Trop J Nat Prod Res, December 2021; 5(12):2063-2067 ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Mercury-Induced Acute Nephrotoxicity in Rats: Treatment with Aqueous Extract of *Pistacia atlantica* (Desf)

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ARTICLE INFO

Article history:
Received 14 February 2021
Revised 06 November 2021
Accepted 04 Deember 2021
Published online 03 January 2022

ABSTRACT

Mercury is known to accumulate in living organisms, causing serious damage. An important characteristic of mercury toxicity is the generation of free radicals. The purpose of this study is to evaluate the protective effect of the aqueous extract of *Pistacia atlantica* against mercury-induced oxidative stress in rats. Mercury chloride ($HgCl_2$) was administered intraperitoneally (at 2.5 mg/kg once per week), and *P. atlantica* was given orally by gavage at a daily dose of 150 mg/kg body weight to rats for 32 days. These results show that $HgCl_2$ caused a significant depletion of the glutathione level and the enzymatic activity of the antioxidant system catalase (CAT), Glutathion peroxidase (GPx,), Glutathion S transferase (GST) at the renal level. These changes were associated with increased lipid peroxidation expressed by a high level of renal Malondialdehyde (MDA) and hydroperoxides (LOOH). However, supplementation with the aqueous extract of *Pistacia atlantica* modified the toxic effects of mercury by reducing lipid peroxidation. These findings may indicate an antioxidant and protective effect of this plant's extract against mercury's harmful effect.

***Keywords*:** Mercury, Liver, Kidney, Redox status, *Pistacia atlantica*, Rat.

Introduction

The increase in industrial and agricultural activities results in several pollutants being released into the environment, mainly into aquatic ecosystems. Metals are among these pollutants commonly found in the environment. Many of these metals induce toxic effects and accumulate easily in tissues.[1] Among these metals, mercury is one of the most dangerous pollutants in the environment and comes from various sources[2]. It is known to accumulate in living organisms, causing serious damage. An important feature of mercury toxicity is the generation of free radicals.[2] The generation of reactive oxygen species (ROS), such as the superoxide anion, singlet oxygen, hydrogen peroxide, and hydroxyl radical, has been one of the underlying agents responsible for tissue damage.[3] Mercury toxicity is also manifested through their interaction with sulfur by reducing molecules containing a free thiol. The reduction of thiols also leads to increased oxidative stress with increased hydrogen peroxide formation and other reactive oxygen species (ROS)[4]. Oxidative stress occurs due to an imbalance between the production of radical (or reactive) oxygen species and the cell's antioxidant capacity. The controlled production of radicals appears to be an essential cell signaling mechanism that maintains the cell's homeostasis.[5] These radicals can lead to protein destruction, DNA denaturation, lipid oxidation.[5]
Consequently, it is very important to reduce and eliminate all sources that generate the risk of free radical production in living organisms.[6] Most plant species in the world possess therapeutic virtues, as they contain active compounds that act directly on the organism. They are used in conventional medicine and herbal medicine and are known to offer advantages that drugs often lack.[5] One of the important plants in Algeria is *Pistacia atlantica* Desf. (Anacardiaceae), this plant has also been used for the treatment of peptic ulcer and hypoglycemic.[6]
Research on natural antioxidants and the exploitation of various secondary metabolites has been particularly studied in recent years. Thus, phenolic compounds, particularly flavonoids, have attracted attention as a potential source of bioactive molecules. The structure of their flavan nucleus is related to their antioxidant capacity. These substances are capable of reducing free radicals. Other mechanisms of free radical oxidation may include enzyme inhibition and chelation of the oligometal that catalyzes the formation of ROS.[6] The present study was carried out to evaluate certain antioxidants' effects on oxidative stress parameters and the damage they can cause to the kidneys during mercury-induced stress. The antioxidant used in this study is the aqueous extract of *Pistacia atlantica*.

