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Ameliorative Effect of *n*-Butanol Fraction of *Phoenix dactylifera* on Mercury-induced Nephrotoxicity in Wistar Rats

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Running Title: *P. dactylifera* and nephroprotection against $HgCl_2$

Abstract

Mercury is a highly toxic substance that poses a serious threat to living organisms. This work evaluated the protective effects of *n*-butanol fraction of *Phoenix dactylifera Linn* (BFPD) on mercury-induced kidney toxicity in Wistar rats. 25 rats were divided into 5 groups containing 5 rats each. Group I was administered 2 ml/kg of distilled water; group II was administered 5 mg/kg of mercury chloride ($HgCl_2$); groups III and IV received 500 mg/kg and 1000 mg/kg of BFPD followed by 5 mg/kg of $HgCl_2$ respectively. Group V was treated with 100 mg/kg of silymarin followed by 5 mg/kg of $HgCl_2$. All administrations were oral and lasted for 2 weeks after which the rats were euthanized and blood and kidney samples were collected for biochemical, histological, and histochemical studies respectively. $HgCl_2$ induced oxidative stress resulting in nephrotoxicity in the rats noticeable by altered levels of Na^{2+}, Ca^{2+}, K^{+}, Cl^{-} and HCO_3^{-}, and activities of SOD and catalase when compared to the control. However, BFPD treatment ameliorated these alterations. The group treated with $HgCl_2$ showed histological variations in the kidney such as dilated Bowman's capsule and glomerular shrinkage while histochemical analysis revealed reduced reactivity to glycogen moiety when compared to the control. Treatment with BFPD protected the histoarchitectural properties of the kidney comparable to the control. In conclusion, BFPD protected the kidney against $HgCl_2$-induced nephrotoxicity in rats due to its antioxidant (flavonoid) properties. Therefore, BFPD may be considered a noble candidate for treating and managing HgCl2-related nephrotoxicity.

Keywords: *Phoenix dactylifera*, Renal toxicity, Mercury chloride, Histochemistry, Oxidative stress.

INTRODUCTION

Mercury is a naturally occurring element that is found in air, water, and in soil. According to the World Health Organization (WHO) [1], mercury is one of the heavy metals posing consequential public health concerns. It is an environmental and industrial pollutant that provokes severe damage to tissues [2,3]. Mercury poisoning can result from inhalation, ingestion, or absorption through the skin and may be highly toxic and corrosive once absorbed into the bloodstream [4]. High exposure to inorganic mercury may result in damage to the gastrointestinal tract and the kidneys [5].

Among all heavy metals, mercury remains the major cause of nephrotoxicity in many parts of the world [6,7]. There is no effective treatment agaist the toxic effects of mercury. The use of chelating agents assists the body's ability to eliminate mercury from the tissues [8]. However, their use is accompanied by adverse effects [9]. Consequently, there is a need to identify natural remedies that could protect the kidneys from environmental toxins such as mercury [10].

The cultural use of medicinal plants is widespread in Africa [11]. Date palm (*Phoenix dactylifera*) fruits contain vitamins and mineral elements and are widely used in traditional medicine for the treatment of various ailments such as sore throat, fever, cystitis, gonorrhea, edema, kidney and abdominal troubles and also, to counteract alcohol intoxication [12, 13]. Several researchers have documented the antioxidant properties of *Phoenix dactylifera* owing to its antioxidant content such as flavonoids [14,-16]. Hence, there's a need to evaluate the potential of *Phoenix dactylifera* as an ameliorative agent following exposure to mercury chloride.

This study evaluated the ameliorative effect of the *n*-butanol fraction of *Phoenix dactylifera* against mercury-induced nephrotoxicity in Wistar rats.

MATERIALS AND METHODS

Experimental Animals

25 male Wistar rats weighing between 130 to 160 g were used. The rats were obtained from the Pharmacology Animal House, Faculty of Pharmaceutical Sciences, Ahmadu Bello University (ABU), Zaria. Wistar rats were kept under standard laboratory conditions in the Animal House of the Department of Human Anatomy, Faculty of Medicine, ABU, Zaria, where they were acclimatized for two weeks before the commencement of the experiments. The rats were fed with rat chow and water *ad libitum* and, were weighed at the beginning, mid-way, and at the end of the experiment.

Plant Material and Fractionation

Dried *Phoenix dactylifera L*. (date palm) fruits were obtained from a local market in Zaria, Kaduna, Nigeria, and authenticated in the Herbarium Unit of the Department of Biological Sciences, Faculty of Life Sciences, Ahmadu Bello University Zaria, where the voucher number 7130 was issued.

Preparation of the *n*-butanol fraction of *Phoenix dactylifera* fruit pulp was conducted in stages involving different solvents of extraction: methanol, ethyl acetate, and *n*-butanol according to the method described by Kriaa et al. [17]. The extraction was conducted according to the method described by Abdul-Wahab et al. [18] which involved manually separating the flesh of dried *Phoenix dactylifera L*. fruits from the pits and pulverized into powder using laboratory mortar and pestle. 500 g of the powder was macerated in 5 liters of methanol in a conical flask. After 36 hours, the solution (mixture of date palm fruit powder and methanol) was filtered using a filter rag and funnel. The filtrate was allowed to settle for a while, thereafter decanted and supernatant collected.

The obtained supernatant was heated to dryness in an evaporating dish (Royal Worcester; made in England) using H-H Thermometer Water Bath (Mc Donald Scientific International – 22050Hzl.0A International Number) at 60ºC. Thus, a semi-solid (pasty) methanol extract was obtained.

Subsequently, the methanol extract obtained was partitioned successively using ethyl acetate followed by n-butanol under similar conditions. Hence, *n*-butanol fraction of *Phoenix dactylifera L.* fruit was obtained. The preparation of n-butanol fraction of *Phoenix dactylifera* fruit was carried out in the Department of Pharmacognosy and Drug Development, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria.

Chemical and Drugs

Mercuric chloride: Fifty (50) grams of mercuric chloride (British Drug Houses (*BDH) Chemicals*, Poole, England) was used to induce nephrotoxicity.
Chloroform (British Drug Houses (*BDH) Chemicals*, Poole, England).

Silymarin (Silybon-70®) (Micro Labs Limited India).

Experimental Design

25 male Wistar rats were divided into five groups of five rats each. Group I served as control and was administered 2 ml/kg distilled water. Group II was administered mercuric chloride ($HgCl_2$) 5 mg/kg (12.5% LD_{50} as reported by Sheikh et al. [19]). Group III was administered 500 mg/kg of *n*-butanol fraction of *Phoenix dactylifera* (BFPD) followed by 5 mg/kg $HgCl_2$. Group IV was administered 1000 mg/kg of BFPD followed by 5 mg/kg $HgCl_2$. Group V was administered 100 mg/kg of Silymarin according to the method Ahmed et al. [20] followed by 5 mg/kg $HgCl_2$. The administrations were via the oral route and lasted for a period of 2 weeks.

Animal Euthanasia and Sample Collection

At the end of the experiment, the rats were euthanized under chloroform anesthesia. The thoracic cavity was dissected and blood samples collected via cardiac puncture into plain sample bottles for biochemical assessments. The abdominal cavity was equally dissected and the kidney was harvested for subsequent studies.

