Available online freely at www.isisn.org

Bioscience Research

Print ISSN: 1811-9506 Online ISSN: 2218-3973

Journal by Innovative Scientific Information & Services Network

RESEARCH ARTICLE BIOSCIENCE RESEARCH, 2021 18(2): 1238-1247. **OPEN ACCESS**

Antioxydant and Hepatoprotective effect of aqueous extract of *Pistacia atlantica* Desf on Rat's exposed to Mercury

Fatiha Benahmed [1,2]*, Fatima Kerroum[3] Sietou BenZakour[1], Aminatou djiré[1] and Omar Kharoubi[1]

[1]Department of Biology, Faculty of Life and Natural Sciences, University of Oran1, Ahmed Ben Bella, 1524 EL M Naouer 31000 Oran, **Algeria**
[2]Department of Biology, University Echahid Ahmed Zabana Relizane, **Algeria**
[3]Research Center of Biotechnology, Constantine, **Algeria**

***Correspondence:** fatbenahmed@hotmail.com **Received 11-02-2021, Revised: 19-03-2021, Accepted: 30-03-2021 e-Published: 10-04-2021**

The purpose of the present study is to evaluate hepato-protective effect of natural products of aqueous extract of *Pistacia atlantica* against oxidative stress induced by toxicity of mercuric chloride in liver of male rats. In the first time, determination of total phenolic and flavonoid contents as well as the antioxidant activity of the leaves extract was carried out. In the second part, Adult Wistar rats weighing between 55 and 65 g were randomly assigned to 3 lots. The animals were administered with mercury chloride ($HgCl_2$) intraperitoneally for 32 days. The group intoxicated receive 2.5 mg/kg of $HgCl_2$, while the treated group receive 2.5 mg/kg of $HgCl_2$ and 150 mg/kg of *P. atlantica*. The tested extract showed a significant antioxidant properties and high phenolic and flavonoid contents. In our experimentation, we obtained a significant change in enzymatic activity. We also observed the significantly a decrease in hepatic glutathione levels, the enzymatic activity of antioxidant system GST and the expression of lipid peroxidation by a higher level of hepatic MDA. In addition, supplementation by aqueous extract of Pistacia atlantica modify the toxic effects of mercury by improving somes disturbances. This results show that the administration of aqueous extract of P. atlantica is a good extract with very promising therapeutic values for the treatment of liver damages

Keywords: Mercury, *Pistacia atlantica*, Hepatoprotective, antioxidants activity, polyphenols, flavonoids

INTRODUCTION

For centuries, herbal medicines have been used by humans to treat their illnesses. Today, they are still the main source of therapeutic substances in developing countries (OMS, 2013). One of the most abundant problems in the biological and medical world is oxidative stress, it comes from an imbalance in redox homeostasis and results in the excessive formation or insufficient suppression of free radicals, this imbalance leads to an overproduction of reactive oxygen species (ROS) capable of causing damage to vital cellular components (lipids, proteins, DNA) leading to many diseases, such as cancer (Belaïch et al.2015; Baldisserotto et al. 2020), to protect themselves from it, the body puts in place a prominent antioxidant defense system (Snezhkina et al. 2019). However, it can develop deficiencies in endogenous antioxidants which require supplementation to maintain optimal bodily function (Aziz et al. 2019). Algeria by its geographical location in the center of the Mediterranean, shelters a rich and diversified vegetation, a large number of plants grow there

spontaneously among which *Pistacia atlantica* or "Betoum" of the family of Anacardiaceaes, widely used in various fields in particular in medicines traditional and in food (Mahjoub et al. 2018). Recently new approaches have demonstrated that plants are capable of having direct impacts on public health and the economy (Bodeker and Graz, 2020), similarly traditional knowledge and scientific reports show that they are rich sources of biologically active compounds among these phenolic compounds considered to be natural antioxidants have attracted particular interest due to their implications in the prevention and treatment of several diseases (El Guiche *et al*., 2015; George et al. 2017). New sources of natural compounds with antioxidant activity are actively sought because synthetic antioxidants present potential toxicological risks (Evenamede et al. 2017; Hęś et al. 2019). It is in this perspective we became interested in *Pistacia atlantica* to assess its antioxidant potential from this goal we noticed that there has been very little phytochemical study on *Pistacia atlantica* in Oran (Algeria). In order to quantitatively characterize the extracts prepared from the leaves of *Pistacia atlantica*, an assay of total polyphenols and flavonoids was carried out.

