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Toxicity of mercury on the brain: ability of extract of *Pistacia atlantica* regulated effect

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ABSTRACT

Objective: The purpose of this study was to evaluate the neuroprotective effect of 150 mg / kg extract of the plant *Pistacia atlantica* against mercury-induced oxidative stress

Methods: Hg was administered intraperitoneally (2,5 mg/kg body weight, one time a week), and *P. atlantica* and were given orally by gavage at a daily dose (150 mg/kg body weight) to rats for 32 days. 24 male adult Albinos Wistar rats were divided into four groups: group 1 Control, group 2 ($HgCl_2$) group 3 (Hg + *P. atlantica*) and group 4 (*P. atlantica*). Paramatrical tests of oxidative stress and histological sections of the cerebral parenchyma. Results: Our results showed that the intraperitoneal injection of mercury chloride $HgCl_2$ causes deleterious effects in the brain resulting in: a failure of redox status by disrupting the antioxidant defense system by a significant decrease in the activity of catalase glutathione peroxidase, glutathione-s-transferase and superoxide dismutase acetylcholinesterase and increase of the activity of the enzyme lactate dehydrogenase. The levels of lipid peroxidation markers were high in TBARS intoxicated rats with protein oxidation increased in the brain intoxicated by. The continuous use of mercury is also at the origin, in brain tissue However, supplementation of *P. atlantica* extract with mercury-treated rats attenuated some of the harmful and toxic effects of this metal. This clearly demonstrates the protective roles of this plant

Keywords: mercury, *Pistacia atlantica*, Wistar rat, brain, antioxidant, neurotoxicity.

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1. INTRODUCTION

Mercury is a highly toxic, redox-active element which represents one of the main agents responsible for environmental pollution[1]. Mercury was recorded as the third most dangerous heavy metal after arsenic and lead, according to the Agency for Toxic Substance and Disease Registry Agency (ATSDR)[2]. It can be found in three different chemical forms; elemental mercury (Hg0), organic mercury (mainly methylmercury), and inorganic mercury (mainly mercuric chloride)[2]. The mercurial exposure is a reality faced by several people around the world[3], due the routes of exposure to the metal be associated with the used in pharmaceuticals products[4], use of cosmetics[5], Mercury is used in various chemical industries. In chlorine production plants, it is widely used for the synthesis of chlorinated compounds and also used in the production of sodium hydroxide. Mercury has various applications including to control weeds, fungi, bacteria and insects[6].

Mercury adversely affects the cellular, pulmonary, haematological, cardiovascular, immunological, neurological and endocrine systems[7]. The central nervous system, kidney, liver and gastrointestinal system are the main target sites of mercury toxicity[8]. In addition, symptoms such as headache, impaired coordination, tremor, diarrhea, abdominal cramps, dermatitis, proteinuria, polyneuropathy and hepatic dysfunction occur as a result of mercury toxicity[2].

The toxicity of mercury can be stopped by using an antioxidant defense mechanism including reducing or eliminating active oxygen species, free radicals, and heavy metals[9].

The herbal medicines are widely used in traditional methods to treat various diseases. Specifically, in recent years, the therapeutic and antioxidant effects of some herbal drugs are taken into account for clinical settings[10]. The genus *Pistacia* (Anacardiaceae) comprising more than 11 species is widely distributed from south-west Asia to north-west Africa. *Pistacia atlantica* is one of the most widely distributed wild species, which is called "Butom" in Algeria, and is the most characteristic plant species of the arid and semi-arid regions of the country[11]. it is a high-altitude tree with a height of 2–7 m. The plant has been naturally spread to the Canary Islands, Mediterranean countries, Syria, the Caucasus, Iran, Afghanistan and Pakistan[12]. This medicinal plant has been used since *Pistacia* ancient times for treatment of gastrointestinal, liver, and kidney diseases. Various biological effects are reported for species, including antioxidant, antimutagenic, antiatherogenic, anthelmintic, antimicrobial, antiviral, anti-inflammatory, antinociceptive, antitumor, and especially antidiabetic properties[13].In our present study, we evaluated the protective effect of the leaves aqueous extract of *P. atlantica* against mercuric chloride-induced neurotoxicity in rats by assessing some.

