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Full Length Research Article

EFFECT OF AQUEOUS AND METHANOLIC EXTRACT OF *PILIOSTIGMA RETICULATUM* ON SODIUM FLUORIDE-INDUCED OXIDATIVE STRESS IN MALE WISTAR RATS

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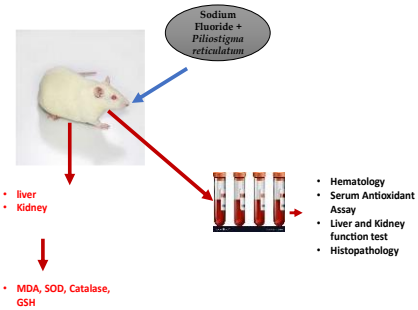
ABSTRACT
Daily exposure to sodium fluoride has been associated with oxidative stress. The effects of both aqueous and methanolic extracts of *Piliostigma reticulatum* on hematology and on oxidative stress markers in cardiac, liver, and kidney tissues were evaluated in this study. Twenty-four adult male Wistar rats were grouped into six groups of four animals each: group 1 (control) received distilled water only, group 2 received aqueous extract of *Piliostigma reticulatum* (APR) (100 mg/kg/day), Group 3 received methanolic extract of *Piliostigma reticulatum* (MPR) (100 mg/kg/day), group 4 received sodium fluoride (NAF) (600 mg/L) + APR, group 5 received NAF (600 mg/L) + MPR and group 6 received NAF alone for 2 weeks. Results from this study show that NAF and treatments with APR and MPR did not significantly alter the hematological parameters in rats. However, there was a significant increase ($P < 0.05$) in cardiac, liver, and kidney MDA. Also, there was a decrease ($P < 0.05$) in cardiac, liver, and kidney catalase, SOD, and GSH. Treatment with both APR and MPR ameliorated the oxidative stress in these organs. Histopathological examination reveals the presence of distortion of hepatic structure with extensive vacuolations in the sodium fluoride-treated group, which was ameliorated with APR and MPR treatments. In conclusion, aqueous and methanolic extracts of *Piliostigma reticulatum* ameliorated cardiac and hepato-renal oxidative stress induced by NAF in Wistar rats.

Keywords: sodium fluoride, oxidative stress, *Piliostigma reticulatum*, aqueous and methanolic extract, histopathology.



Graphical abstract

INTRODUCTION

Sodium fluoride occurs ubiquitously and is one of the elements needed by humans in trace amounts for maintaining dental health and hygiene (Yadav, 2018). Apart from its use in the manufacture of toothpastes and mouth washes industrially, it is used as a part of the raw materials for the production of some pesticides, insecticides, anticoagulants, pesticides, steel and glass fiber (Mohammed and Al-Okaily, 2017; Kashyap *et al.,* 2021; Le and Le, 2023). Animals and humans are exposed to sodium fluoride from sources such as fluoride-treated drinking water, cosmetic products, aerosols, beverages, vegetables, and food grains grown on agricultural lands that were treated with pesticides containing sodium fluoride (Kashyap *et al*., 2021). Although its use in households and industries is widespread, a lot of disadvantages have been reported (Kashyap et al., 2021). Fluoride toxicosis causes impairment of liver and kidney function (Mohammed and Al-Okaily, 2017; Dharmaratne, 2019). Fluoride toxicosis also induces myocardial injury. Myocardial damage is accompanied by the accumulation of myocardial enzymes, disruption of the arrangement of myocardial fibers, and excessive oxidative stress (Tian *et al*., 2023; Hou *et al*., 2024). Excess Sodium fluoride intake has been linked with various pathological conditions (neuro-inflammatory, neurodegenerative changes, and memory impairment) in the brain of Wistar rats (Perera *et al*., 2018). Oxidative stress has been implicated in the pathophysiology of the effect of sodium fluoride on these organs (Tian *et al*., 2023; Hou *et al*., 2024). Oxidative stress occurs when there is an increase in reactive oxygenated species beyond the capacity of antioxidant defense mechanisms of the body. This increase causes disruptions in biomolecules, which ultimately contribute to many disease conditions (Pisoschi *et al.,* 2020). Sodium fluoride causes oxidative stress by inducing mitochondrial swelling and affecting enzymes of cellular respiration. The net result in a decrease in adenosine triphosphate concentration, which in turn induces production of hydrogen peroxide and reactive oxygen species (Gupta *et al*., 2013; Labib *et al*., 2022). Previous studies reveal that sodium fluoride causes increase in systolic blood pressure, diastolic blood pressure, mean arterial pressure, malondialdehyde, protein carbonyl, myeloperoxidase, advanced oxidative protein products, together with significant reductions in glutathione peroxidase, superoxide dismutase, catalase, glutathione reductase, reduced glutathione, and nitric oxide (NO) bioavailability (Oyagbemi *et al*., 2018; Adetunji *et al*., 2023). Fluoride toxicosis also causes an increase in serum alanine aminotransferase, aspartate

aminotransferase, alkaline phosphatase, and lactate dehydrogenase activity (Azab *et al*., 2018). In the kidneys, sodium fluoride induces oxidative stress (Azab *et al*., 2018). Decreased antioxidant enzyme activity and increased malondialdehyde levels in the brain due to sodium fluoride exposure have been reported (Basha et al., 2011). *Piliostigma reticulatum* (Synonym: *Bauhinia reticulata*) is a leguminous medium-sized plant belonging to the family Caesalpiniaceae. It is a plant that occurs in the Sahelosudanian region of Africa from Senegal, Mauritania to Sudan and Nigeria (Fayanju *et al*., 2022). It is a tree, occurring up to 30ft in height with an evergreen, dense, spreading crown (Daniels *et al*., 2021). *Piliostigma reticulatum* is known to possess antioxidant properties due to its phytochemical composition, particularly it richness in tannins, flavonoids, and alkaloids contained in its leaves and stem bark (Boualam *et al.,* 2021; Daniel and Taye, 2021). The ameliorative effects of the aqueous and methanolic extract of *Piliostigma reticulatum* to tackle sodium fluoride-induced oxidative stress in the rat heart, liver, and kidney are yet to be studied. This study aims to evaluate the antioxidant effect of the aqueous and methanolic extracts of *Piliostigma reticulatum* against soium fluoride induced oxidative stress.