Mercury toxicity is known to affect the redox state of target tissues by increasing the production of free radicals, producing by oxidative stress (Benahmed et al. 2020). The clinical use of mercury is limited by the onset of severe damage in gut, liver organs (Benahmed et al. 2020; Hallal et al. 2016), Hence, single doses of $HgCl_2$ have been used to induce hepatotoxicity in experimental animals (Benahmed et al. Hazelhoff and Torres, 2018).Mercury is the sixth most abundant toxic element found in the earth's crust as an elemental form that is released into the environment through the use of both natural and anthropogenic sources. (Hazelhoff and Torres, 2018) estimates that there is a risk health associated with mercury exposure in 19 million people worldwide, with an assessment of disease burden of 1.5 million DALYs (Budnik, and Casteleyn. 2019). The liver is a major site of metabolism for mercury and it can accumulate in the liver, resulting in severe hepatic damages (Hazelhoff MH and Torres AM, 2018).

Studies have revealed that mercuric chloride caused histopathological and ultrastructural lesions in the liver evidenced by periportal fatty degeneration and cell necrosis (Benahmed et al.2020). The toxic effects of divalent mercury can be prevented by antioxidant defense mechanisms including reducing or eliminating active oxygen species, free radicals, and heavy metals (Toul et al. 2017). Many researchers have reported that most edible plants are therapeutic foods that exhibit numerous biological properties such as antibacterial, anticancer, antiviral activities and antioxidant properties (Bodeker et Graz, 2020),

The choice of these substances lies in the fact that they play key roles in many biological activities (Wang et al. 2018; Zhou et al. 2019). For the quantitative evaluation of our aqueous extract of *P.atlantica*, an assay of polyphenols and flavonoids was performed in our study.

Through this study, we investigated some effects of exposure to inorganic mercury in the function of the liver, on the other hand, several efforts have made to use the alternatives drugs as the treatment by natural sources such as des medicinal plants for reducing the damages in liver (Xu et al*.* 2017)

The aim of this present research was to evaluate hepatoprotective effect of the leaves aqueous extract of *P. atlantica* against mercuric chloride-induced in rats by assessing the antioxidant parameters.

MATERIALS AND METHODS

2.1. Chemicals

All of the chemicals and reagents used in the experiments were of analytical grade. β-carotene, reducing power, DMSO, BHT, NBT, Ascorbic acid were purchased from Sigma-Aldrich Chemical Company (St. Louis, MO).

2.2. 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).

.2.2.1. Phytochemical analysis of plant.

For phenolics, the total phenolic contents were determined according the Folin–Ciocalteu spectrophotometric method described by (Singleton et al. 1999) with slight modifications. Briefly, an aliquot of the extract solution of extract (0.125 mg/mL; 0.2 mL) was mixed with Folin–Ciocalteu solution (1 mL, 1:10 v/v) and the

aqueous solution of sodium carbonate (75 g/L, 0.8 mL). The tubes were vortexed and incubated for 2h in the dark at room temperature. Absorbance was then measured at 765 nm using UV–vis spectrophotometer (Jasco, V-530). Gallic acid was used to calculate the standard. Curve (10, 30, 50, 70, 90, 110, 130, 150 µg/mL) and the results were expressed as mg of gallic acid equivalents (GAE) per g of dried weight.