2. MATERIALS AND METHODS

2.1. Plant Material and Preparation of Aqueous Extract

Leaves of *P. atlantica* Desf used in this study were collected from Oran (Algeria) in October 2018. The plant material was authenticated in the botanic laboratory, University of Oran1. After the leaves were cleaned and air-dried, they ground to a fine powder and extracted with distilled water (1: 10, w/v) under the heat conditions (60 °C) during 60 min. The mixture was filtered. The obtained decoction was frozen and then lyophilized (freeze-dryer christalpha 2-4 lsc d 37520, Germany).

2.2 *Animals and experimental design*

For our study, a total of 24 rats (55 ± 10) g the protocol processes using laboratory animals were in accordance with the Guide for the Care and Use of Laboratory Animals (8th Edition, 2011) and approved by the University's Scientific Committee. Wistar strain rats were randomized to a treatment regimen using Mercury Chloride at 2.5 mg/kg body weight (bw), per 125 mg/Kg with the plant extract for a total duration of 04 weeks or 32 days, the animals were then divided into groups that met the following criteria:

❖ ***Group 1***: Control group (Control1) consisting of 6 rats receiving daily drinking water by gavage and intraperitoneal injection of 0.9% NaCl 3 times per week for 4 weeks, i.e., 32 days.2 **Group 2**: Intoxicated group ($HgCl_2$) treated with Mercury Chloride 2.5 mg/kg bw administered once per week by intraperitoneal injection for 4 weeks; the number of subjects was also of the order of 6 rats. **Group 3**: Group intoxicated with Mercury Chloride 2.5 mg/kg bw injected into the peritoneum once a week, treated with the plant 150mg/Kg ($HgCl_2$+*P. atlantica*) administered by daily gavage to 6 animals over a period of 32 days and **Groupe 4** treated with the *P. atlantica* 150mg/Kg

2.3. Tissue simple preparation

At the end of each treatment period the groups of rats are sacrificed by intraperitoneal injection of a 10% chloral solution (Penthobarbital) at a rate of 3 mL/kg body weight (b.w.) The animals were weighed before sacrifice, once sacrificed, the cranial cavity is opened. The brain was freed of adipose tissue, rinsed with a fresh 0.9% NaCl solution, weighed and then stored in the freezer at -80°C (CHRIST ALPHA 2-4 LSC D-37520 ALS Angelantoni Life sciences) for the determination of oxidative stress parameters; part of these organs was fixed in 37% formalin diluted 1/10th to make histological sections.

2.3.1. Preparation of tissue homogenates:

The brains of the different groups studied were used. After grinding and homogenizing the tissue (0.5g) in a phosphate buffer (PBS 0.1 mol/l, pH=7.4) to which 0.3 mol/l sucrose and 0.08 mol/l potassium chloride (KCl) (5ml) were added using a WiseTis® homogenizer (HG-15A) while maintaining a temperature of 4°C, the homogenate obtained is centrifuged at 7600 rpm for 10mn at 4°C, once the supernatant is recovered, it is in turn centrifuged at 12000 rpm for 10mn to remove cellular debris. Then, aliquots of the supernatant obtained are collected in Eppendorf tubes and stored at -80°C while waiting to determine the oxidative stress parameters.

2.3.2. Measurement of lipid peroxidation (LPO), Glutathione reduced GSH and antioxidant enzyme activity

2.3.2.1. Lipid peroxidation (LPO) levels, reduced GSH and antioxidant enzyme activities

2.3.2.2. *LPO levels [thiobarbituric acid reactive substances (TBARS)]*

Lipid peroxidation was assessed by TBARS assay using the Ohkawa *et al.*[14]method. MDA is one of the end products formed during the decomposition of polyunsaturated fatty acids (PUFA) meditated by free radicals. The level of lipid peroxidation was evaluated with the TBARS (Thiobarbituric reactive species) test.