MATERIALS AND METHODS

Experimental Animals

Twenty-four (24) adult male Wistar rats were purchased from the Department of Veterinary Pharmacology and Toxicology, Faculty of Veterinary Medicine, Usmanu Danfodiyo University, Sokoto, and kept at the animal house of the Veterinary Teaching Hospital, Faculty of Veterinary Medicine, Usmanu Danfodiyo University, Sokoto. The experimental animals were acclimatized for 10 days by housing in plastic cages (4 rats per cage) with access to water and standard pelleted feed ad libitum.

Chemicals

Commercial grade sodium fluoride (Guangdong Guangua Sci-Tech Chemical Co. Limited, China) was obtained from a reputable chemical Store in Zaria, Kaduna State, Nigeria. *Piliostigma reticulatum* leaf was sourced from Kwalkwalawa village of Usmanu Danfodiyo University, Sokoto. The leaves were authenticated at the herbarium unit, Department of Botany, UDUS. The leaves were assigned a voucher number as UDUH/ANS/0742.

Plant extracts preparation

Aqueous Extraction:

One hundred (100) g of the *Piliostigma reticulatum leaves* was soaked in 1 litre of water in a clean container and allowed to stay for 24 hours. It was then filtered using a Muslin cloth and then put in a drying cabinet at 45-60ºC for evaporation and concentration.
The percentage yield is calculated as:

$$\% \text{ yield} = \frac{W3-W1}{W2} \text{ X } 100$$

Where W1 = Weight of empty plate (35.118g)
W2 = Weight of sample (500g)
W3 = Weight of plate + extract after drying (75.198g)

$$\% \text{ yield} = \frac{75.198-35.118}{500} \text{ X } 100$$

% yield = 8.016

Methanolic Extraction:

This was done as described by Abdulkadir *et al*. (2022). One hundred (100) g of *Piliostigma reticulatum* leaves was soaked in 1 litre of methanol in a clean container for 3 days. The container was shaken every 24 hours for a homogenous mixture. After that, it was filtered using a muslin cloth and then put in a drying cabinet at 45-60ºC for subsequent evaporation and concentration.
The percentage yield was calculated as:

$$\% \text{ yield} = \frac{W3-W1}{W2} \text{ X } 100$$

Where W1 = Weight of empty plate (53.117g)
W2 = Weight of sample (250g)
W3 = Weight of plate + extract after drying (78.017g)

$$\% \text{ yield} = \frac{78.017-53.117}{250} \text{ X } 100$$

% yield = 9.96

Animal Treatments

The animal experiments in the present study were performed under the approval of the institutional Animal Care and Use Committee of Usmanu Danfodiyo University, Sokoto, and were assigned the number UDUS/IACUC/2024/R06

Experimental Design

The experimental rats were randomly divided into 6 groups of 4 rats each as follows:
Group I: Control [1ml/day]
Group II: APR (Aqueous *Piliostigma reticulatum*) [100mg /kg/day]
Group III: MPR (*Methanolic Piliostigma reticulatum*) [100mg/kg/day]
Group IV: [NAF 600 mg/L] + MPR [100mg/kg/day]
Group V: [NAF 600 mg/L] +APR [100mg/kg/day]
Group VI: [NAF 600 mg/L] (Oyagbemi *et al*, 2018).
The treatments were done orally daily for 2 weeks (14 days), and weights were recorded weekly. The animals were sacrificed at the end of the experiment (on day 15) following light ketamine/xylazine anesthesia. Blood, heart, liver, and kidney samples were collected for hematological, biochemical, and histopathological analysis.

Blood Sample Collection

At the end of the experiment, blood samples (7mls) were drawn via cardiac puncture for the determination of the effect of treatments on hematological and serum biochemical analysis

Hematology

Three (3) mls of the blood samples collected were put into EDTA sample bottles. It was then processed at the clinical pathology laboratory of Veterinary Teaching Hospital, Usmanu Danfodiyo University, Sokoto. using an automated hemoanalyzer (HB 7021, China).

Serum Biochemical Analysis

Two (2) mls of the blood samples collected were put in clean phlebotomy tubes and allowed to clot. The clotted blood was centrifuged at 4,000 rpm for 10 minutes, and serum was collected into Eppendorf tubes and stored at -20°C until use. From the collected serum sample, serum oxidative stress parameters were analyzed using specific methods. Malondialdehyde (MDA) was analyzed using the method of Draper and Hadley (1990), glutathione peroxidase (GSH) was analyzed using the method described by Patterson and Lazarow (1955), Superoxide dismutase (SOD) and catalase were analyzed using the pyrogallol autooxidation method (Li, 2012).

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Liver and kidney function test

Two (2) mls of the blood samples for kidney and liver function analysis were transferred into clean phlebotomy tubes and allowed to clot. The clotted blood was centrifuged at 4,000 rpm for 10 min, and serum was collected into Eppendorf tubes and stored at -20 °C until use. From the collected serum sample, ALT, AST, ALP, Urea, and Creatinine were determined using Randox commercial kits as described by Reitman and Frankel (1957).

Tissue Antioxidant Analysis

Fresh tissue samples from the liver and kidney were weighed and homogenized in ice-cold saline using a glass homogenizer. Individually, each homogenate was centrifuged at 10,000 x g for 20 min. and the supernatant was collected for biochemical analysis.
The supernatant obtained from homogenization and centrifugation of liver and kidney samples was used for the estimation of catalase activity using the method of Claiborne (1985), glutathione peroxidase activity was determined as described by Rotruck *et al*. (1973), and superoxide dismutase was determined by measuring the inhibition of auto-oxidation of epinephrine at pH 10.2 as described by Misra and Fridovich (1972). The MDA level was calculated as described by Varshney and Kale (1970).