Phytochemical Screening

Qualitative phytochemical screening for secondary metabolites contained in n-butanol fraction of Phoenix dactylifera was conducted according the method of Trease and Evans [21] in the Department of Pharmacognosy and Drug Development, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria.

Body and Organ Weights

Before euthanasia absolute body weights of the animals were measured at the beginning (Initial Weight) and, at the end (Final Weight) of the experiment. Percentage weight change was computed as described by Agbon et al. [22], and means were

compared between the groups. The kidney was also weighed. Relative organ weight (Organosomatic index) was computed according to the method of Rahardjo et al. [23], as described by Agbon et al. [21] [(organ weight/ final body weight) ×100] and values obtained were analyzed and compared between the group.

Histological and Histochemical Studies

The harvested organ (kidney) was fixed in 10% buffered formal saline and processed using routine histological techniques for light microscopic examination. Processed histological sections were stained with Hematoxylin and Eosin (H&E) to demonstrate general histoarchitectural features. Sections were equally stained using histochemical stain (Periodic Acid Schiff) to demonstrate glycogen moiety. Histological tissue processing was carried out in the Histology Unit of the Department of Human Anatomy, ABU, Zaria.

Image Analysis - Quantification of PAS Reactivity

PAS-stained micrographs were quantified for PAS reactivity to glycogen moiety as described by Amber et al. [24] which involved measuring staining intensity using a computer running image analysis software (ImageJ® NIH, US) according to the manufacturer's specifications. The ImageJ® region of interest (ROI) manager tool for analysis of specific areas of the micrographs was employed[25]. The mean gray values for three ROIs were obtained and means were computed and analyzed.

Biochemical Studies

Biochemical assessments were conducted using the collected blood samples to assay for kidney function and oxidative stress biological markers. Kidney function was assayed through serum kidney electrolytes (Sodium - Na^+, Potassium - K^+, Chlorine - Cl^-, Hydrocarbonate - HCO_3^- and Calcium – Ca^{2+}) using the ELITE analyzer machine which analyzed the samples and reports values obtained. Oxidative stress biomarkers were assayed through lipid peroxide levels and antioxidant enzyme activity namely: malondialdehyde - MDA, superoxide dismutase – SOD, and catalase – CAT. Biochemical assessments were conducted in the Department of Chemical Pathology, Faculty of Basic Clinical Sciences, Ahmadu Bello University Teaching Hospital, Shika.

Data Analysis

Data obtained were expressed as mean ± S.E.M. Data was analyzed using GraphPad Prism (*version 9.3*). The presence of significant differences among means of the groups was determined using a one-way analysis of variance (ANOVA) with the *Tukey post hoc test*. Paired sample *t–test* was employed for the comparisons of means as appropriate. Values were considered significant when $p < 0.05$.

RESULTS

Phytochemical analyses

Qualitative phytochemical screening of n-butanol fraction of Phoenix dactylifera fruit pulp indicating the presence of secondary metabolites was carried out (Table 1). A yield of 12.44% (62.2 g) was obtained for n-butanol fraction.

Table 1: Phytochemical constituents of *n*-butanol fraction of *Phoenix dactylifera*

Constituents	Inference
Alkaloid	-
Anthraquinone	-
Cardiac glycoside	+
Flavonoid	+
Saponin	+
Steroid and tripenone	+
Tannins	+

+ = Present
- = Absent

Physical Observation

The physical activities of experimental animals including agility and behavioral patterns especially feeding habits were observed during the period of administration. Wistar rats in the control group were observed to exhibit normal movement and playfulness, whereas rats in the treatment groups exhibited reduced activities such as sluggishness and loss of appetite especially in the mercury ($HgCl_2$) treated group.

Body Weight and Organosomatic Index

The absolute body weights of the Wistar rats were measured and the initial and final weights were compared. The result showed that weight increase was observed in all treated groups with a remarkable increase in the control, BFPD, and silymarin-treated groups (**Figure 1a**). Weight change assessment revealed no significant difference in all treated groups when compared to the Control (**Figure 1b)**

The organosomatic index (relative organ weight) of the kidney revealed no significant difference in all treated groups as compared to the control (**Figure 1c**).

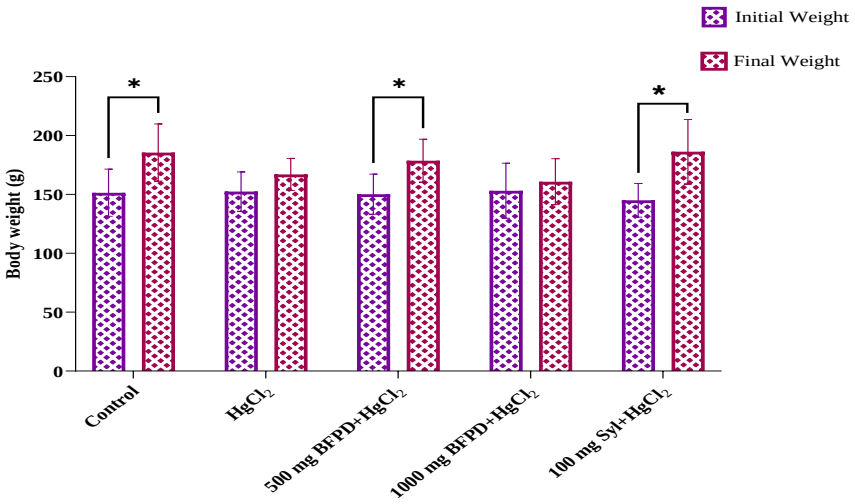

Figure 1a: Comparison of absolute body weight of Wistar rats

n= 5, mean ± SEM, Paired sample t-test, *=$p < 0.05$ when compared to control.

$HgCl_2$=Mercury chloride (5 mg/kg), *n*-BFPD=*n*-Butanol fraction of *Phoenix dactylifera* (500 mg/kg; 1000 mg/kg), Syl=silymarin (100 mg/kg)

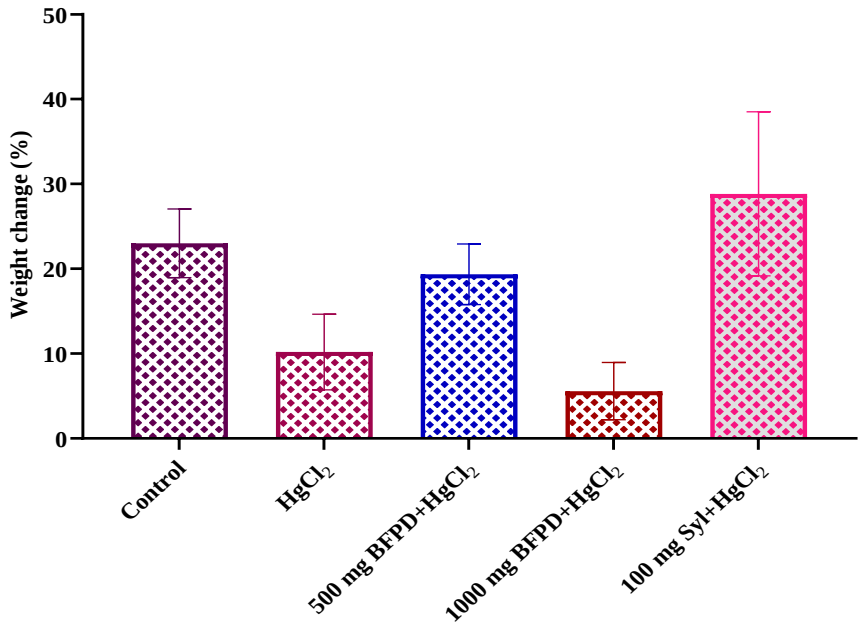

Figure 1b: Effect of *n*-BFPD on percentage (%) weight change of Wistar rats

n= 5, mean ± SEM, ANOVA, *p* is not significant when compared across the group.

