules had a clear outline, and the epithelial cells were arranged regularly. Swollen glomeruli, cracked capillary network, renal tubules were extensively lesion, the epithelial cells became necrotic, a large number of cells accumulated in the lumen and were positive for eosin, renal interstitial congestion was more serious, and more inflammatory cells infiltrated into the interstitium were all observed in the sodium fluoride only treated group. Studies have shown that fluoride toxicity can lead to cell death, apoptosis, and/or necrosis both *in vivo* and *in vitro*. Zhan et al. (2006) indicated that supplemental fluoride treatment caused severe renal histological changes as well as increased renal cell apoptosis. Necrosis has been observed as a primary mechanism of cell death in the presence of relatively high fluoride concentrations (Barbier et al., 2010). However, *Cyperus esculentus* extract reversed the histopathological damages inflicted by sodium fluoride. This can be attributed to the phytochemical composition of *Cyperus esculentus* reported by Oguwike et al. (2017).

4. Conclusion

This study provided direct evidence for the altered body-organ weight, antioxidant–oxidant balance, liver and kidney function indices, pro-inflammatory biomarkers, and caspase 3 activity and histology in the liver and kidney following fluoride intoxication. This study also demonstrated the direct beneficial effect of *Cyperus esculentus* in preventing hepato-renal damage induced by fluoride. Based on the data provided in this study, further studies on clinical trials and toxicological parameters of *Cyperus esculentus* and related natural products are well merited.

CRediT authorship contribution statement

Sunday Aderemi Adelakun: Conceptualization, Methodology, Validation, Writing – review & editing, Investigation, Writing – original draft. **Babatunde Ogunlade:** Methodology, Project administration, Supervision, Investigation. **Obinna Peter Fidelis:** Formal analysis, Investigation, Writing – original draft. **Oluwafemi Abidemi Adedotun:** Writing – original draft, Investigation.

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Sunday Aderemi Adelakun: Conceptualization, Methodology, Validation, Writing – review & editing, Investigation, Writing – original draft. **Babatunde Ogunlade:** Methodology, Project administration, Supervision, Investigation. **Obinna Peter Fidelis:** Formal analysis, Investigation, Writing – original draft. **Oluwafemi Abidemi Adedotun:** Writing – original draft, Investigation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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