Histopathology

The histopathological examinations of the whole liver and kidney of the rats were conducted in accordance with the method described by Luna (1960). 20 Sections (5 µm thick) of each liver and kidney were cut with a microtome and stained with haematoxylin and eosin (H & E) for histopathological examination under a light microscope (Olympus - CX 41, Japan) at x 400 magnification

Statistical analysis

Data obtained from this experiment were expressed as Mean ± SD and subjected to a one-way analysis of variance test with Tukey's post hoc test for comparison. Statistical analysis was performed with the aid of GraphPad Prism (version 8.0). $P < 0.05$ was considered significant.

RESULTS

The study revealed that there were no significant changes ($P > 0.05$) in the hematological parameters following subacute oral administration of aqueous and methanolic extracts of *Piliostigma reticulatum* (Table 1). However, for the serum markers of hepato-renal damage, the mean ± SD serum total protein and ALP concentrations were significantly higher ($P < 0.05$) in all the treatment groups compared to the control (Table 2). For the serum AST, ALT, urea, and creatinine concentrations, the NAF + APR, NAF + MPR, and NAF groups were significantly higher ($P < 0.05$) compared to the control. Although the NAF + APR treatment group was significantly higher ($P < 0.05$) compared to the NAF + MPR groups in the serum liver and kidney biochemical tests (Table 2).

Table 1: Effect of subacute oral administration of aqueous and methanolic extracts of *Piliostigma reticulatum* on hematological parameters in Wistar rats.

PARAMETER	CONTROL	APR	MPR	NAF + APR	NAF + MPR	NAF
WBC (10^9/L)	15.89 ± 1.20	20.42 ± 2.96	15.85 ± 0.22	15.79 ± 0.58	16.10 ± 0.35	19.85 ± 1.29
RBC (10^6/µL)	7.11 ± 0.82	6.59 ± 0.39	6.52 ± 0.36	7.03 ± 0.10[a]	6.81 ± 0.14	6.08 ± 0.25
Hb (g/dL)	260.50 ± 26.50	239.70 ± 7.22	245.00 ± 8.53	261.8 ± 5.84	251.7 ± 5.67	240.0 ± 23.00
PCV (%)	43.80 ± 1.75	38.01 ± 1.90	39.11 ± 1.23	41.40 ± 1.00	39.30 ± 1.55	37.40 ± 3.28
Neutrophil (%)	75.30 ± 1.53	73.72 ± 0.49	75.13 ± 0.31	75.8 ± 0.52	74.50 ± 0.54	74.46 ± 1.31
Lymphocytes (%)	11.53± 0.83	12.51 ± 0.33	11.59 ± 0.16	11.23 ± 0.28	11.99 ± 0.33	12.12 ± 0.89
Eosinophils (%)	7.89 ± 0.42	8.26 ± 0.11	7.96 ± 0.01	7.78 ± 0.14	8.10 ± 0.12	8.06 ± 0.26
Monocytes (%)	5.26 ± 0.28	5.51 ± 0.12	5.31 ± 0.06	5.21 ± 0.10	5.39 ± 0.07	5.37 ± 0.17
Basophil (%)	ND	ND	ND	ND	ND	ND
MCV (fL)	58.85 ± 2.45	54.93 ± 0.64	57.38 ± 2.23	56.1 ± 0.63	54.93 ± 1.32	58.60 ± 2.80
MCH (Pg)	36.63 ± 0.70	36.55 ± 1.24	37.78 ± 1.02	56.80 ± 0.63	37.03 ± 0.63	39.40 ± 2.19
MCHC (g/L)	623.50 ± 37.50	664.30± 14.88	659.00± 16.39	663.80 ± 8.87	674.80 ± 12.42	672.50 ± 5.50
PLT (10^9/L)	339.50 ± 48.50	335.30± 37.92	304.30± 74.13	391.8 ± 50.93	396.3 ± 66.03	373.50± 37.50

Values are expressed as mean ± SD, WBC (White blood cell), RBC (Red blood cell), Hb (Hemoglobin), PCV (Packed cell volume), MCV (Mean corpuscular volume), MCH (Mean corpuscular hemoglobin), MCHC (Mean corpuscular hemoglobin concentration), PLT (Platelet).

Table 2: Effect of subacute oral administration of aqueous and methanolic extracts of *Piliostigma reticulatum* on serum makers of hepato-renal damage in Wistar rats.

PARAMETER	CONTROL	APR	MPR	NAF + APR	NAF + MPR	NAF
Total Protein (g/dl)	1.23 ± 0.03[a]	3.158 ± 0.04[b]	4.81 ± 0.21[b]	4.83 ± 0.03[b]	5.56 ± 0.09[b]	6.95 ± 0.13[b]
ALP	14.90 ± 0.85[a]	27.43 ± 3.56[b]	28.3 ± 1.59[c]	23.9 ± 3.41[c]	33.2 ± 1.68[c]	64.8 ± 3.9[c]
AST	24.32 ± 1.82[a]	24.6 ± 0.74[a]	27.9 ± 0.49[a]	34.2 ± 2.81[b]	64.73 ± 0.90[c]	124.75±1.34[c]
ALT	31.34 ± 0.74[a]	60.2 ± 0.42[b]	65.05 ± 5.86[b]	67.73 ± 5.13[b]	100.72 ± 2.19[c]	145.60±2.5[d]
Urea	4.097 ± 0.13[a]	7.92 ± 0.22[b]	10.48 ± 0.24[b]	12.24 ± 0.41[c]	20.75 ± 0.13[c]	45.20 ±0.74[d]

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Creatinine	6.24 ± 0.03[a]	10.70 ± 0.04[b]	11.74 ± 0.02[b]	13.99 ± 0.23[b]	15.38 ± 0.02[c]	17.73± 0.02[c]

Values are expressed as mean ± SD Data with different alphabets ([a,b,c,d]) are Significant (P< 0.05) different from control. ALP (Alkaline Phosphatase), AST (Aspartate Aminotransferase), ALT (Alanine Aminotransferase).