Materials and Methods

Plant material and preparation of aqueous extract
The leaves of *P. atlantica* Desf used in this study were collected from Oran (Algeria) in October 2018. The plant's identification was confirmed at the department of Botany of Ahmed Ben Bella University 1 (Oran, Algeria) where a specimen (voucher No. LB 2370) was kept. After the leaves were cleaned and air-dried, they were ground to a fine powder and extracted with distilled water (1: 10, w/v) at 60°C) for 60 min. The mixture was filtered, and the extract obtained frozen and then lyophilized (Freeze-dryer Christalpha 2-4 LSC d 37520, Germany).

Animals and experimental design
A total of 24 male Wistar rats with weight of 65 ± 10 g were obtained from the Department of Biology, Faculty of Nature and Life Sciences, Oran 1 University. This study was approved by our Institutional Ethical Committee for Animal Research (agreement number 45/DGLPAG/DVA. SDA.14). The General Guidelines for the Use of Living Animals in Scientific Investigations Council of European

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Citation: B, Omar K, El azhari M. Mercury-Induced Acute Nephrotoxicity in Rats: Treatment with Aqueous Extract of *Pistacia atlantica* (Desf). Trop J Nat Prod Res. 2021; 5(12):2063-2067. doi.org/10.26538/tjnpr/v5i12.3

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Communities were followed (Council of European Communities, 1986). The animals were allowed free access to tap water and rodent chow, housed under standard conditions with a 12/12 h light-dark cycle at 25 ± 2°C, controlled humidity (60 ± 5%), and air circulation.
The rats were randomly divided into three equal groups (six rats in each group). They received the treatment as follows: Group I served as control (T), receiving an intraperitoneal injection of saline solution (NaCl; 0.9%) once a week for 32 days. Group II consisted of Hg intoxicated rats which were given a dose of 2.5 mg/kg body weight (b.wt.) of $HgCl_2$ by intraperitoneal injection for four weeks. Group III serving as treated group ($HgCl_2$+*P.at*) received $HgCl_2$ (2.5 mg/kg body weight) intraperitoneally and were given (150 mg/kg b.wt) aqueous extract of *P. atlantica (P.at)* orally for 32 days. No deaths or toxicity symptoms were recorded after oral administration of single doses of the lyophilized tested extract at any dose level up to the highest dose tested to ensure its safety. After the end of treatment, the experimental animals (rats) were sacrificed by decapitation (solution of chloral, 3%) to obtain blood and kidney tissues.

Determination of antioxidant markers in kidney tissues
The adult rat kidney from the different studied groups was removed and rapidly dissected. After crushing and homogenization, the tissues were placed in a PBS buffer (0.1 mol/L; pH=7.4) supplemented with sucrose (0.3 mol/L) and potassium chloride (0.08 mol/L) using a WiseTis® homogenizer (HG-15A; Germany) and maintained at a temperature of 4°C. The homogenate was centrifuged at 7600 rpm for 10 minutes at 4°C to obtain supernatant, which was further centrifuged at 12000 rpm for 10 minutes to remove cellular debris and was then stored at -80°C.
Lipid peroxidation (LPO) levels in the kidney were assessed by TBARS (thiobarbituric acid reactive substances) assay using the method described by Ohkawa *et al*.[7] Reduced glutathione levels (GSH) were determined by the colourimetric method based on the reducing properties of thiol groups (SH) described by Ellman.[8] Renal tissue protein content was assessed using the method of Lowry *et al*. [9] Determination of catalase activity (CAT) was done by the method of Aebi *et al*.[10] Glutathione peroxidase (GPx) was assessed by the method of Rotruck *et al*. [11] Glutathione S-transferase (GST) was determined by the method described by Habig *et al*. [12] and superoxide dismutase (SOD) assayed was done by the method of Marklund and Marklund.[13] The results were expressed as Units/mg protein (mmol H_2O_2 degraded/mg of protein, nmol /mg proteins, µmol/mg proteins/min, µmol/mg of proteins.