2.3.2.3. Reduced GSH levels

Reduced GSH was determined using a colorimetric technique as described by Sedlak and Lindsay[15]. The high solubility of the tris buffer provides a favourable medium for the GSH reaction to proceed which reduces the DTNB (also called Ellman's reagent) by producing the yellow chromophore TNB (2-nitro-5 thiobenzoic acid), which has a maximum absorbance at 412 nm, and an oxidized glutathione-TNB adduct (GS-TNB). The rate of formation of TNB, is proportional to the concentration of GSH in the sample. In brief, 1 mL of cerebrum supernatant (homogenate) is prepared after treatment with 1 mL of 50% trichloroacetic acid-distilled water (1:4), and the supernatant obtained after centrifugation at 2400 r/min for 15 min was mixed with 0.02 mL of 0.01 mmol/L DTNB and an amount of Tris buffer (0.4 mol/L, pH 8.5). Total GSH content was expressed as nanomoles of GSH per milligram of protein.

2.4. Effect of treatment on antioxidant enzymes activities in brain

2.4.1. *Determination of CAT (EC 1.11.16) levels*

CAT was assayed by the method of Aebi[16], 250 µL of cerebrum homogenates and 250 µL of 0.03 mol/L H_2O_2 (prepared in phosphate buffer, 0.066 mol/ L, pH 7.0) were added in a cuvette. After incubation for 5 min, TiOSO4 was added to the mixture and absorbance was directly measured against phosphate buffer as a blank 420nm, and one unit of CAT is equal to 1 mmol H_2O_2 degraded/ mg of protein.

2.4.2. Activity of GPx (EC 1.11.1.9)

GPx activity in brain tissues was assessed by the method of Rotruck *et al.*[17]. Glutathione peroxidase (GPX) present in the homogenate catalysis the reduction of a hydrogen

peroxide (H2O2) by oxidising reduced glutathione (GSH) added at known concentration to form glutathione disulphide (GSSG). The reaction is stopped at a fixed time (t) by addition of TCA the strong acid causing denaturation and aggregation of the GPx and the thiol group (R-SH) of the remaining GSH cysteine is detected by the Ellman reagent giving the chromogenic product TNB which is measured at a wavelength of 340 nm allowing determination of the peroxidase activity. Briefly the reaction mixture contained 0.2 mL of Tris–HCl buffer (0.4 mol/L, pH 7.0), 0.2 mL of reduced GSH (1 mmol/L), 0.1 mL of sodium azide

2.4.3. Superoxide dismutase (EC 1.15.1.1)

The assay technique of the Marklund and Marklund[18] is performed in a buffer (Tris Hcl 50mM and EDTA 10 mM) at pH 8.2, with a cerebrum homogenate fraction and pyrogallol (15 mM) and the change in absorbance is monitored for three minutes at a wavelength of 440 nm. Results are expressed in U of SOD / mg protein.

2.4.4. Determination of lactate dehydrogenase (LDH)

We used kits (Biolabo, French). The decrease in absorbance due to the conversion of NADH to NAD+ in the presence of pyruvate is directly proportional to the LDH activity in the brain homogenate, absorbance is measured at 340 nm.

2.4.5. Estimation of tissue AChE

AChE The method for the determination of acetylcholinesterase (AChE) according to the method Ellman's *et al.*[19] consists of providing the enzyme with a substrate acetylthiocholine, the hydrolysis of which by acetycholinesterase in the homogenate releases acetic acid and thiocholine, the latter having an SH thiol group capable of easily cleaving the DTNB which gives the yellow TNB absorbed at 410 nm.

2.4.6. Determination of total tissue protein contents

The determination of total protein at the brain tissue level was performed using the method of Lowry *et al.*[20]. using bovine serum albumin as a standard, and necessary dilutions were realized to get the correct concentrations of the proteins present in tissues.