Serum malonaldehyde (MDA) concentration.

The effect of aqueous and methanolic extracts of *Piliostigma reticuatum* on serum MDA concentration in Wistar rats is shown in Figure 1. Serum MDA concentrations were significantly elevated (P < 0.05) in the NAF-treated rats compared to the control, APR, MPR, NAF + APR, and NAF + MPR groups. Treatments with the extracts (NAF + APR and NAF + MPR) reduced the serum MDA concentration

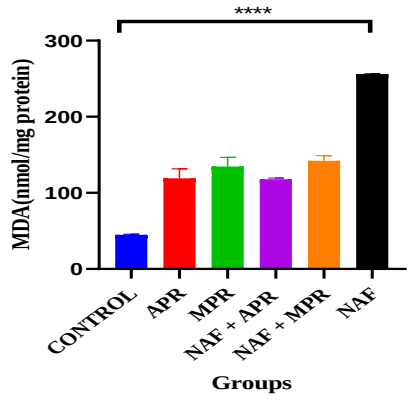

Figure 1: Effect of subacute oral administration of *Piliostigma reticulatum* on serum MDA concentration in Wistar rats. ****significant difference at p < 0.05

Serum glutathione (GSH) levels

The effect of aqueous and methanolic extracts of *Piliostigma reticulatum* on mean ± SD serum GSH levels in Wistar rats is shown in Figure 2. There was no significant difference (p > 0.05) between the control, APR, MPR, and NAF + APR treatment groups. However, the mean serum GSH levels in the NAF + MPR and NAF-treated groups were significantly lower compared to the control (P < 0.05).

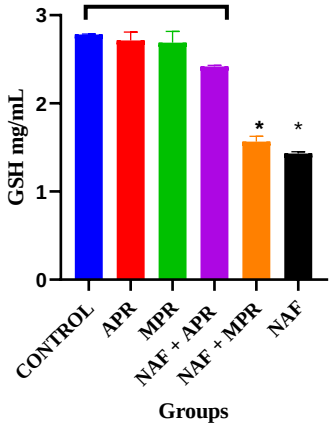

Figure 2: Effect of subacute oral administration of *Piliostigma reticulatum* on serum GSH activity in Wistar rats. ** significant difference at p < 0.05

Serum catalase activity

The effect of aqueous and methanolic extracts of *Piliostigma reticulatum* on mean ± SD serum catalase activity in Wistar rats is shown in Figure 3. Serum catalase activity was significantly lower (P < 0.0003) in the NAF treatment group compared to the control. Although treatment with the extracts improved the serum catalase activity in the APR, MPR, NAF + APR, and NAF + MPR groups, it was significantly lower compared to the control (P = 0.0004, 0.0008, 0.0004, and 0.0009, respectively).

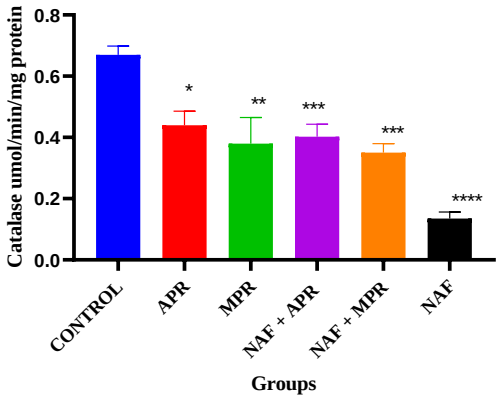

Figure 3: Effect of subacute oral administration of *Piliostigma reticulatum* on serum catalase activity in Wistar rats. *,**, ***, **** significant difference at p < 0.05

Serum superoxide dismutase (SOD) levels

Serum SOD levels were significantly lower (P < 0.05) in the APR, MPR, NAF + APR, NAF + MPR, and NAF treatment groups compared to the control (P = 0.018 for APR and P = 0.0001 for MPR, NAF + APR, NAF + MPR, and NAF) (Figure 4).

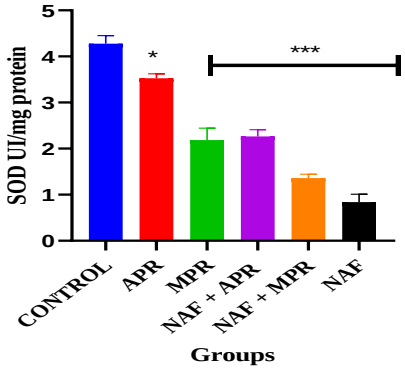

Figure 4: Effect of subacute oral administration of *Piliostigma reticulatum* on serum SOD activity in Wistar rats. *** significant difference at p < 0.05

Cardiac malondialdehyde (MDA) concentration

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The effect of aqueous and methanolic extracts of *Piliostigma reticulatum* on mean ± SD cardiac MDA concentration in Wistar rats is shown in Figure 5. Cardiac MDA concentration was significantly higher (P < 0.0001) in the NAF-treated groups compared to the control, APR, MPR, NAF + APR, and NAF + MPR treated groups.

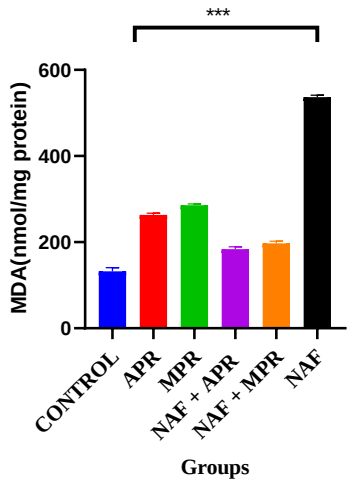

Figure 5: Effect of subacute oral administration of *Piliostigma reticulatum* on cardiac MDA concentration in Wistar rats. *** significant difference at p < 0.05

Cardiac catalase activity

Cardiac catalase activity was significantly lower (P < 0.05) in the APR, MPR, NAF + APR, NAF + MPR, and NAF-treated groups compared to the control. The APR treatment group was significantly higher (P > 0.05) than the MPR-treated rats. Furthermore, the NAF-treated rats had significantly lower (P < 0.05) catalase activity compared to the NAF + APR and NAF + MPR groups (Figure 6).