Statistical analysis
The results were represented as mean values ± standard error (mean ± SE). Data were analyzed by SPSS (Statistical Packages for Social Science, version 23.0, IBM Corporation, New York, USA) using one-way analysis of variance (ANOVA) followed by Least Significant Difference test (LSD) with p = 0.05, for comparison of various treatments. A student's t-test was used to determine the significant difference among two different.

Results and Discussion

Intoxication with $HgCl_2$ is associated with increased production of reactive oxygen species (ROS), which interacts with sensitive biological macromolecules leading to lipid peroxidation, DNA damage (mutagenesis), and protein oxidation.[14] Studies have also revealed that it is associated with nephrotoxicity.[15]
The study results show a significant decrease in GSH of -55.55% at the renal level in the mercury chloride poisoned lot compared to the control (Figure 1) due to direct mercury attacks on thiol groups of this protein. The toxicity of $HgCl_2$ is attributed to Hg's high affinity for thiol, which allows it to deplete cellular GSH and damage thiol proteins and enzymes.[16] The study results also show a significant decrease in lipid peroxidation of -56.23% at the renal level in mercury poisoned rats treated with *Pistacia atlantica* extract compared to those treated with mercury chloride (Figure 2). There was also a significant decrease in the levels of TBARS in the mercury intoxicated group treated with the aqueous extract of *Pistacia atlantica*. This effect may be due to flavonoids in the extract known to prevent lipid peroxidation due to their high propensity to scavenge free radicals.

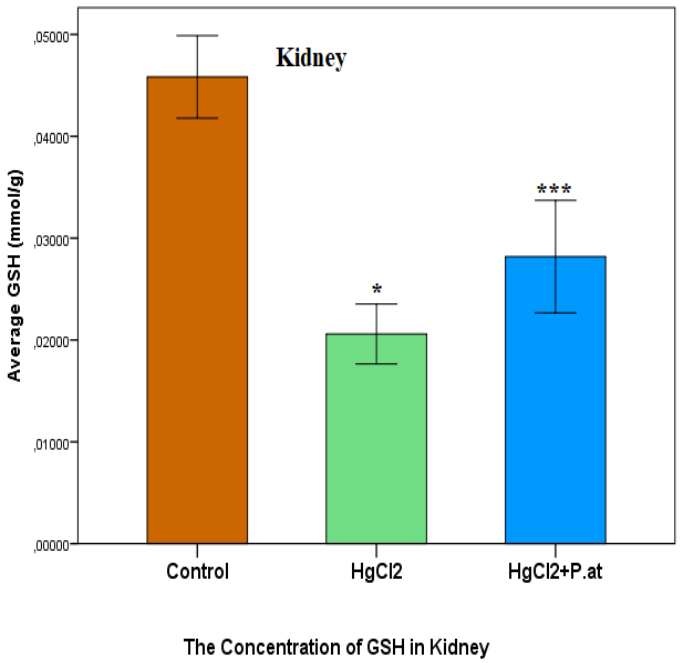

Figure 1: Effects of *P. atlantica* on GSH level (nmol /mg proteins) in kidneys.
The results are represented by the mean ± standard deviation (Mean ± SE). *P* <0.05 (*) = indicates a significant difference in the poisoned rats compared to controls. (***) = indicates a significant difference in the poisoned rats treated with the aqueous extract of *P.at* compared to the control rats *p* <0.05.