2.5. Histopathological studies [haematoxylin and eosin (H&E) staining]

Samples (brain) from each group were selected, transversely cut and fixed in 10% buffered formaldehyde solution, then conserved in paraffin. Four micrometre tissue sections were realized and dried at adequate temperature to get Paraffin removed from the glass slides. The next step was to rehydrate sections then stain them with haematoxylin and eosin as nuclear and cytoplasmic stains. The sections were analyzed using Leica®DM5000B microscope and photographed with Leica EC3 digital camera

3 STATISTICAL ANALYSIS

The results were represented as mean values ± standard error (Means ± ES). 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 α = 0.05, for comparison of various treatments. A student's t-test was used to determine the significant difference among two different.

4. RESULTS

4.1. Effect of treatment on lipid peroxidation and GSH contents in cerebrum

Changes in TBARS and GSH levels were illustrated in Figures 1, and a significant increase in TBARS levels by **+66.85%** in cerebrum of intoxicated rats was noted when compared to controls. A highly significant (P <0.001) increase was also noted in cerebellum of exposed rats, and these results were accompanied by a reduction in GSH levels in cerebrum of Hg treated rats (**-32.43%**) in comparison with those of controls. The co-administration of *P. atlantica* and $HgCl_2$ decreased the TBARS production by a rate of **-56.36**%. This treatment alleviated significantly GSH levels in brain regions (+167.46**%)** when compared to intoxicated rats. The plant extract showed more efficient results in term of restoring normal values of some altered parameters than the chelation strategy did.

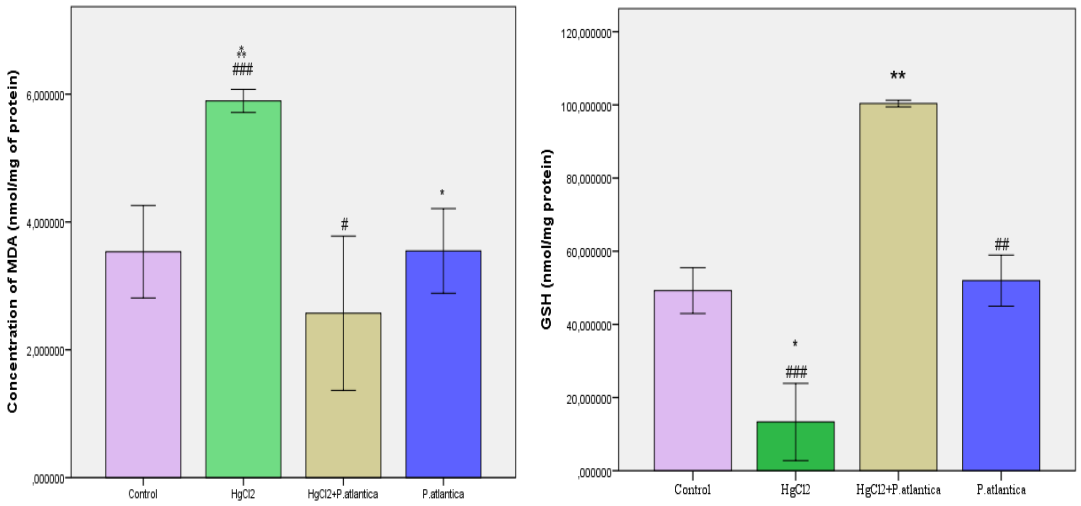

Figure 1: Effects of *P. atlantica* on TBARS level (nmol/mg of proteins) and GSH (nmol /mg proteins) level. The results are represented by the mean ± standard deviation (Means ± SD). P <0.001 (⁂) = indicates a significant difference in the poisoned rats compared to controls. (###) = indicates a significant 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<0,01

4.2. Effect of treatment on antioxidant enzymes activities in cerebrum

Exposure to $HgCl_2$ produced significant changes in the cerebrum redox status. A very significant decrease (P< 0.01) in CAT, GPx, GST and SOD activity, activities was recorded in intoxicated group compared to controls (Figures 2). Oral administration of aqueous *P. atlantica* extract during mercury exposure showed an amelioration in CAT, GPx, GSt, and SOD, by significantly increasing their values (65.98%, 100.35%, 100.48% and 85.71%) respectively.