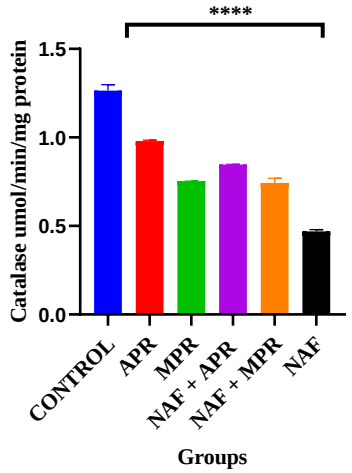

Figure 6: Effect of subacute oral administration of *Piliostigma reticulatum* on cardiac catalase activity in Wistar rats. **** significant difference at p < 0.05

Cardiac superoxide dismutase (SOD) levels

Cardiac SOD levels were significantly lower (P < 0.05) in the APR, MPR, NAF + APR, NAF + MPR, and NAF treatment groups compared to the control (P = 0.012 for APR and P = 0.0001 for MPR, NAF + APR, NAF + MPR, and NAF) (Figure 7).

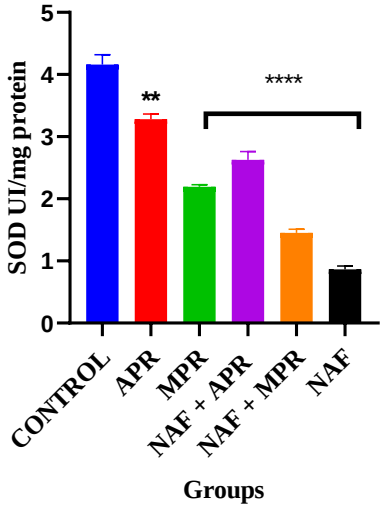

Figure 7: Effect of subacute oral administration of *Piliostigma reticulatum* on cardiac SOD activity in Wistar rats. **** significant difference at p < 0.05

Cardiac glutathione (GSH) levels

Cardiac GSH levels were significantly lower (P < 0.05) in the APR, MPR, NAF + APR, NAF + MPR, and NAF groups compared to the control (Figure 8). Treatment with both aqueous and methanolic extracts of *Piliostigma reticulatum* increased the cardiac GSH concentrations compared to the control.

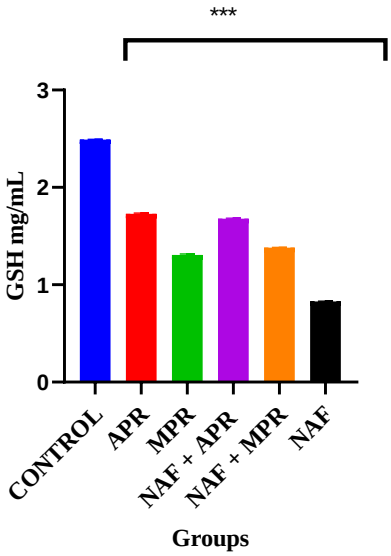

Figure 8: Effect of subacute oral administration of *Piliostigma reticulatum* on cardiac GSH activity in Wistar rats. *** significant difference at p < 0.05

Effect of Treatments on Hepato-renal Antioxidant Activity

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The effect of aqueous and methanolic extracts of *Piliostigma reticulatum* on mean ± SD MDA concentration in the liver of Wistar rats is shown in Figure 9. Liver MDA concentration was significantly higher ($P < 0.05$) in the NAF group compared to the control. Although the MDA concentration in the APR, MPR, NAF + APR, and NAF + MPR was significantly higher than the control, it ameliorated the effect of treatment with NAF.

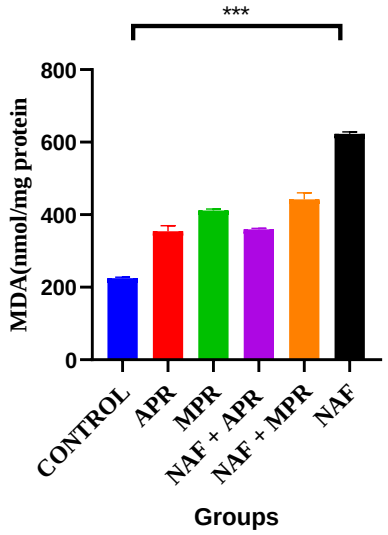

Figure 9: Effect of acute oral administration of *Piliostigma reticulatum* on liver MDA activity in Wistar rats. *** significant difference at $p < 0.05$

The effect of subacute administration of aqueous and methanolic extracts of *Piliostigma reticulatum* on kidney MDA concentration in Wistar rats is shown in Figure 10. Kidney MDA concentration was significantly higher ($P < 0.05$) in the APR, MPR, NAF + APR, NAF + MPR, and NAF groups compared to the control.

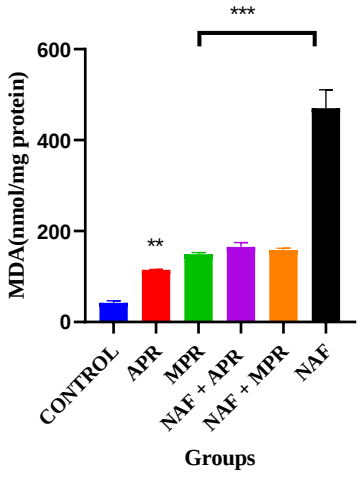

Figure 10: Effect of acute oral administration of *Piliostigma reticulatum* on serum kidney MDA activity in Wistar rats. **, *** significant difference at $p < 0.05$

Liver and kidney GSH concentration was significantly higher ($P < 0.05$) in the control group compared to the APR, MPR, NAF + APR, NAF + MPR, and NAF groups.(Figures 11 and 12).