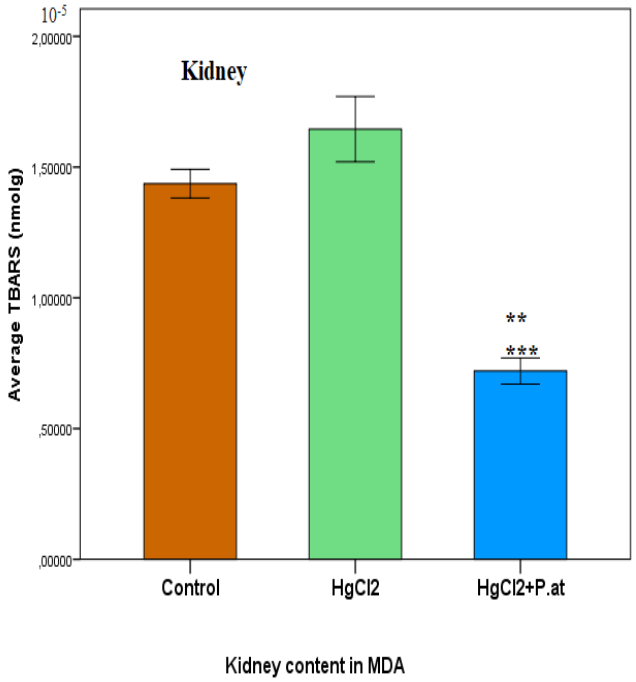

Figure 2: Effects of *P. atlantica* on TBARS level (* 10^{-5} nmol/g) in kidneys.
The results are represented by the mean ± standard deviation (Mean ± SE). *P* <0.05 (*) = indicates a significant difference in the poisoned rats compared to controls. (**) = indicates a significant difference in mercury-poisoned rats treated with the aqueous extract of *P. at* compared to mercury-poisoned rats. (***) = indicates a significant difference in the poisoned rats treated with the aqueous extract of *P. atlantica* compared to the control rats ($p < 0.05$).

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Table 1: Changes in blood elements in the treatment groups

Variables	Group I N = 10	Group II N = 10	Group III N = 10	F test	P-value	LSD for difference in between groups
Haemoglobin (mg/dL)	12.5 ± 0.67^{NS}	12.3 ± 0.8**	12.4 ± 0.88**	0.13	0.88 NS	
Haemoglobin after 1 month	12.2 ± 0.69^{NS}	9.6 ± 0.58**	13.2 ± 0.75**	76.5	<0.001 HS	P1 < 0.001 P2 = 0.004 P3 < 0.001
Platelets	341.7 ± 68.7$^{\#}$	335.5 ± 73.2$^{\#\#}$	362.7 ± 44.98$^{\#\#}$	0.51	0.61 NS	
Platelets after 1 month (L)	332.5 ± 72.1$^{\#}$	166.8 ± 51.1$^{\#\#}$	538 ± 75.8$^{\#\#}$	76.5	<0.001 HS	P1 < 0.001 P2 < 0.001 P3 < 0.001
WBCs	8.26 ± 1.5^{N}	7.83 ± 1.8^{N}	7.79 ± 1.7^{N}	0.24	0.79 NS	
WBCs after 1 month	7.86 ± 1.7^{N}	4.57 ± 1.11**	9.43 ± 1.7**	26.3	<0.001 HS	P1 < 0.001 P2 = 0.03 P3 < 0.001
Lymphocytes	3.31 ± 0.62^{N}	3.25 ± 0.81$^{\#\#}$	3.22 ± 0.71$^{\#\#}$	0.04	0.96 NS	
Lymphocytes after 1 month	3.31 ± 0.63^{N}	1.93 ± 0.81$^{\#\#}$	3.91 ± 0.3$^{\#\#}$	27.1	<0.001 HS	P1 < 0.001 P2 = 0.04 P3 < 0.001
Neutrophil	5.22 ± 1.2^{N}	5.37 ± 1.14$^{¥¥}$	5.53 ± 0.96$^{¥¥}$	0.19	0.83 NS	
Neutrophil after 1 m.	5.25 ± 1.16^{N}	2.33 ± 0.12$^{¥¥}$	6.34 ± 0.62$^{¥¥}$	43.5	<0.001 HS	P1 < 0.001 P2 = 0.02 P3 < 0.001