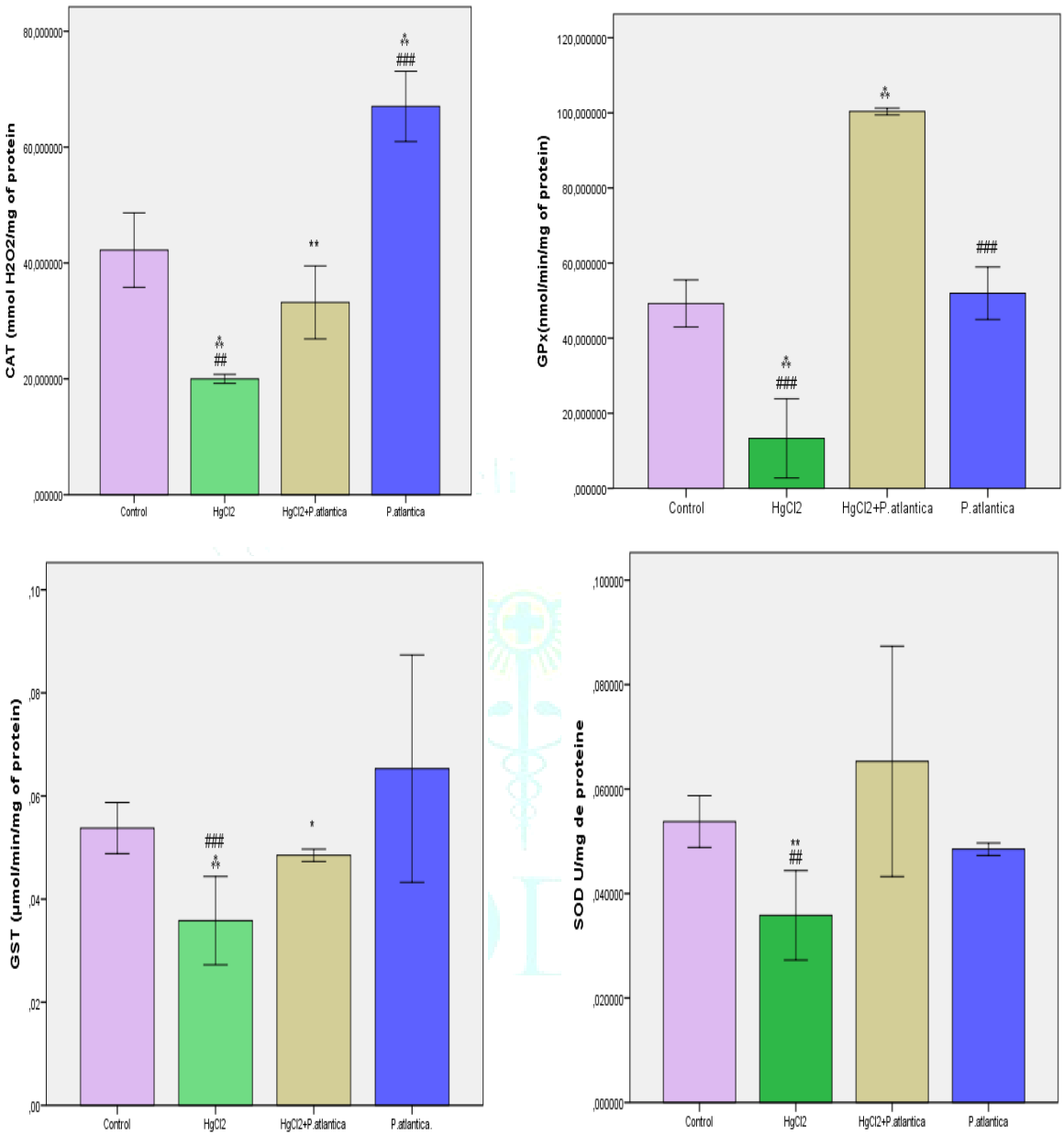

Figure 2**:** Effects of *P. atlantica* on CAT activity (mmol H_2O_2/mg of protein), GPx activity (nmol /mn/mg proteins), GST activity (µmol /mn/mg proteins) SOD activity (U/mg of proteins) The results are represented by the mean ± standard deviation. P <0.001 (⁂) = indicates a significant difference 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<0,01

4.2. Effect of treatment on LDH activities in cerebrum

The increase in LDH level in brains poisoned by $HgCl_2$ (349.51 ± 24.71 IU / g) compared to the control and decrease by $HgCl_2$ + *P. atlantica* in brain when compared to those in Hg treated group. (Figure 3).