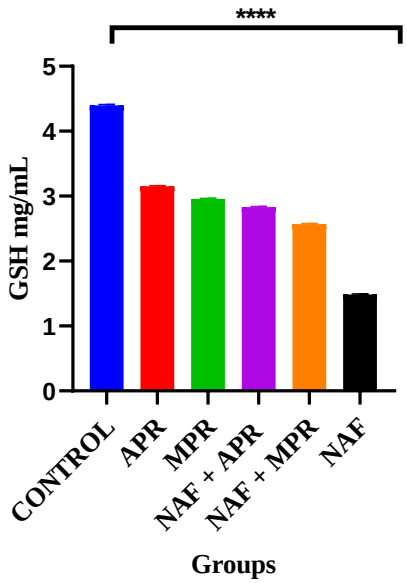

Figure 11: Effect of acute oral administration of *Piliostigma reticulatum* on liver glutathione activity in Wistar rats. **** significant difference at $p < 0.05$

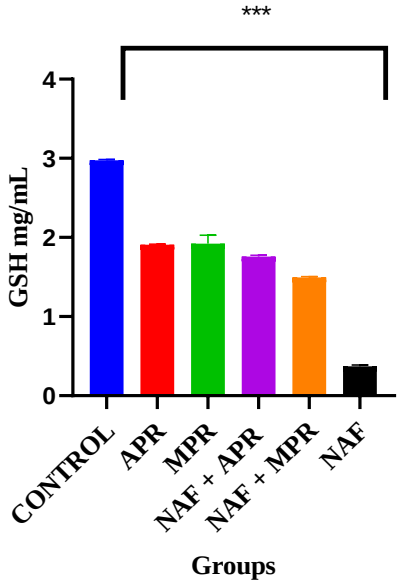

Figure 12: Effect of acute oral administration of *Piliostigma reticulatum* on kidney glutathione concentration in Wistar rats. *** significant difference at $p < 0.05$

The effect of treatment with aqueous and methanolic extracts of *Piliostigma reticulatum* on liver catalase levels in Wistar rats is shown in Figure 13. Liver catalase activity was significantly higher ($P < 0.05$) in the control group compared to the APR, MPR, NAF + APR, NAF + MPR, and NAF groups. Furthermore, kidney catalase

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concentration was significantly higher (P < 0.05) in the control group compared to the APR, MPR, NAF + APR, NAF + MPR, and NAF groups (Figure 14).

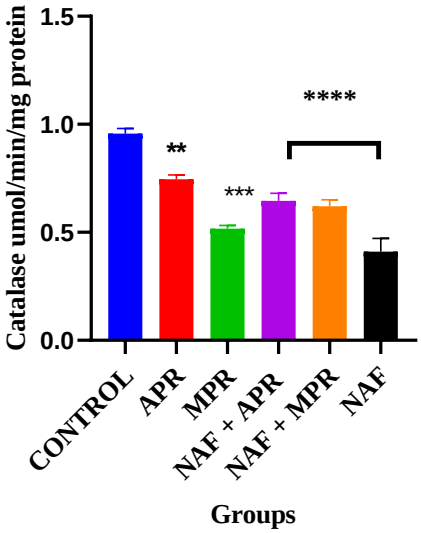

Figure 13: Effect of subacute oral administration of *Piliostigma reticulatum* on liver catalase activity in Wistar rats. **,***,**** significant difference at p < 0.05

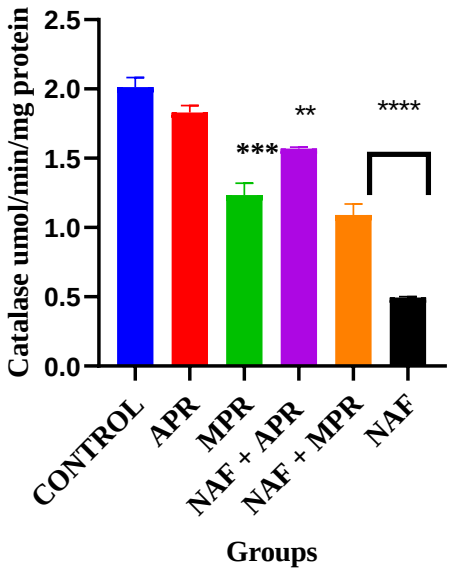

Figure 14: Effect of subacute oral administration of *Piliostigma reticulatum* on kidney catalase activity in Wistar rats. **,***,**** significant difference at p < 0.05

Liver and kidney SOD concentrations were significantly higher (P < 0.05) in the control group compared to the APR, MPR, NAF + APR, NAF + MPR, and NAF groups. Treatments in the NAF + APR and NAF + MPR reversed the reduction in SOD seen in the NAF group (Figures 15 and 16)

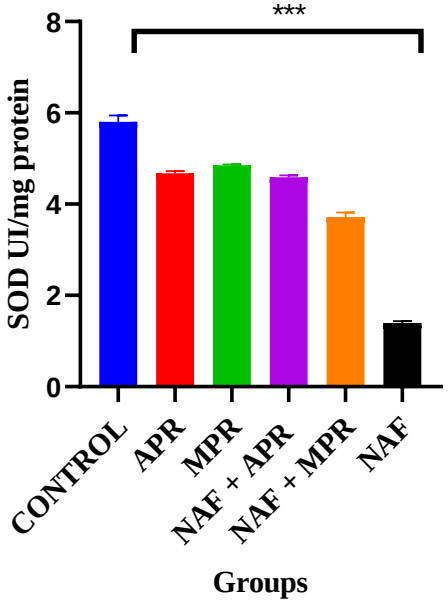

Figure 15: Effect of subacute oral administration of *Piliostigma reticulatum* on liver SOD activity in Wistar rats. *** significant difference at p < 0.05