Concerning flavonoids, the procedure performed using the method of the mercury chloride colorimetric method described by (Djeridane et al., 2006). an aliquot of the extract solution (0.125 mg/mL; 1 mL) was mixed with equal volume of AlCl3 solution (2% in methanol). Then the mixture was properly mixed and the intensity of pink color was measured at 430 nm. Quercetin was used to calculate the standard curve (5, 10, 15, 20, 30, 40, 50 µg/mL) and the flavonoid contents were expressed as mg of quercetin equivalents (QE) per g DW.

2.2.2. In vitro Antioxidant potential assays

2.2.2.1. Antioxidant activity with the β-carotene bleaching method

The antioxidant activity was evaluated with the β -carotene bleaching protocol according to the method of (Nachvak et al., 2018). 0.5 mg of β-carotene was solubilized in 1 mL of chloroform, then 25 µL of linoleic acid and 200 mg of Tween 40 were added. After, the chloroform in the mixture was evaporated; 50 mL of oxygenated ultra-pure water was added with vigorous stirring. To 40 µL of the sample at different concentrations was added 160 µL of β carotene/linoleic acid mixture, followed by initial absorbance measured at 470 nm using a 96-well microplate reader. After incubation of the plate for 2 h at 50°C., the absorbance was measured again. DMSO was used as a control. BHT was used as standard. The anti-oxidant activity was calculated using the following equation:

Antioxidant activity % = [1 – (As t=0 – As t=120/Act=0 –Act=120)] x 100.

Where: As t=0: absorbance of the sample at t = 0, As t=120: absorbance of the sample at t = 2h, Ac t=0: absorbance of control. at t = 0, Ac t=120: Absorbance of control at t = 2h.

2.2.2.2. Scavenging of superoxide radical by alkaline DMSO method

The superoxide radical scavenging ability was measured in terms of inhibition of generation of O2− using alkaline DMSO according to (Rajamanikandan et al., 2011). The reaction mixture consisted of 40 µL of *P. atlantica* at varying concentrations (0-4mg/mL in DMSO), 130 µL of alkaline DMSO (1 mL DMSO containing, 5 mM NaOH in 0.1 mL water) and 30 µL NBT (1 mg/mL in DMSO). The mixture was incubated at 25 °C for 5 min, and absorbance was measured at 560 nm using a 96-well microplate reader. Ascorbic acid was used as positive control. The scavenging activity is determined using the equation:

Scavenging activity= (A sample- A control)/ (A sample) x 100

2.2.2.3.Hydroxyl radical scavenging activity

Scavenging activity of hydroxyl radical of the extract was measured according to the method of (Rana et al. 2011) 200 µL of the final reaction solution consisted of aliquots (80 µL) of various concentrations of *P. atlantica*, 24 µL $FeSO_4$ (8 mmol/L), 20 µL of H_2O_2 (20 mmol/L) and 80 µL sodium salicylate (3mmol/L). The reaction mixture was incubated for 1 h at 37 C. L-Ascorbic acids were used as the standard.The color development was measured at 560 nm using a 96-well microplate reader against a blank. The scavenging activity is determined using the formula:

Scavenging activity= (A control - A sample)/(A control) x 100

2.2.2.4. ABTS free radical scavenging activity

The decolorization test of 2,2'-azinobis- (3-ethylbenzothiazoline-6-sulfonic acid (ABTS) free radical cations was carried out using an improved method of O¨ ztu¨rk et al with slight modifications A stock solution of ABTS+ was produced by the oxidation of ABTS 7.0 mM in water and 2.5 mM potassium persulfate for 16 h in the dark at room temperature, after that, the solution ABTS+ was diluted with absolute ethanol to an absorbance of 0.8-0.9 at 734 nm before being used in the test. Then, 160 *µ*L of ABTS+ solution was added to 40 *µ*L of *β*-glucans in DMSO at different concentrations. After 10 min, the absorbance was monitored at 734 nm by using a 96-well microplate reader. DMSO was used as a control, while BHA was used as standard. Results were expressed as IC50.The capability to scavenge the ABTS+ radical was calculated using the following equation:

ABTS radical-Scavenging activity= (A control - A sample)/(A control) x 100

2.2.2.4. DPPH free radical scavenging activity

The radical scavenging activity of *β*-glucans was determined using DPPH assay according to Blois. The decrease in the absorption of the DPPH solution after the addition of an antioxidant was measured at 517nm.The reaction requires preparing a mixture of 40 *µ*L of *β*-glucans at various concentrations (0.0625–4 mg/mL) with 160 *µ*L DPPH solution (0.02% prepared in DMSO). For each concentration, a blank is prepared by mixing40 *µ*L of samples with 160 DMSO. A control containing 40 *µ*L DMSO and 160 *µ*L DPPH is also prepared. Alpha-tocopherol (4mg/mL) was used as standard. After incubation for 30 min in the dark, the decrease in the absorption of the DPPH solution was measured at 517nm using a 96- well microplate reader (Enspire Multimode plate reader, Perkin Elmer). Scavenging activity was expressed as IC50 (Concentration in *µ*g/mL of *β*-glucans that reduces the absorbance of DPPH by 50%). The anti-radical activity is determined using the formula:

DPPH radical scavenging activity (%) = (A control - A sample)/(A control) x 100

2.3. Determination of Hepato-protective Effect in Vivo

2.3.1. Animals and Experimental Design

Twenty-four adult male rats of Wistar strain (weighted 60 ± 10 g) were obtained from the animal facility of the Department of Biology at the Faculty of Nature and Life Sciences, Oran 1 University. The experimental animal procedures were conducted in accordance with the Regulations of the Guide for the Care and Use of Laboratory Animals (8th edition, 2011) and approved by the scientific committee of the university. The animals were allowed free access to tap water and rodent chow, housed under the standard conditions with 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 negative control (control) receiving an intraperitoneal injection of saline solution (0.9% NaCl); once a week for 32 days. Groupe II serving as inorganic mercury intoxicated group ($HgCl_2$) received 2.5 mg/kg body weight (b.wt.) by intraperitoneal injection for four weeks. Group III serving as Inorganic Mercury-treated group ($HgCl_2$+*P.atlantica*) received $HgCl_2$ poisoning doses same as intoxicated group and treated by aqueous extract of *P. atlantica* (150 mg/kg b.wt., orally administration by force-feeding) daily for 32 days. They have recorded no deaths or any symptoms of toxicity 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.

The period of experimentation was 4 weeks under standards laboratory conditions. All the animals received the corresponding doses of the respective test solution daily for 4 consecutive weeks. All the groups were fasted for 24 h allowed to receive only water and libitum. After the end of treatment, rats were scarified by decapitation (solution of chloral, 3%) for obtaining blood and liver tissue.

2.3.2. Determination of antioxidant makers in liver tissues

The adult rat livers from the different studied groups were removed and rapidly dissected. After crushing and homogenization, the tissues 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 temperature of 4°C. The obtained homogenate was centrifuged at 7600 rpm for 10 minutes at 4°C to obtain supernatant which centrifuged at 12000 rpm for 10 minutes to remove cellular debris and stored at -80°C prior to examine the oxidative stress parameters presented by: Lipid peroxidation (LPO) levels assessed by TBARS assay using the method described by Ohkawa et al. (1979) in the liver. Tissue protein content at the hepatic tissues was performed using the method of Lowry et al. (1951) and determination the activity glutathione S-transferase (GST) (EC: 2.5.1.18) described by Habig et al. (1974), The results were expressed as Units/mg protein (mmol H_2O_2 degraded/ mg of protein, µmol/mg proteins/min,) respectively.

2.4. 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 $\alpha = 0.05$, for comparison of various treatments. A student's t-test was used to determine the significant difference among two different.

RESULTS

3.1. Phytochemical analysis of plant

Total phenolic contents (TPC) and flavonoid contents (TFC) of aqueous extract of P. atlantica indicated in table 01 demonstrated the very highest levels by 391.9 ± 28 and 59.3 ± 0.8 mg/g, respectively.