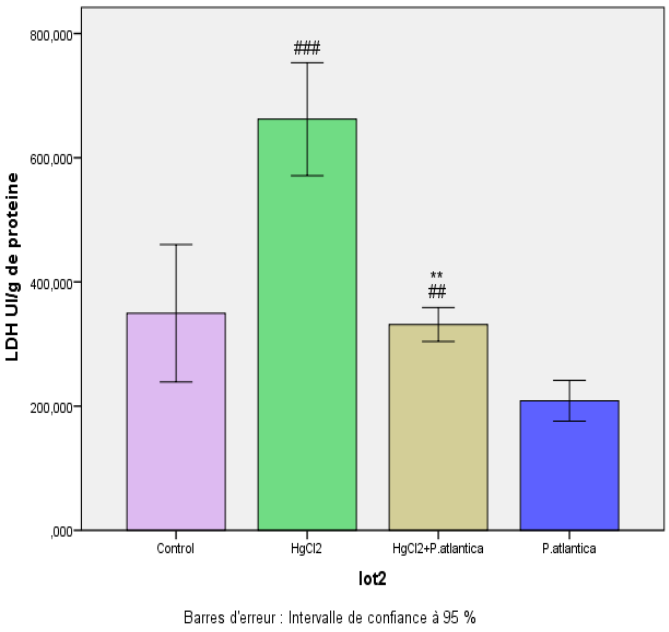

Figure 3: Effects of *P. atlantica* on LDH activity (U/g of proteins). The results are represented by the mean ± standard deviation. P <0.001 (⁂) = indicates a significant difference in the poisoned rats compared to controls. (###) = indicates a significant difference in mercury-poisoned rats treated 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<0,01

4.3. Effect of treatment on AChE activity

Administration of mercuric chloride to rats produced a significant (P<0.05) decrease in AChE brain activity of (-76.51%) compared to control rats. However, treatment with ($HgCl_2$ + *P. atlantica)* (150 mg/kg) improved the increase of AChE compared to intoxicated rats. (Figure 4).

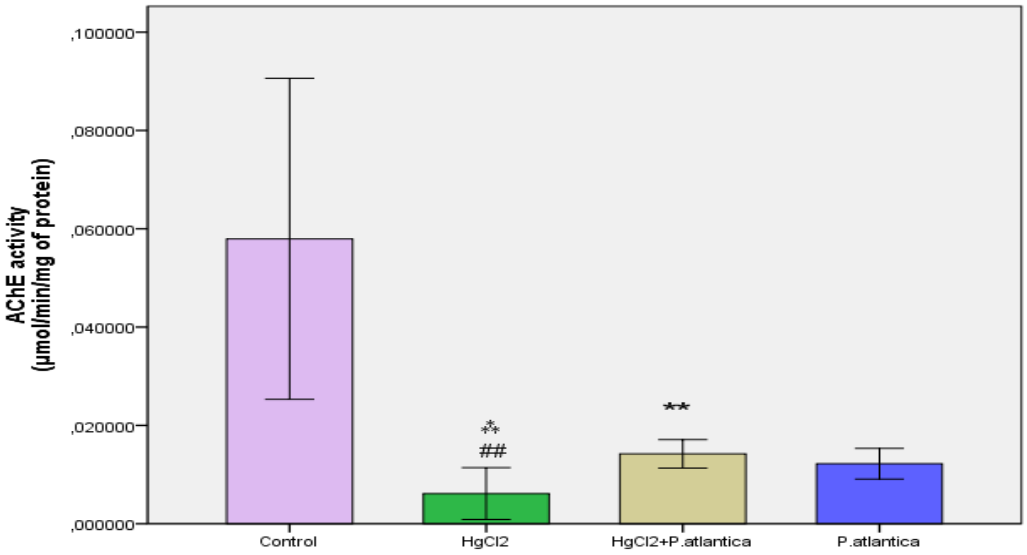

Figure 4**:** Effects of *P. atlantica* on AChE activity (µmol/mn/mg of proteins). The results are represented by the mean ± standard deviation. P <0.001 (⁂) = indicates a significant difference in the poisoned rats compared to controls. (###) = indicates a significant difference in mercury-poisoned 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<0,01