*The values in the upper row for each parameter represent the baseline values. All data are expressed as Mean ± SD
The P-value for change in Hb within groups by paired t-test within each group NS means non-significant ** means highly significant P1<0.001
The P-value for change in platelets within groups by paired t-test within each group # means significant P1<0.05 ## means highly significant P<0.001
The P-value for change between groups of WBCs N means non-significant
The P-value for change in WBCs within groups^{N} means non-significant P>0.05 ** means highly significant P<0.001
The P-value for change in lymph within groups$^{\#}$ groups^{N} means non-significant P>0.05 $^{\#\#}$ means highly significant P<0.001
The P-value for change in neutrophil within groups^{N} means non-significant P>0.05 $^{¥¥}$ means highly significant P<0.001
P1: for the difference between group I and II, P2: for the difference between group I and III, P3: for the difference between group II and I

Lipid peroxidation is mostly initiated by hydrogen abstraction leading to oxidative damage of polyunsaturated fatty acids (PUFA) in macromolecules. Flavonoids have been shown to reduce highly oxidizing free radicals such as superoxide, peroxyl, alkoxyl, and hydroxyl radicals through different mechanisms by donating a hydrogen atom to free radicals and metal ions chelation associated with the inhibition of free radical generation. Chelation of metal ions by flavonoids has been considered to prevent lipid peroxidation by limiting the metal ion's access to lipid hydroperoxides (LOOH).[17-19]
As shown in Figure 3, a significant increase (P <0.05) in the Hydroperoxyde level in the kidney tissue was observed in the intoxicated rats when compared to the control groups. The Hg chloride treatment induced significantly decreased (P<0.05) of catalase (Figure 4) was noted at the renal level in the $HgCl_2$ group and in group $HgCl_2$+*P.at* compared to the control that may be explained by the highest renal mercury load according to Agarwal *et al.*[20] The highest concentration of mercury was observed in the kidneys followed by liver and blood. The reducing activity of this enzyme maybe due by direct attack through its binding to thiol groups on its active site.[18] The result shows that there were no significant differences in terms of SOD activities in the kidney tissue of intoxicated and treated rats (Figure 5). There was a significant decrease in kidney function of -45.03% (GPx) and -60.52% (GST) activity in the intoxicated group compared to the control. Similarly, there was a significant (p < 0.05) decrease in kidney GPx and GST activity in the group treated with *Pistacia atlantica* extract in the kidney (-49.04 and -61% respectively) (Figures 6 and 7). The decrease in GST and GPx may also be explained by the reduction in the rate of reduced GSH glutathione from its reduction to oxidized GSSG. GSH is essential for the reactions of GST and GPx enzymes. This report is consistent with that of Mleiki *et al.*[19] who proposed that GST inhibition has been attributed to the direct action of the metal on the enzyme or indirectly via the production of ROS that interacts directly with the enzyme leading to the depletion of its substrate (GSH), and the downward regulation of GST genes through different mechanisms.

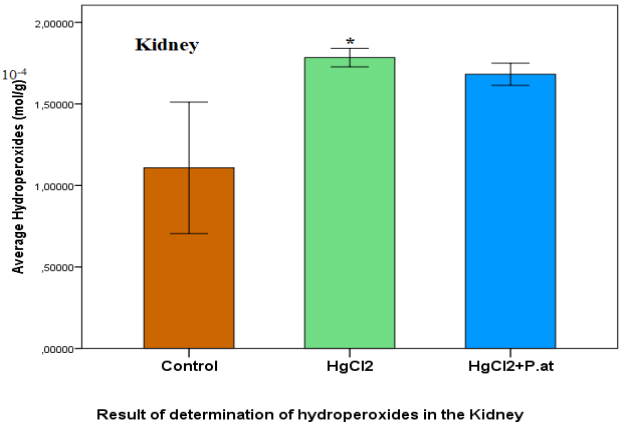

Figure 3: Effects of *P. atlantica* on hydroperoxide level (10^{-4} nmol/g) in kidney.
The results are represented as the mean ± standard deviation (Mean ± SE). P <0.05 (*) = indicates a significant difference in the poisoned rats compared to controls.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