3.2. In vitro Antioxidant potential assays

The result of our extract study's shows a higher antioxidant capacity with lower EC50 values of 16.46 ± 1.46, 96.42 ± 5.75, 152.56 ±4.29, 7.25±0.21, 11.12±0.29 and 10.32±0.3 µg/mL measured by Reducing power, DMSO and β -carotene assays, DPPH, CUPRAC, ABTS+ respectively (table 01).

3.3. Determination of Hepato-protective Effect in Vivo

3.3.1. Determination of Antioxidant Makers in Liver Tissues

Determination of lipid peroxidation:

TBARS are a marker of lipid peroxidation, reflecting a pro-oxidative effect. Lipid peroxidation increased significantly by 18.33% in the liver at mercury chloride poisoned group compared to the control group; the mercury poisoned rats treated with *Pistacia atlantica* extract compared to those treated with mercury chloride decrease. (Figure01).

GST

In liver, GST activity is not significantly different in the INT+PL groups compared to the control

Table 01: Antioxidant activity of aqueous extract of *P. atlantica* leaves

		Positive Control			
Antioxidant activity (EC50 values, µg/mL)		α-Tocopherol	Ascorbic acid	BHT[b]	BHA[b]
Reducing Antioxidant Power	16.46 ± 1.46	34.93±2.38	6.77 ± 1.15	//	//
DMSO	96.42 ± 5.75	31.52±2.22	7.59 ± 1.16	//	//
β-carotene linoleic acid bleaching assay	152.56 ±4.29	//	//	0.91±0.01	1.05±0.03
DPPH	7.25±0.21	//	//	6.82±0.49	//
CUPRAC	11.12±0.29	//	//	9.62±0.87	3.64±0.19
ABTS+	10.32±0.3	//	//	1.59±0.03	1.03±0.00
Phenolic compounds (mg/g)					
TPC	391.9 ± 28				
TFC	59.27 ± 0.8				

** BHA: standard for antioxidant activity. The antioxidant activity was expressed as EC50 values (mean ± SD), what means that higher values correspond to lower reducing power or antioxidant potential. EC50: extract concentration corresponding to 50% of antioxidant activity or 0.5 of absorbance in Cupric Reducing Antioxidant Power assay. Total phenolic content (TPC as mg gallic acid/g Dry weight), total flavonoids contents (TFC as mg quercetin/g Dry weight).*

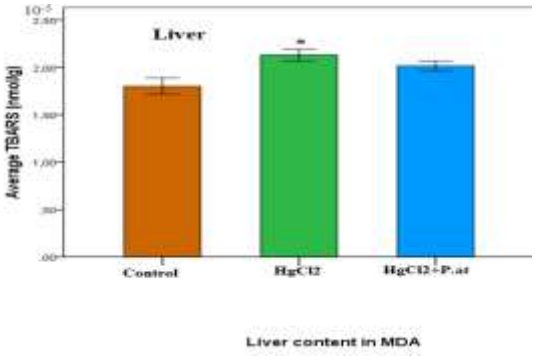

Figure 1: The hepatoprotective effect of *P.atl* on $HgCl_2$-induced oxidative stress in liver tissues. LPO (TBARS) content in liver of rats. Data are expressed as mean ± SD, n = 6. * and *** significantly different ($p < 0.05$) from control and $HgCl_2$-treated group, respectively