4.4 Effect of treatment on brain histopathological changes

Pathological changes in the brain of rats intoxicated by $HgCl_2$ degeneration neuronal necrosis, gliosis, fragmentation of myeline and axonal degeneration on cuts located intact in the control group. these changes were reduced to a minimum in the + $HgCl_2$+*P. atlantica (Figure 9).*

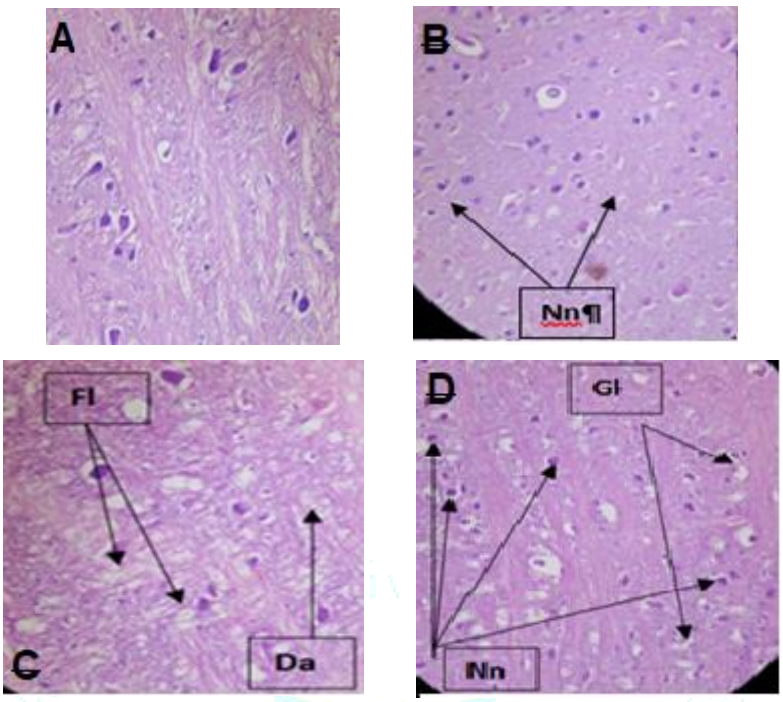

Figure 5: Effects of *P. atlantica* on HgCl2-induced histological changes in brain of control and experimental rats. A (control): section of cerebral (A) showing normal histo-architecture (H&E, 20×); B (HgCl2 +*P. atlantica*:) showing very reduced neuronal necrosis ; C and D (HgCl2: 2.5mg/Kg Sections of cerebral showing neuronal necrosis gliosis, oedeme, fragmentation of myeline and axonal degeneration. Gl: gliose, Om : Oedeme, Nn : neuronale Necrosis, Fl: fragmentation of myeline, Da : axonal degeneration