$HgCl_2$=Mercury chloride (5 mg/kg), n-BFPD=*n*-Butanol fraction of *Phoenix dactylifera* (500 mg/kg; 1000 mg/kg), Syl=silymarin (100 mg/kg)

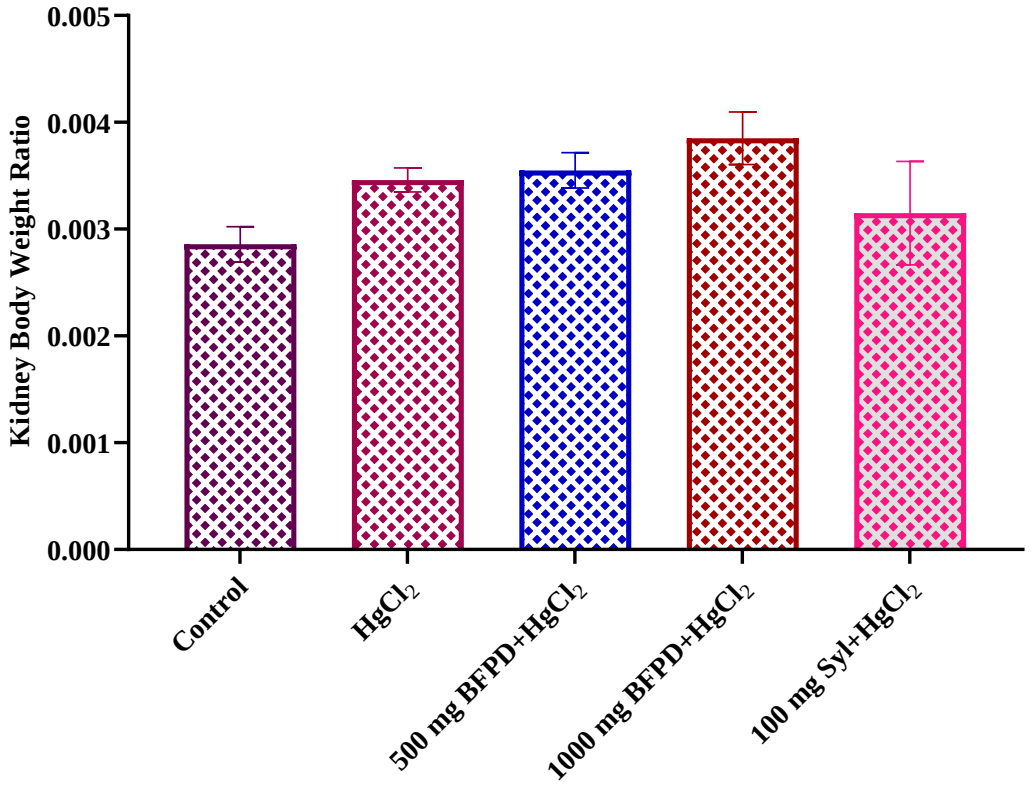

Figure 1c: Effects of *n*-BFPD kidney body weight ratio of experimental Wistar rats

n= 5, mean ± SEM, ANOVA, *p* is not significant when compared across the group.

$HgCl_2$=Mercury chloride (5 mg/kg), *n*-BFPD =*n*-Butanol fraction of *Phoenix dactylifera* (500 mg/kg; 1000 mg/kg), Syl=silymarin (100 mg/kg)

Histological results

Histological examination of the kidney sections of the control group revealed normal histoarchitectural features of the kidney as evidenced by the characteristic appearance of the outer renal cortex and an inner renal medullar. The renal cortex demonstrated renal corpuscle (Bowman's capsule, Bowman's space, glomerulus (anastomosing capillaries)), blood vessels, proximal convoluted tubule, and distal convoluted tubules (Figure 2; Plate A).

The $HgCl_2$–treated group revealed distortion of the histoarchitectural features of the kidney as dilated Bowman's space and shrunken glomerulus when compared to the control ((Figure 2; Plate B). The *n*-BFPD + $HgCl_2$ showed preserved histoarchitectural features of the kidney when compared to the control ((Figure 2; Plate C and D). Normal histoarchitectural features of the kidney were observed in Silymarin + $HgCl_2$ treated group when compared to the control ((Figure 2; Plate E).

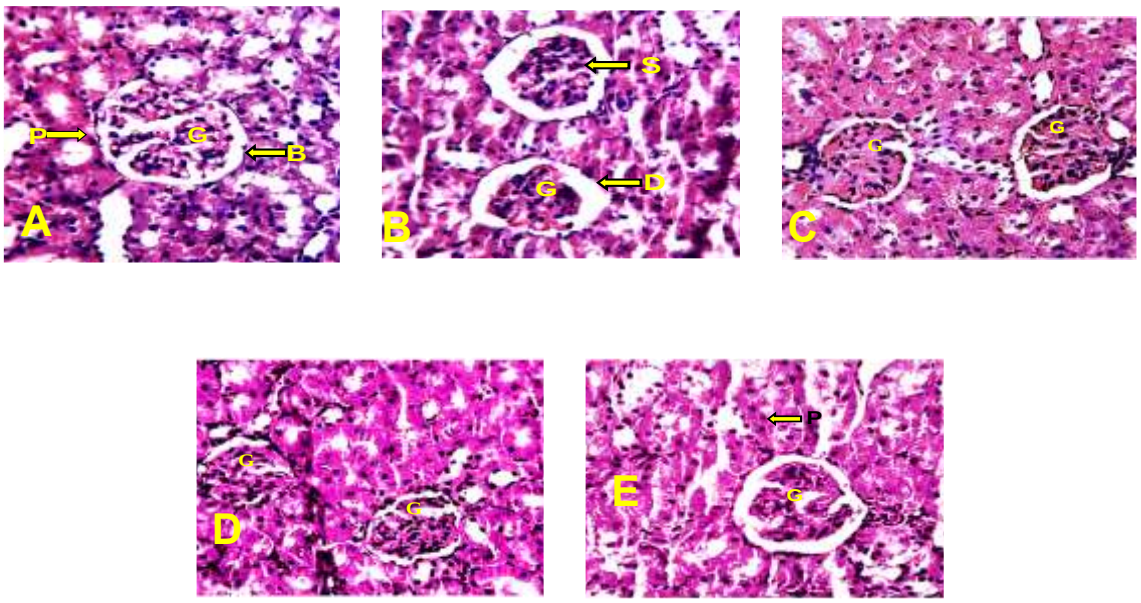

Figure 2: The kidney section of Wistar rats (H & E x250)

Plate A: Photomicrograph of the transverse section of kidney of Wistar rats administered 2 ml/kg H_20 (control group) with normal histoarchitectural features. G: Glomerulus; B: Bowman's space; P: Podocytes.

Plate B: Photomicrograph of the transverse section of kidney of Wistar rats administered $HgCl_2$ with distorted histoarchitectural changes. G: glomerular, D: Dilated Bowman's space, S: Shrunken glomerulus.