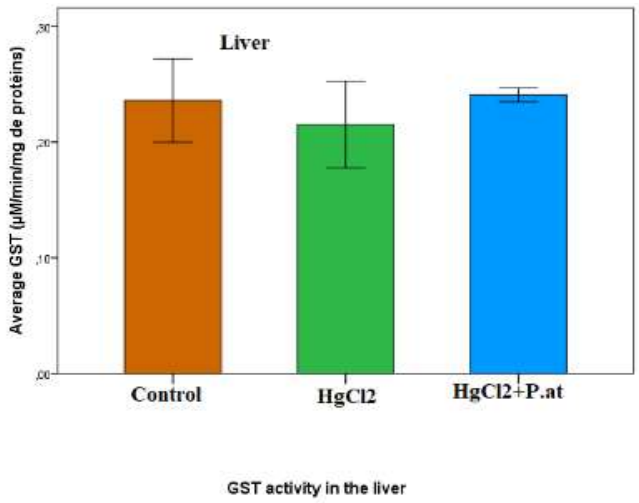

Figure 02: The hepatoprotective effect of *P.atl* on $HgCl_2$-induced oxidative stress in liver tissues, GST activity (µmol/mg of proteins). The results are represented by the mean ± standard deviation (Moy ± SEM). P <0.05 (*) = indicates a significant difference in the poisoned rats compared to controls. (**) = 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.

DISCUSSION

The total polyphenol content of the extract shall be calculated with reference to the gallic acid calibration curve and expressed in milligram gallic acid equivalent per gram of extract (mg GGE/g extract).The total polyphenol content of our sample extract which is consistent with the results of Hashemi et al. (2017) which is 269 mg EAG/g extract (Southwest Iran). The variability of its results is strongly dependent on the region of growth of the species (geographical aspect) (Konieczynski et al. 2016) but also on the low specificity of the reagent of "Folin-ciocalteu" which is the main disadvantage of this colorimetric assay (Roslan et al. 2019). The quantitative estimation of total flavonoids using the method of aluminum trichloride ($AlCl_3$) showed that this extract is relatively rich in flavonoids with a rate of 59.27±0.8 mg EC/g extract (table). This result was obtained from the calibration curve established with known concentrations of catechin. Comparing our results with other results obtained on the same plant variety in different extracts, we find that the flavonoid content of our sample is very high compared to those of Bakka et al. (2019) who found a level of 0.0407±0.000 mg EC/g of chloroformic extract in the same axis Khiya et al. (2019) found a level of 129.15 mg EC/g of ethanolic extract which is much higher than our results. This difference in content can be explained by the studies of Mbaïhougadobé et al., (2017) who stated that the total flavonoid content is variable and differs from one plant to another and from one extract to another. The results obtained in table 1 suggest that the antioxidant capacity of P.atlantica is related to its content of total polyphenols and total flavonoids. The results of the evaluation of antioxidant activity by the DPPH test are expressed in IC50. The IC50 is the concentration of antioxidants necessary to reduce 50% of the DPPH radical (Awa et al., 2018). Given that the IC50 of our extract (7.25±0.21 µg/mL) is significant compared to the standards (BHT, BHA) (6.82±0.49 µg/mL) this would mean that it has significantly reduced the DPPH, therefore has a good antioxidant activity, His results are superior compared to those of Achili et al., (2020) on the methanolic extract which determined an IC50 of 2.87±0.16 µg/ml which is also higher than those of Karimi et al. (2020) whose IC50 is equal to 1.54±0.12 µg/ml of ethyl acetate extract. Rigane et al. (2016) found an IC50 of 32±0.01 µg/ml, this concentration is higher than our result but lower in antioxidant activity. Several factors can influence the IC50 values, such as the solvent used, the concentration of the DPPH solution and the incubation period (Sharma and Baht, 2009).

The IC50 value is inversely related to the antioxidant capacity of a compound; the lower the