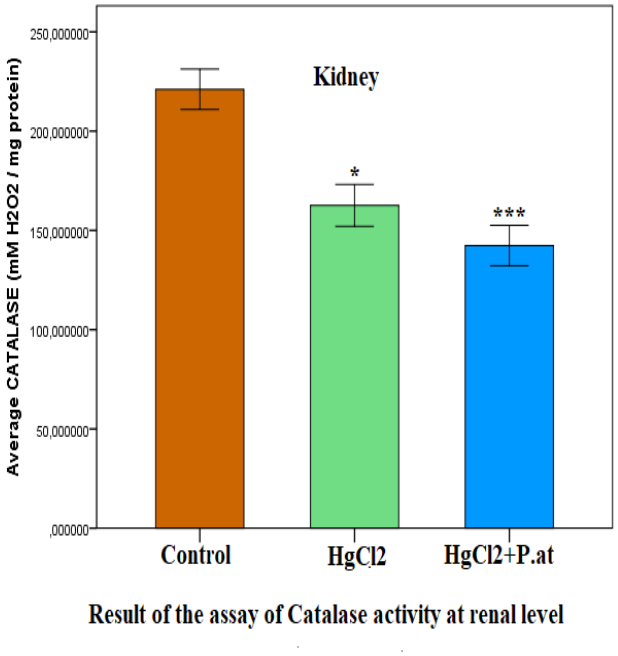

Figure 4: Effects of *P. atlantica* on CAT level (mmol H_2O_2/mg of protein) in kidney.
The results are represented by the mean ± standard deviation (Mean ± SE). p <0.05 (*) = indicates a significant difference in the poisoned rats compared to controls. (***) = indicates a significant difference in the poisoned rats treated with the aqueous extract of *P.at* compared to the control rats $p < 0.05$.

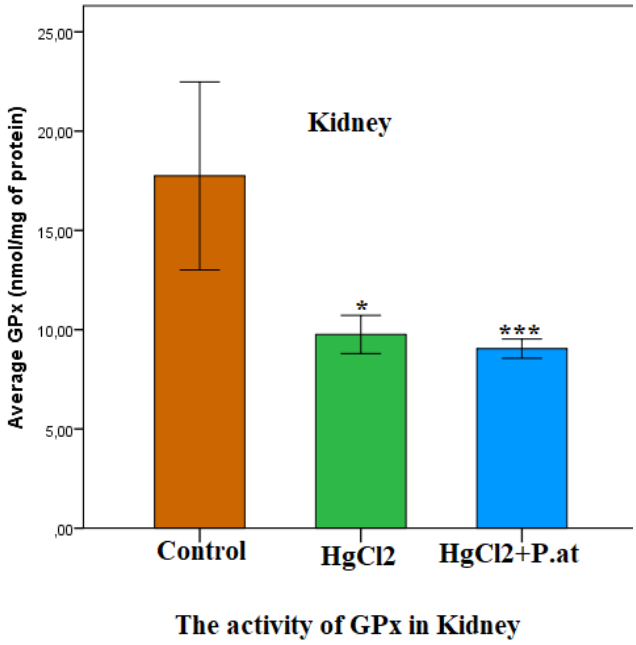

Figure 6: Effects of *P. atlantica* on GPx level (nmol /mg proteins) in kidney.
The results are represented by the mean ± standard deviation (Mean ± SE). $p < 0.05$ (*) = indicates a significant difference in the poisoned rats compared to controls. (***) = indicates a significant difference in the poisoned rats treated with the aqueous extract of *P.at* compared to the control rats $p < 0.05$.