Plate C: Photomicrograph of the transverse section of kidney of Wistar rats administered 500 mg/kg *n*-BFPD and $HgCl_2$ with mild histoarchitectural distortion. G: Glomerulus.

Plate D: Photomicrograph of the transverse section of kidney of Wistar rats administered 1000 mg/kg *n*-BFPD and $HgCl_2$ with normal histoarchitectural features. G: glomerulus.

Plate E: Photomicrograph of the transverse section of kidney of Wistar rats administered Silymarin and $HgCl_2$ with mild distortions in the histoarchitectural features. G: Glomerulus, P: Podocytes.

Histochemical results

PAS staining for glycogen moiety revealed positive reactivity to PAS demonstrating the presence of membrane and cytoplasmic glycogen moiety in podocytes of the control group (Figure 3; Plate A). In the $HgCl_2$ –treated group, reduced reactivity to PAS was observed indicating depletion of glycogen moiety of the podocytes as compared to the control (Figure 3; Plates B). The *n*-BFPD + $HgCl_2$ and silymarin + $HgCl_2$ treated groups revealed PAS-positive stain intensity comparable to the control (Figure 3; Plates C - E).

Quantification of kidney PAS reactivity decreased nonsignificantly in all treated groups except *n*-BFPD (1000 mg/kg) + $HgCl_2$ when compared to the control (Figure 4).

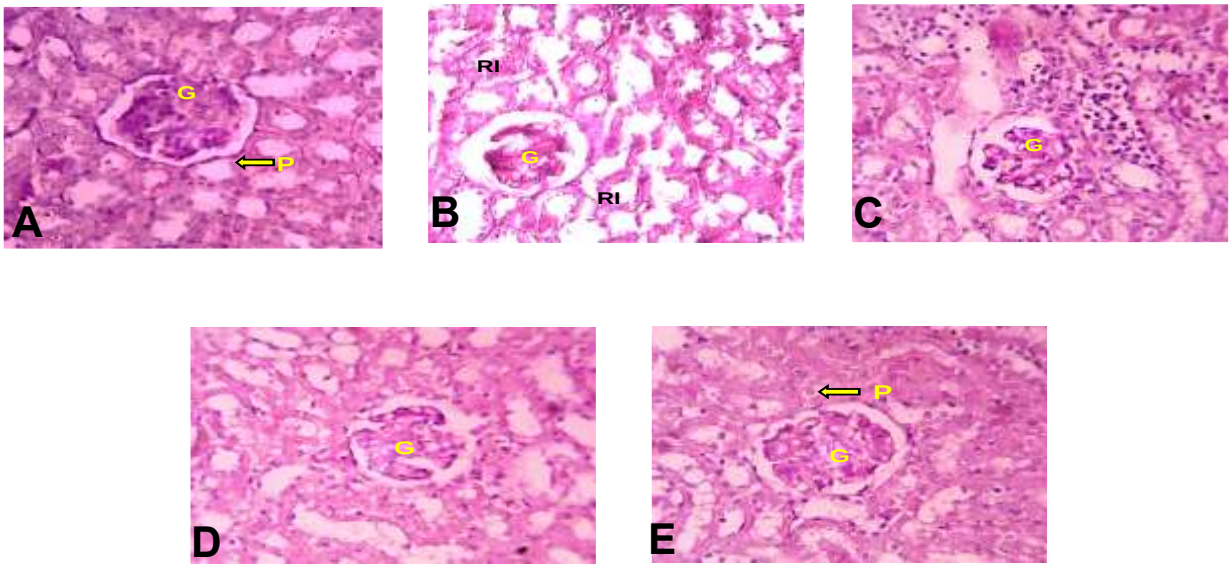

Figure 3: The kidney section of Wistar rats (PAS x250)

Plate A: Photomicrograph of the transverse section of kidney of Wistar rats administered 2 ml/kg H_2O (control group) with normal staining intensity. P: Podocytes; G: glomerulus.

Plate B: Photomicrograph of the transverse section of kidney of Wistar rats administered $HgCl_2$ showing reduced PAS stain intensity. G: glomerulus; RI: Reduced Stain Intensity.

Plate C: Photomicrograph of the transverse section of kidney of Wistar rats administered 500 mg/kg *n*-BFPD and $HgCl_2$ with mild PAS stain intensity. G: glomerulus.

Plate D: Photomicrograph of the transverse section of kidney of Wistar rats administered 1000 mg/kg *n*-BFPD and $HgCl_2$ with normal PAS stain intensity. G: glomerulus.

Plate E: Photomicrograph of the transverse section of kidney of Wistar rats administered 100 mg/kg Silymarin and $HgCl_2$ showing normal PAS stain intensity. G: glomerulus; P; podocytes.

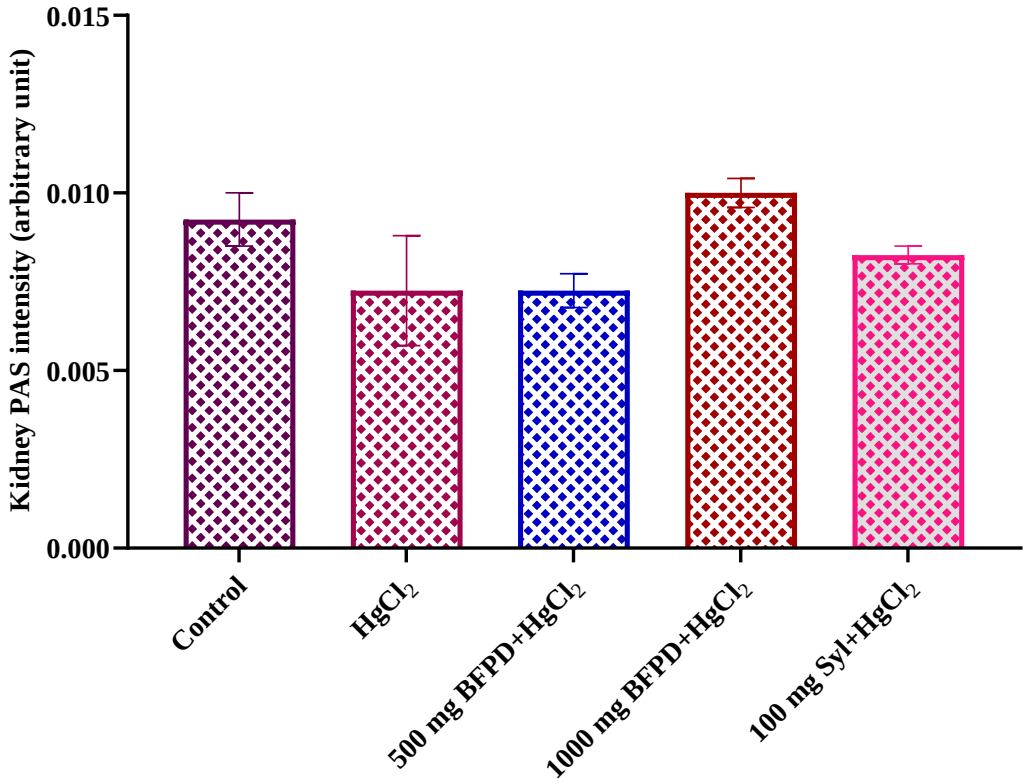

Figure 4: Effect of *n*-BFPD on kidney PAS intensity of Wistar rats

n= 5, mean ± SEM, ANOVA, *p* is nonsignificant when compared across the groups.