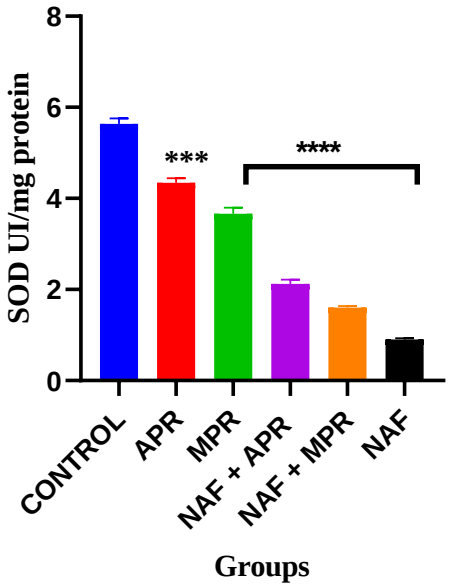

Figure 16: Effect of subacute oral administration of *Piliostigma reticulatum* on kidney SOD activity in Wistar rats. *** significant difference at p < 0.05

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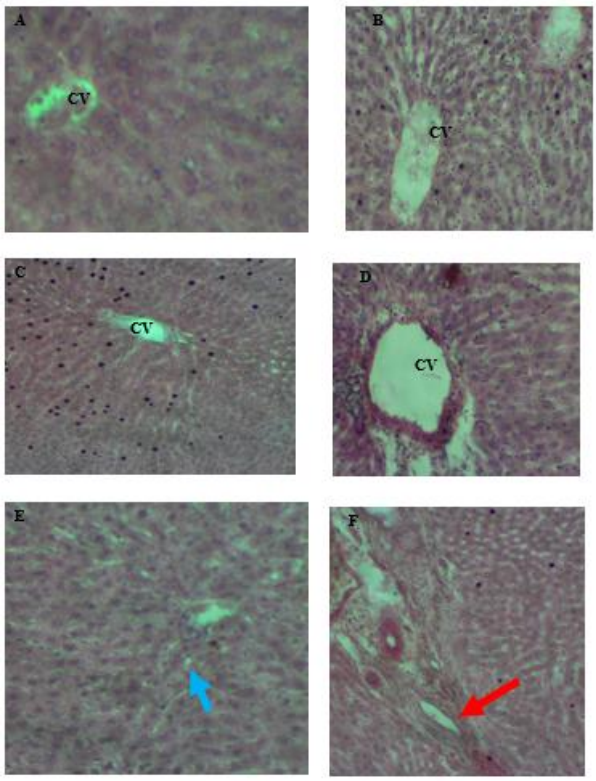

Figure 17: Photomicrographs of sections of the liver of Wistar treated with (A) control and (B) Aqueous extract of *Piliostigma reticulatum*, showing normal liver architecture. (C: Methanolic extract of *Piliostigma reticulatum* and D: treatment with NAF and APR showing extensive vacuolations. E: Treatment with NAF + MPR showing sinusoidal dilation with vacuolations. F: treatment *reticulatum* showing normal kidney architecture. (D: treatment with NAF and APR, E: treatment with NAF + MPR, and F: treatment with NAF alone, showing granular dystrophy of the renal tubules (H and E X 10).

DISCUSSION

Excessive sodium fluoride intake has been reported to induce oxidative stress in vital organs of the body, such as the heart, liver, and kidney (Azab *et al*., 2018; Oyagbemi *et al*., 2018; Caglayan *et al*., 2021). Although not significant, the decrease in RBC, PCV, and Hb in the NAF may be due to their decreased production of bone marrow, which may result in anemia and low hemoglobin production (Abbas *et al.,* 2017). It has also been reported that NAF causes a decrease of RBC, which may affect their Hb-carrying capacity, consequently lowering Hb content (Abbas *et al*., 2017). Furthermore, both the aqueous and the methanolic extract of *Piliostigma reticulatum* showed no significant difference in the PCV, Hb, and RBC compared to the control. Other studies have explained that this may be due to the presence of saponin in *P. reticulatum* (Abdulkadir *et al.,* 2022). Saponins cause programmed cell death (eryptosis) in red blood cells by increasing calcium ion entry into cells, thereby causing cell shrinkage and cell membrane scrambling (Bissinger *et al*., 2014).

The significantly increased total protein, ALT, AST, and ALP in the NAF-treated group is an indication that sodium fluoride causes hepatocyte damage, in agreement with the works of Azab *et al.* with NAF showing distortion of hepatic structure with extensive vacuolations (Red arrow = vacuolation; blue arrow= sinusoidal dilation) (H and E X 10).

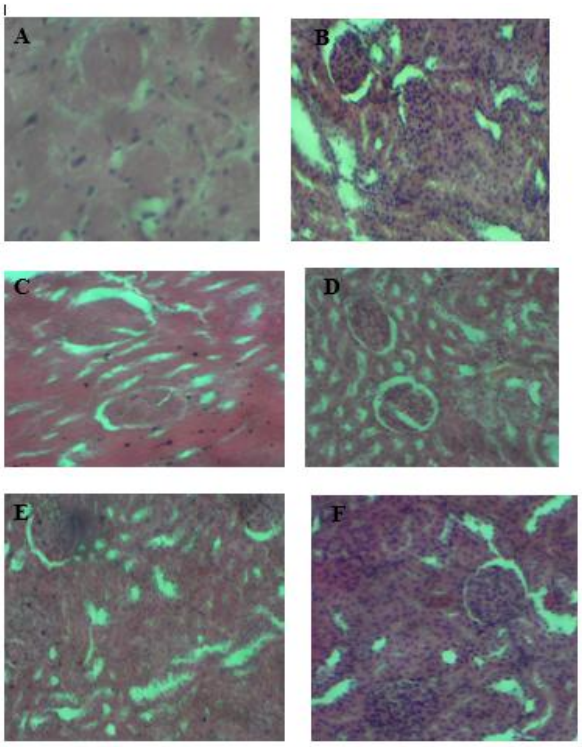

Figure 18: Photomicrographs of the sections of the kidney of Wistar treated with (A) control and (B: Aqueous extract of *Piliostigma reticulatum*) and (C: Methanolic extract of *Piliostigma*