5. DISCUSSION

Nerve cells and especially astrocytes are sensitive to damage caused by an excess of ROS such as $O_2°^-$, H_2O_2, NO et HO°. These ROS responsible for cellular dysfunction, therefore for the disruption of nerve functions and the occurrence of neurodegenerative diseases[21]. The present results corroborate the previous results which demonstrated that exposure to mercury stimulated the generation of ROS. Our results agree with further studies[22, 23]. the combination of Hg and the *P. atlantica* extract in our study showed a reduction in TBARS levels in the brain. The GSH antioxidant system is an important target in mediating the neurotoxicity of mercury[24]. In this study, the administration of mercury led to the depletion of glutathione (GSH) content in the brain. From a molecular point of view, the decrease in GSH levels, which can occur following the formation of the GS-HgCH3 complex will lead to an increase in the generation of reactive species and oxidative damage in a plethora of biomolecules (acids nucleic acids, lipids and proteins)[25]. The evaluation of GSH levels in the brain tissue recorded a significant decrease in rats poisoned by $HgCl_2$. Our result is in agreement with several studies[26, 27]. The extract of *P. atlantica* administered in group 3 poisoned by Hg (Hg + *P. atlantica*) reveals an increase in the level of GSH comparing to the $HgCl_2$. We have demonstrated a decrease in antioxidant enzymes (SOD, GPx, GST and catalase) in the brain intoxicated by mercury chloride $HgCl_2$. The decrease in enzyme activity was a consequence of direct inhibitory effects, probably linked to Hg-selenol interactions[28, 22]. Several researchers have approved the inhibitory and reducing effect of antioxidant enzymes during mercury chloride $HgCl_2$ intoxication in the brain[26]. The significant increase in the level of glutathione-s transferase, catalase, superoxide dismutase and peroxidase in the brain treated with the extract of *P. atlantica* compared to the $HgCl_2$ is carried out thanks to the presence of phenolic compounds in general and the flavonoids which could directly neutralize reactive oxygen metabolites due to the presence of different antioxidant substances. Our result agrees with work on burned rats receiving 300 µL / kg / day of *P. atlantica* oil for 14 days marked a significant increase in the levels of antioxidant enzymes SOD, GPX, and VEGF (Vascular Endothelial Growth Factor)[29]. AChE is a complex protein that has an active center, It belongs to the family of hydrolases and it is expressed in the central nervous system and muscles, its role is to hydrolyze the neurotransmitter acetylcholine in order to complete the transmission of the nerve impulse and thus restore the excitability of the cholinergic synapses[30]. The administration of $HgCl_2$ leads to a reduction in acetylcholine esterase levels in the brain of the rats of the second group compared to the rats of the first group controls. which confirms the study of Dahalan and al.,[31]. It was found that all the metal ions that Ag2 +, Cd 2 +, Cu 2 + Hg2 + Pb2 + and Zn2 + significantly inhibited the activity of AChE but with different percentages of inhibition. Inhibition of acetylcholine esterase activity in the brain treated with *P. atlantica* ($HgCl_2$+ *P. atlantica*) comparing to the addict what is consistent with the work of Nadeem *et al.,*[32]. *P. atlantica* is mainly characterized by monoterpenoids such as α-pinene which have AChE

inhibitory and antioxidant activities. In addition, previous studies have shown that limonene is a potent inhibitor of AChE as well as β-phellandrene[33].

As a result, lactate dehydrogenase (LDH) is a cytosolic enzyme, which will be released and its activity is measured in order to assess cell death, in particular primary necrosis and secondary necrosis (necrosis following apoptosis). The increase in LDH level in brains poisoned by $HgCl_2$ (349.51 ± 24.71 IU / g) compared to the control, which confirms studies showing a high LDH level in the brain tissue[34]. From the observation of the histological sections at the level of the cerebral parenchyma that we carried out reveals that the toxicity of mercury was manifested by unequivocal tissue damage. Neurodegenerations have been characterized by morphological changes such as neuronal loss and vacuolation[35] these neurodegenerative changes in the brain could invariably affect the learning, memory and hearing capacities associated with its functions. Our results also agree with Akintunde and Babaita[35]. The protective effect against brain bonds induced by mercury was approved by the *Pistacia* extract which reduced neuronal necrosis and prevented other pathologies such as gliosis degenerate axon which is consistent with the work of Liu et al.,[36], This reduction in ROS is due to secondary metabolites such as: flavonoids and tannins which is found in the extracts of the leaves of *P. atlantica.*

In conclusion, the results of the present study indicate that *P. atlantica* demonstrated significantly higher levels of rescue of $HgCl_2$-induced neurotoxicity and oxidative damage, histopathological changes and inhibition of AChE.

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Conflict of interest statement

The authors report no conflict of interest.

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