$HgCl_2$=Mercury chloride (5 mg/kg), *n*-BFPD =*n*-Butanol fraction of *Phoenix dactylifera* (500 mg/kg; 1000 mg/kg), Syl=silymarin (100 mg/kg).

Biochemical results

Kidney electrolytes

Evaluation of kidney serum electrolytes of Wistar rats revealed no significant differences in the levels of Na^{2+}, Cl^-, HCO_3^-, K^+ and Ca^{2+} in the treated groups when compared to the control group (Figures 5a-5e). Silymarin + $HgCl_2$-treated group revealed significant decrease in Ca^{2+} level relative to the $HgCl_2$-treated group (Figure 5e).

Oxidative Stress Biomarkers

Assessment of oxidative stress biomarkers associated with serum of Wistar rats revealed nonsignificant decrease in SOD activities in all the treated groups relative to the control (Figure 6a). CAT activity decreased significantly in $HgCl_2$-treated group in relative to the control (Figure 6b). MDA activity increased nonsignificantly in all treated groups as compared to the control (Figure 7).

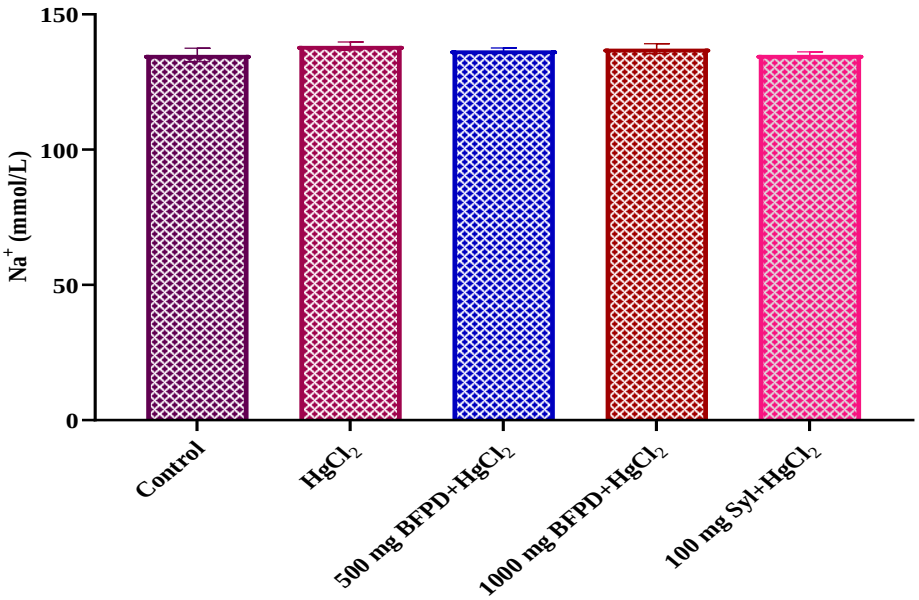

Figure 5a: Effect of *n*-BFPD on kidney sodium concentration of Wistar rats

n= 5, mean ± SEM, one way ANOVA, *p* is nonsignificant when compared across the group.

$HgCl_2$=Mercury chloride (5 mg/kg), *n*-BFPD =*n*-Butanol fraction of *Phoenix dactylifera* (500 mg/kg; 1000 mg/kg), Syl=silymarin (100 mg/kg)

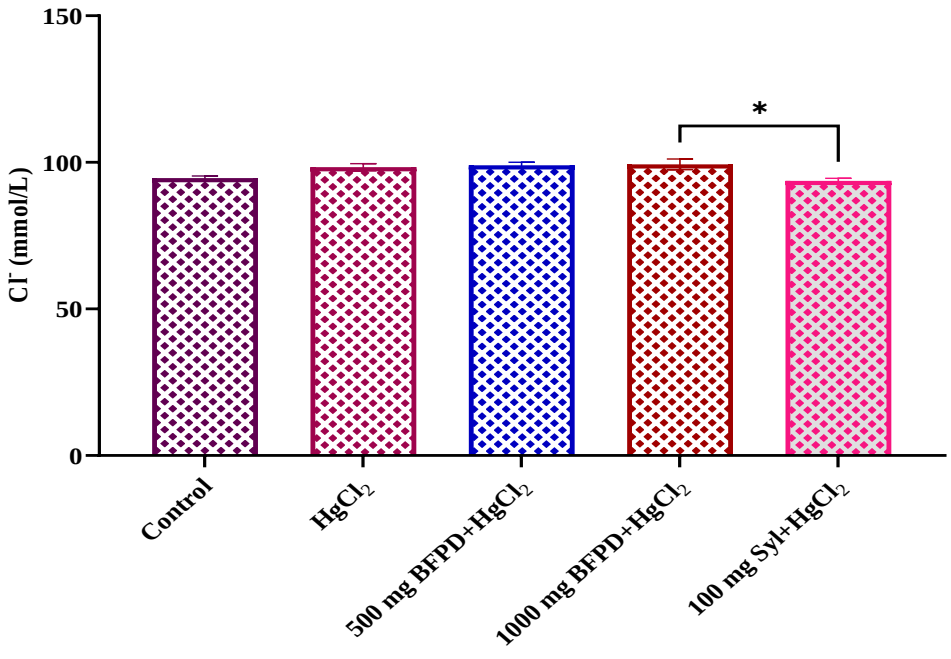

Figure 5b: Effect of *n*-BFPD on kidney serum chlorine concentration of Wistar rats

n= 5, mean ± SEM, one way ANOVA, *=*p* is significant when 1000 mg n-bfpd was compared to Silymarin group.

$HgCl_2$=Mercury chloride (5 mg/kg), *n*-BFPD =*n*-Butanol fraction of *Phoenix dactylifera* (500 mg/kg; 1000 mg/kg), Syl=silymarin (100 mg/kg)