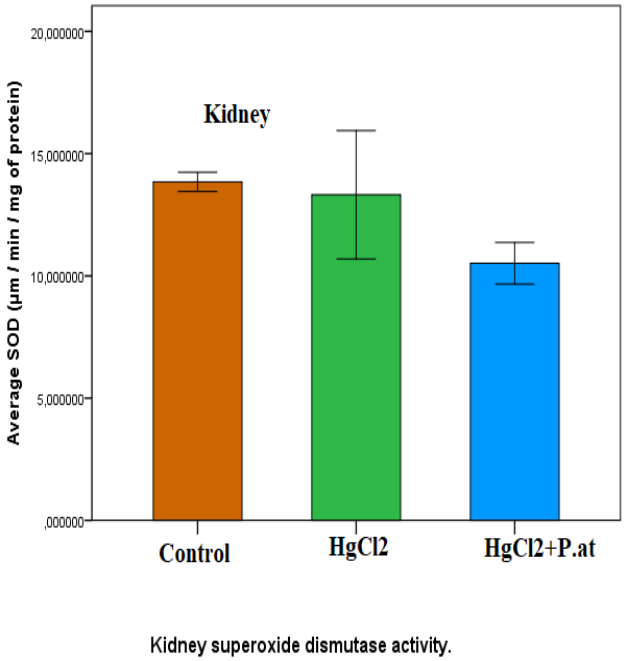

Figure 5: Effects of *P. atlantica* on SOD level (µmol/mg of proteins) in kidney.
The results are represented by the mean ± standard deviation (Mean ± SE). p <0.05 (*) = indicates a significant difference in the poisoned rats compared to controls. (***) = indicates a significant difference in the poisoned rats treated with the aqueous extract of *P.at* compared to the control rats p <0.05.

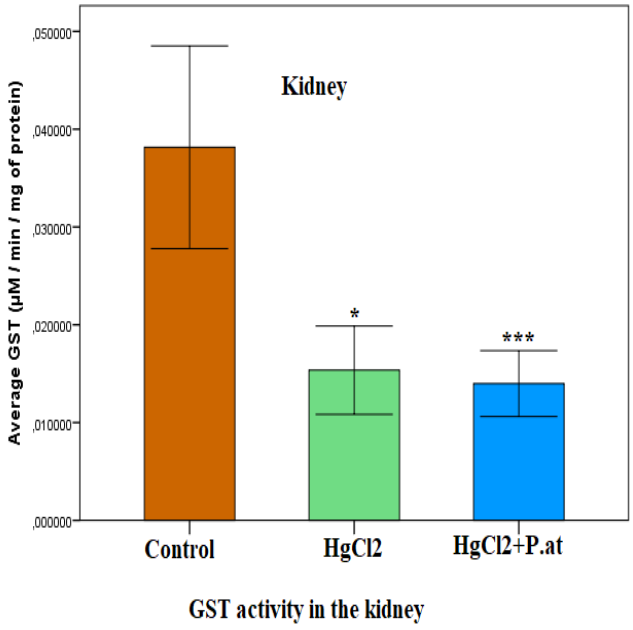

Figure 7: Effects of *P. atlantica* on GST level (µmol/mg of proteins) in kidney.
The results are represented by the mean ± standard deviation (Mean ± SE). $p < 0.05$ (*) = indicates a significant difference in the poisoned rats compared to controls. (**) = indicates a significant difference in mercury-poisoned rats treated with the aqueous extract of *P. at* compared to mercury-poisoned rats. (***) = indicates a significant difference in the poisoned rats treated with the aqueous extract of *P.at* compared to the control rats $p < 0.05$.

The decrease in GST at the renal level induces an increase in GSH levels. Perhaps because the plant has effectively eliminated xenobiotics and lipid peroxidation through flavonoids associated with lipid peroxidation's effective inhibition, leading to a decrease in the GST. It may also be due to the kidney's sensitivity to mercury, knowing that it is the first organ of mercury accumulation, leading to an imbalance in certain enzymes' activity. In the intoxicated mercury group treated with *Pistacia atlantica* extract, no difference was recorded, suggesting that the effect of mercury is still present; these results do not agree with those of Agarwal *et al*.[20]

Conclusion

This study shows that *Pistacia atlantic* leaf extract did not significantly change renal functions indices in $HgCl_2$ treated rats.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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