(2018), Sewelam *et al*. (2017), and Caglayan *et al*. (2021). Reduction in the serum concentration of these enzymes in the NAF + APR and NAF + MPR groups shows *that Piliostigma reticulatum* ameliorated the damaging effect of NAF on the liver. As the primary organ of elimination and retention of fluoride, the kidney is sensitive to the toxicity of fluoride (Luo *et al.,* 2017). The integrity of the kidneys was evaluated in this study via serum creatinine and urea levels. NAF administration significantly increased BUN and creatinine levels compared to control and other treatment groups. The reduction seen in the NAF + APR and NAF + MPR shows that these extracts of *Piliostigma reticulatum* ameliorated the damage to the kidneys induced by NAF administration

Sufficient data suggest that NAF causes deleterious effects on the heart, with oxidative stress being the pinpointed mechanism of toxicity (Oyagbemi *et al*., 2018; Srinivas *et al*., 2021).

The association between sodium fluoride and changes in oxidative parameters is an important indicator of the toxicity of sodium fluoride on cellular mechanisms (Miranda *et al*., 2018) The great importance of evaluating oxidative stress markers such as MDA, SOD, GSH and catalase in the peripheral blood is that this site is a useful source of biomarkers, as can be easily obtained and evaluated in experimental subjects (Miranda et al., 2018). Malondialdehyde (MDA) is the final product of polyunsaturated lipid peroxidation in the cells. An increase in free radicals causes excessive production of MDA. In the present study, serum MDA

was increased in the NAF group compared to the control, suggesting oxidative stress (Oyagbemi et al., 2017). First line antioxidants evaluated in this study (SOD, catalase, and GSH) were significantly reduced in the serum of the NAF group compared to the control, which is also an indication that sodium fluoride induces oxidative stress, as reported by Oyagbemi *et al*., (2018); Srinivas *et al*. (2021). Though this reduction may not necessarily be as a result of the increase in pro-oxidative species caused by sodium fluoride exposure, but also from failures in the repair and replacement system of the body (Miranda *et al*., 2018)

Treatment with aqueous and methanolic extracts of *P. reticulatum* led to significant increases in the concentrations of these serum antioxidants of the Wistar rats, with the aqueous extract treatment (NAF + APR) showing increased concentrations compared to the methanolic extract (MPR + NAF). These results indicate the potential of aqueous and methanolic extracts of *P. reticulatum* in combating oxidative stress induced by sodium fluoride. This is in agreement with Beppe *et al.* (2023), who documented that *P. reticulatum* protected rats against monosodium glutamate-induced oxidative stress.

In the present study, there was a significant increase in the cardiac MDA concentration of the NAF group compared to the control. This shows increased lipid peroxication in the cardiac tissue of the Wistar rats. This is in agreement with Nabavi et al. (2012), and it was explained that this may be a result of iron release, which takes part in participates in Fenton-type reactions. Rats in the APR + NAF group and NAF + MPR group showed decreased MDA concentration, implying the extracts ameliorated oxidative stress. Similarly, as was seen in the serum first line antioxidants (SOD, GSH, and catalase) evaluated from the heart tissue of the Wistar rats were significantly reduced in the NAF group compared to the control, which also is an indication that sodium fluoride induces oxidative stress as reported by Oyagbemi *et al.* (2016) and Srinivas *et al.* (2021). In the present study, the significant decrease in the levels of GSH observed in NAF-treated rats suggests that the non-protein sulfhydryl groups and protein-bound sulfhydryl groups could alter each other via thiol disulphide reactions (Nabavi *et al*., 2012)

Treatment with aqueous and methanolic extracts of *P. reticulatum* led to significant increases in the concentrations of these antioxidants in the cardiac tissue of Wistar rats. These results indicate the potential of aqueous and methanolic extracts of *P. reticulatum* in combating oxidative stress induced by sodium fluoride in the heart tissue.

NAF causes oxidative stress and apoptosis in the liver. This oxidative stress is usually accompanied by increasing reactive oxygen species, which leads to increased MDA levels (Azab *et al.,* 2018). In this study, the liver and kidney MDA concentration was significantly increased, and this is in agreement with Caglayan *et al*. (2021), who reported that following NAF administration, liver and kidney MDA were significantly increased, and liver SOD, GSH, and catalase were significantly reduced. NAF causes cellular apoptosis in the liver and kidneys via an oxidative injury-dependent pathway that results in increased lipid peroxidation in cells, thus causing mitochondrial dysfunction and the activation of the downstream pathways (Adelakun *et al.,*2022; Caglayan *et al*., 2021). The antioxidant effect of aqueous and methanolic extracts of *Piliostigma reticulatum* in the liver and kidneys was demonstrated by the decreased MDA and increased liver SOD, GSH, and catalase. There were no histopathological changes seen in the heart tissue for all treated groups, possibly due to the short duration of administration. However, in the liver, oxidative stress-related changes such as sinusoidal dilation, enlarged portal vein, and vacuolations were seen in the group treated with NAF alone. These changes were ameliorated by the administration of aqueous and methanolic extracts of *Piliostigma reticulatum*.

CONCLUSION

Results from this study show that subacute administration of sodium fluoride at 600mg/L in Wistar rats causes oxidative changes in the serum, liver, and kidney of rats. It increases MDA concentration and reduces the free radical scavenging effect of antioxidant enzymes (catalase, GSH, and SOD). The results of this work also show that administration of aqueous and methanolic extract of *Piliostigma reticulatum* ameliorated the oxidative damage induced by sodium fluoride in the liver and kidneys of Wistar rats.

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