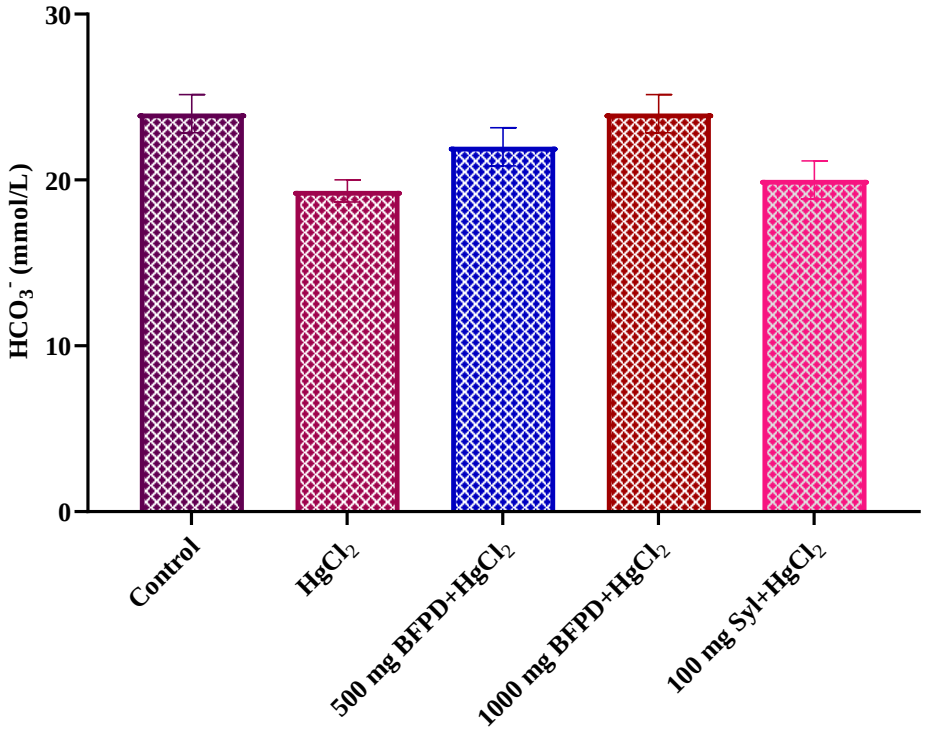

Figure 5c: Effect of *n*-BFPD on kidney serum bicarbonate concentration of Wistar rats
n= 5, mean ± SEM, one way ANOVA, *p* is nonsignificant when compared across the group.
$HgCl_2$=Mercury chloride (5 mg/kg), *n*-BFPD =*n*-Butanol fraction of *Phoenix dactylifera* (500 mg/kg; 1000 mg/kg), Syl=silymarin (100 mg/kg)

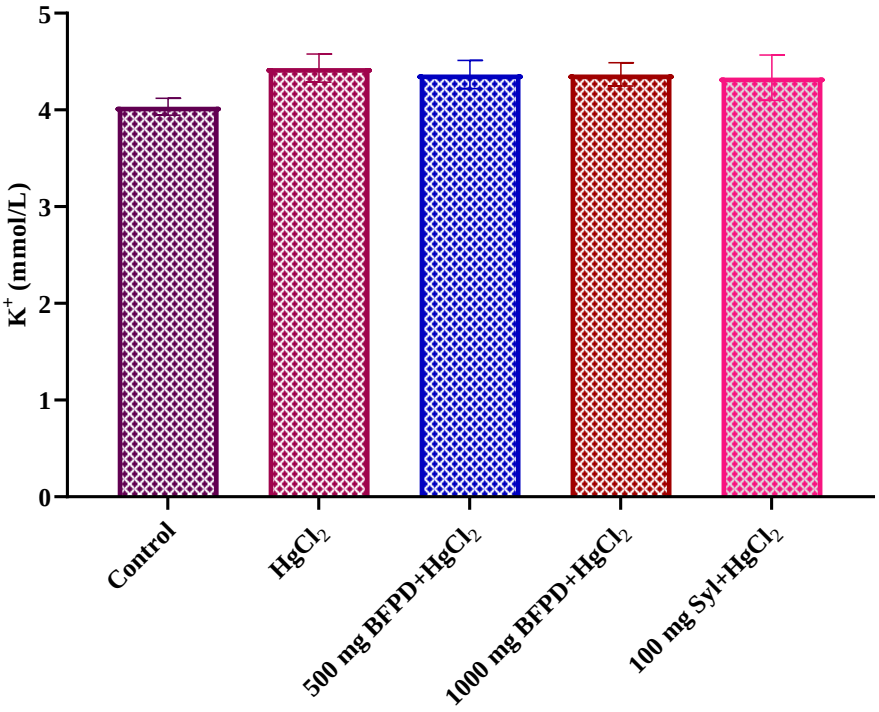

Figure 5d: Effect of *n*-BFPD on kidney serum potassium concentration of Wistar rats
n= 5, mean ± SEM, one way ANOVA, *p* is nonsignificant when compared across the group.
$HgCl_2$=Mercury chloride (5 mg/kg), *n*-BFPD =*n*-Butanol fraction of *Phoenix dactylifera* (500 mg/kg; 1000 mg/kg), Syl=silymarin (100 mg/kg)

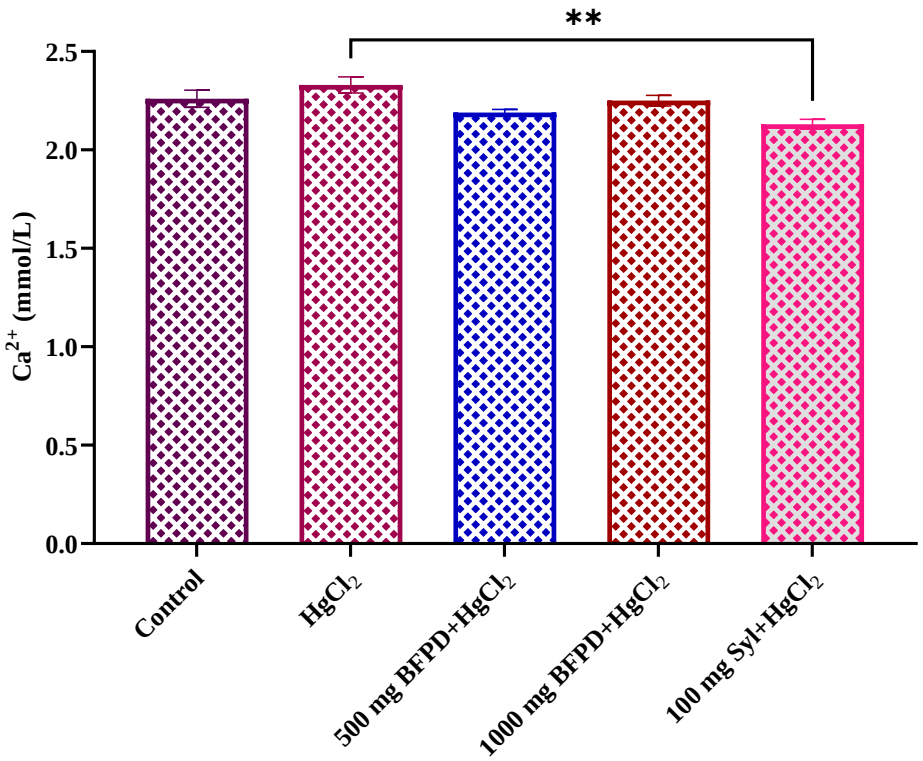

Figure 5e: Effect of *n*-BFPD on kidney serum calcium concentration of Wistar rats
n=5, mean ± SEM, one way ANOVA, *=*p* is significant when $HgCl_2$ group was compared to Silymarin group.
$HgCl_2$=Mercury chloride (5 mg/kg), *n*-BFPD =*n*-Butanol fraction of *Phoenix dactylifera* (500 mg/kg; 1000 mg/kg), Syl=silymarin (100 mg/kg)

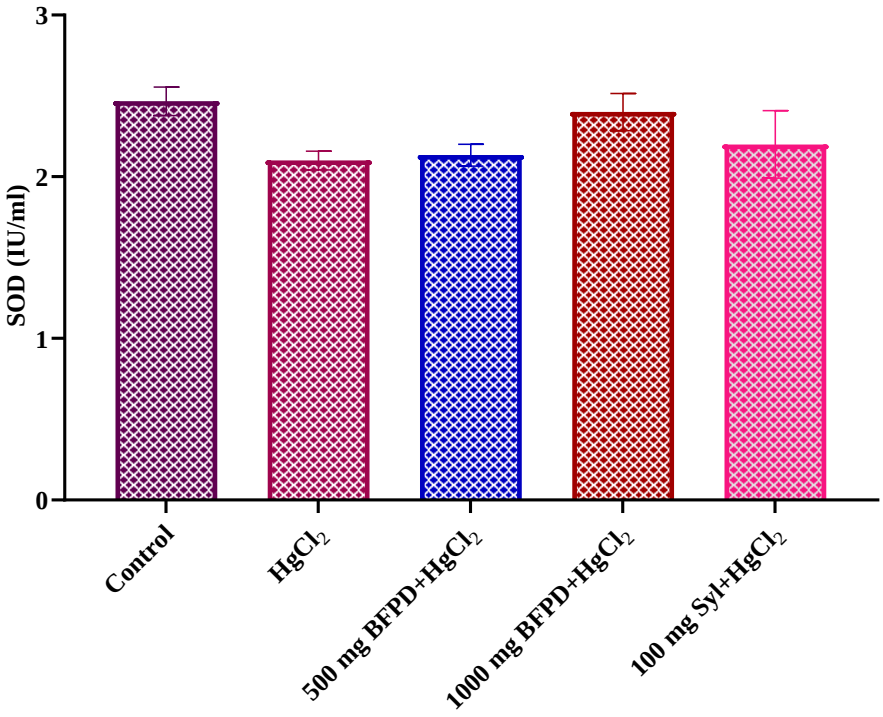

Figure 6a: Effect of *n*-BFPD on superoxide dismutase concentration of Wistar rats
n= 5, mean ± SEM, one way ANOVA, *p* is nonsignificant when compared across the group.
$HgCl_2$=Mercury chloride (5 mg/kg), *n*-BFPD =*n*-Butanol fraction of *Phoenix dactylifera* (500 mg/kg; 1000 mg/kg), Syl=silymarin (100 mg/kg)

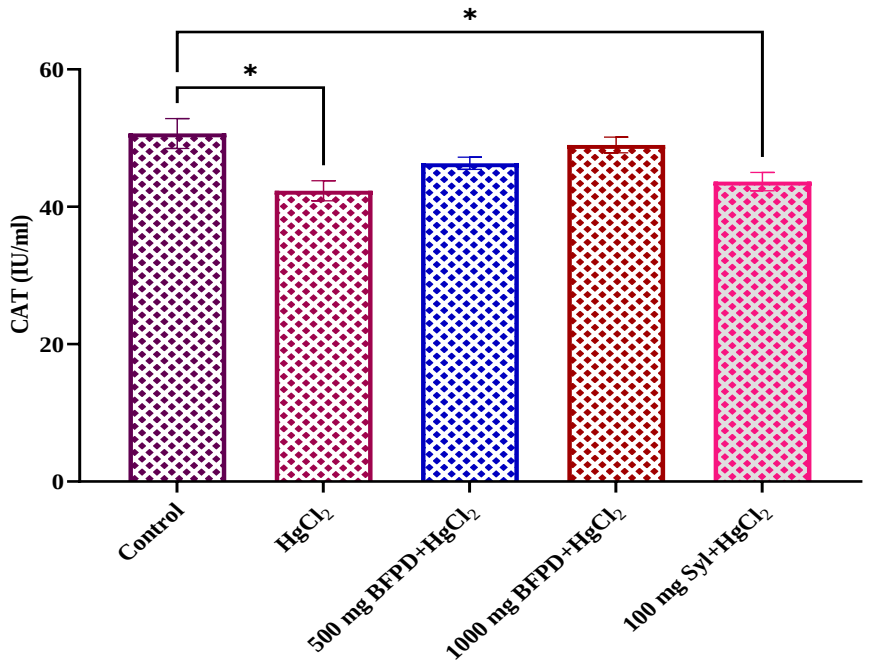

Figure 6b: Effect of *n*-BFPD on catalase concentration of Wistar rats
n= 5, mean ± SEM, one way ANOVA, *=*p* is significant when control was compared to $HgCl_2$ group and when control was compared to Silymarin group.
$HgCl_2$=Mercury chloride (5 mg/kg), *n*-BFPD =*n*-Butanol fraction of *Phoenix dactylifera* (500 mg/kg; 1000 mg/kg), Syl=silymarin (100 mg/kg)

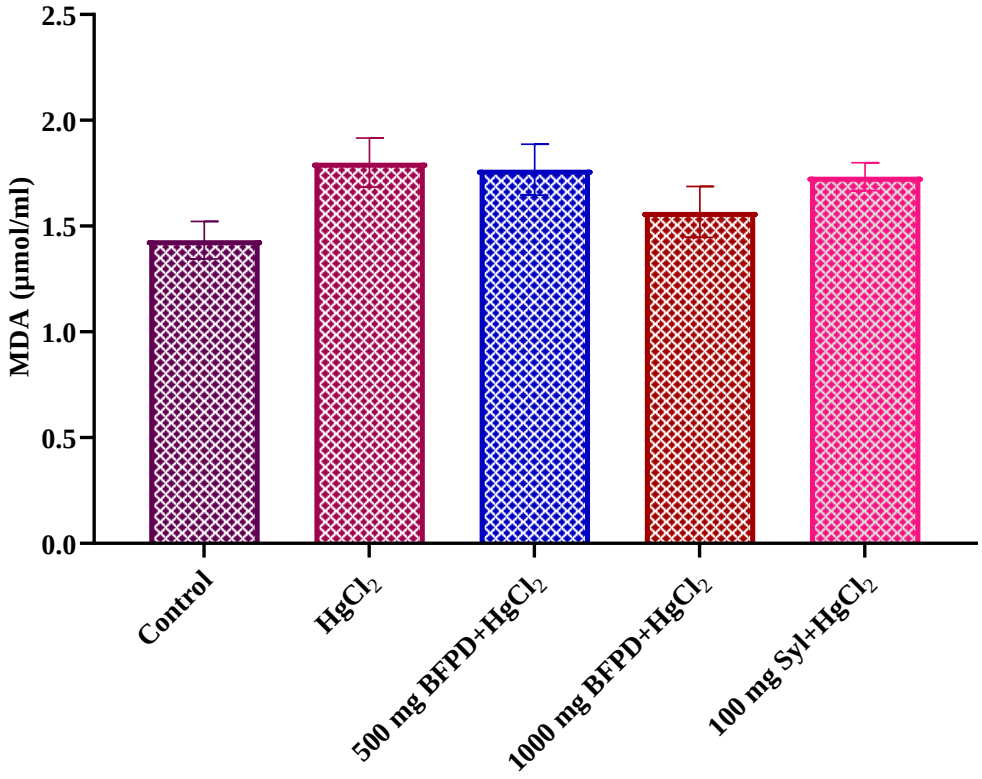

Figure 7: Effect of *n*-BFPD on malondialdehyde concentration of Wistar rats
n= 5, mean ± SEM, one way ANOVA, *p* is nonsignificant when compared across the group.
$HgCl_2$=Mercury chloride (5 mg/kg), *n*-BFPD =*n*-Butanol fraction of *Phoenix dactylifera* (500 mg/kg; 1000 mg/kg), Syl=silymarin (100 mg/kg)

DISCUSSION

In this work, phytochemical analysis of *n*-butanol fraction of *Phoenix dactylifera* (BFPD) was carried out, and its protective effect on mercury-induced kidney toxicity in Wistar rats was assessed using physical observations, histological, histochemical, and biochemical assessments. Phytochemical analysis of BFPD revealed the presence of flavonoids, saponins, and tannins among others which have been reported to possess nephroprotective actions [26-28] both in vivo and in vitro models.

Physically observed behavior are important markers for animal well-being, especially rodents[29]. Poor physical activity such as sluggishness and aggression were observed in the mercury-treated group when compared to the control group, this could be the consequence of toxicity leading to loss of appetite and improper assimilation of food. This finding is in line with the report of Ansar and AlGhosoon [29], and Amber et al. [23] who observed rats exposed to mercuric chloride triggered physiological changes including reduced agility and poor feeding. Agbon et al. [22]. reported reduced physical activity following treatment with mercuric chloride in rats.

The absolute body weight of an animal has been reported to be an important indicator of the health status of that animal [30]. A lower absolute body weight change observed in the $HgCl_2$-treated group when compared to the control could be attributed to $HgCl_2$-induced toxicity. This accords with the works of Thomas et al. [31] and Jadhav et al. [32] who observed that the body weight of rats treated with $HgCl_2$ decreased significantly when compared to the control.

Based on the results of this study, no significant differences in relative kidney to body weight ratio (organosomatic index) were observed when compared to the control. This indicates that the treatment probably did not alter relative kidney body-weight ratio values. These findings agree with Ajibade et al. [33] who reported no significant difference in organ-body weight ratio when exposed to $HgCl_2$. Similarly, Amber et al. [23] observed that oral administration of $HgCl_2$ and plant extract did not affect the organ-body weight ratio of Wistar rats.

Among all heavy metals, mercuric chloride remains the major cause of nephrotoxicity in many parts of the world [6,7]. The kidney is the first target organ for mercury accumulation and toxicity [34,35]. In fact, in a very short time (1 hour), 50% of an administered dose of mercury is present in the kidney [34,35]. In the $HgCl_2$-treated group, alterations such as dilated Bowman's space and shrunken glomerulus were observed in this study when compared to the control. This agrees with Oda and El-Ashmawy [36] and Amber et al. [23] who reported necrotic tubules, glomerular atrophy, and dilatation of Bowman's space after mercury administration.

Phoenix dactylifera is reported to possess various pharmacological activities including anti-inflammatory, nephroprotective, antioxidant activity among others [37,38]. Their high therapeutic effects have increased their use, encouraged by the growing consumer concern for health. Silymarin is a poliphenolic compound extracted from Silibum marianum and Cynara cardunculus seeds and fruits. It acts as an antioxidant checkmating free radical induced tissues damage, inhibits lipid peroxidation, and alters drug-induced histopathological changes [39,40].

The groups exposed to *n*-BFPD and silymarin followed by $HgCl_2$ administration showed relatively normal histoarchitecture similar to that of the control. This could be the result of the antioxidant potential of *n*-BFPD which is in line with Hounkpatin et al. [37] who suggested that antioxidants could contribute to the treatment of mercury poisoning.

Mercury chloride treated group showed less reaction to PAS stain suggestive of glycogen depletion in the podocytes when compared to the control. This agrees with Zhang et al. [41]. A relatively normal PAS staining intensity observed in *n*-BFPD and silymarin-treated groups similar to the control concurs with [42,43] and Amber et al. [23] who reported that the nephroprotective potential of plants extracts could be attributed to its phytoconstituents such as flavonoids. These flavonoids are antioxidants that donate electrons to free radicals thereby stabilizing and preventing them from undergoing long chain reactions leading to oxidative stress.

The kidney is responsible for fluid and electrolyte balance in the body, alterations in electrolyte (Na^{2+}, Ca^{2+}, K^+, Cl^- and $HCO3^-$) concentrations in the serum has been linked to renal injury. Acute or chronic toxicity could lead to changes in electrolyte balance within the body [44,45]. This study demonstrated slight increased concentration of Na^{2+}, Ca^{2+}, K^+, Cl^- in $HgCl^2$-treated group when compared to control. This could be attributed to impaired renal tubular function caused by $HgCl^2$ related toxicity. This result is consistent with that of Amber et al. [23] that reported alterations in the kidney electrolytes when exposed to inorganic mercury in an experimental animal model.

n-Butanol Fraction of Phoenix dactylifera and silymarin treated groups showed nonsignificant decrease in concentrations of Na^{2+}, Ca^{2+} and K^+ in all treated groups when compared to $HgCl_2$-treated group which suggests some level of attenuation attributed to the antioxidant properties they possess. However, a

slight elevation of Cl^- was observed in *n*-BFPD treated groups as compared to control, while silymarin treated group was modulated towards normal. HCO_3^- concentration decreased in $HgCl_2$-treated group compared to control and increased in *n*-BFPD and silymarin treated groups compared to $HgCl_2$-treated group. Similar findings were reported by [46,47] who observed decrease in serum electrolyte concentrations post $HgCl_2$ exposure.

Relative to oxidative stress bio-markers, it was noted that $HgCl_2$ diminished the activities of enzymatic antioxidants such as SOD and CAT (with a remarkable decline in CAT activity) in kidney tissues, whereas oxidative stress biomarkers (MDA contents) which is the end product of lipid peroxidation was increased compared to the control group. These findings are in agreement with the reports of Manju and Jagadeesan [48] and Nabil et al. [49] who implied that high accumulation of toxic metabolites in the system resulted from $HgCl_2$-induced oxidative stress. Goudarzi et al. [50] equally documented the reduction of antioxidant enzymes as a result of exposure to environmental toxins.

Conversely, co-administration of BFPD + $HgCl_2$ and silymarin + $HgCl_2$ modulated SOD, CAT, and MDA activities slightly toward normal. This indicates that the ameliorative potential of *n*-BFPD could be associated with antioxidant activity aimed against $HgCl_2$-triggered toxicity owing to oxidative stress. These observations concur with Ahmed et al. [23] and Goudarzi et al. [50] who stated that plant extracts inhibit oxidative stress by decreasing lipid peroxidation in drug-induced toxicity. Agbon et al. [22] related the protective potential of *P. dactylifera* to its antioxidant properties.

Phytonutrients with antioxidant activities including polyphenolics; especially flavonoids, have been associated with renal protection in experimental animal models. Flavonoids are beneficial phytochemical constituents of *P. dactylifera* reported to play a critical role as free radical scavengers or antioxidants in biological systems [51, 52]. The most emphasized antioxidant property of flavonoids is derived from their ability to directly scavenge the reactive oxygen species; flavonoids can chelate free radicals immediately by donating a hydrogen atom or by single-electron transfer or binding to metal ions in the human body to prevent them from being accessible for oxidation [53, 54].

CONCLUSION

n-butanol fraction of *Phoenix dactylifera L*. possesses the potential to protect against $HgCl_2$-induced alterations in the kidney of Wistar rats. The nephron-protective effect of the *n*-butanol fraction of *Phoenix dactylifera L*. is dose-dependent, as 1000 mg/kg proved to be more effective than 500 mg/kg. The nephron-protective efficacy of the *n*-butanol fraction of *Phoenix dactylifera L*. is comparable to the reference drug (silymarin). Nephro-protection could be attributed to the constituent antioxidant properties especially flavonoids in *n*-BFPD.

Therefore, the *n*-butanol fraction of *Phoenix dactylifera L*. may be a novel candidate for treating and managing kidney-induced mercury toxicity.

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