value, the higher the antioxidant activity (Bouyahya et al. 2017). The reducing power of our extract has been estimated using the copper reduction test measured at an absorbance of 450nm, the results are grouped in the table 1 with BHT and BHA standards.As a comparison, our extract to show an absorbance (11.12±0.29 µg/mL) which is close to those of the reference antioxidant which is 9.62±0.87 (BHT) and 3.64±0.19 (BHA). In previous studies, the CUPRAC method has been little used to determine the antioxidant capacity of the leaves of P. atlantica, however Achili et al.,(2020) found results in the order of 4.98±0.34 µg/mL for its ethyl acetate extract, which is lower than our result. According to Luminita, (2015) the antioxidant capacity of an extract should reflect the capacity of the lipophilic and hydrophilic fractions so the CUPRAC method shows versatility in the determination of hydrophilic and lipophilic antioxidants, because the CUPRAC chromophore, copper (I) bis (neocuproin) chelate has a unipositive charge with less ion-dipole interactions with water and the chelated rings are essentially hydrophobic. Thus, it is compatible with aqueous and organic solvents as well as alcohol-water mixtures (Apak et al. 2016).The results of the ABTS+ radical absorbance inhibition tests. By the aqueous extract of the leaves of P.atlantica are indicate that the extract has a high reducing potential of the ABTS+ radical, its IC50 is low (IC50=10.32±0.3 µg/mL). However this capacity is quite higher than that of BHT and BHA which are very pure. Our results gave an antioxidant efficacy evaluated by the ABTS method that is much better than those recorded by Rigane et al.(2016) which is 300±0.01 µg/mL for its aqueous extract, while Achili et al. (2020) recorded a better antioxidant activity (2.76±0.17 µg/mL) of methanolic extract. The differences observed in the ABTS test may be due to the solvent used, in addition to an ecological implication (Dawidowicz and Andrzed,2013).The inhibitory potential obtained with DDPH is lower than that measured with the ABTS+ radical. This could be related to the presence in the extract of Pistacia atlantica of substances that have absorption bands at the same wavelength as the DPPH radical, resulting in an increase in optical density and a decrease in inhibitory potential (Dieng et al. 2017).

The antioxidant activities of the extract of Pistacia atlantica determined by DPPH, CUPRAC and ABTS+. were found to be strongly correlated with total polyphenols and flavonoids evaluated respectively by the tests of "Folin-Ciocalteu" and aluminum trichloride. The antioxidant activities of natural compounds are frequently accompanied by an antiproliferative property (Et-Touys et al.2019).

Mercury toxicity is known to affect the redox state of target tissues by increasing the production of free radicals, producing by oxidative stress (Abd Elghani, 2020). The clinical use of mercury is limited by the onset of severe damage in gut, liver organs (Abd Elghani, 2020), Hence, single doses of HgCl2 have been used to induce hepatotoxicity in experimental animals (Benahmed et al. 2020 and Hazelhoff and Torres, 2018).

Through this study, we investigated some effects of exposure to inorganic mercury in the function of the liver; on the other hand, several efforts have made to use the alternatives drugs as the treatment by natural sources such as des medicinal plants for reducing the damages in liver (Xu et al.2017)

The present results corroborate the previous results which demonstrated that exposure to mercury stimulated the generation of ROS. Our results agree with further studies (Joshi et al. 2017)**.** The combination of Hg and the P. atlantica extract in our study showed a reduction in TBARS levels in the liver and increase in the level of GST comparing to the $HgCl_2$. Norasteh et al. 2020 revealed that Pistacia atlantica reduced the free radicals and prevented liver damage by decreasing the level of MDA and increasing the level of antioxidant superoxide dismutase and catalase (Tolooei and Mirzaei, 2015).

CONCLUSION

Quantitatively our results lead us to conclude that the aqueous extract of *Pistacia atlantica* contains a considerable amount of total polyphenols and flavonoids which confirms its antioxidant activity and this has been shown by the results obtained in different tests: DDPH, ABTS, and CUPRAC. Our study showed that this extract has a good activity which could be due to compounds considered as antioxidant agents and the rats intoxicated and treated by *P. atlantica* induce a benefic effect on liver tissues, and restore the antioxidant, biochemical parameters to normally. These finding could be related to the mixtures of bioactivities of *P. atlantica*, in addition to the free radical scavenging activity of *P. atlantica* due to its contents of the high level of total phenolic and flavonoids. The aqueous extract of *P. atlantica* leaves possesses the obvious hepatoprotective capability and has an encouraging effect in the treatment of acute liver

damages induced by mercury. This extract is considered as a very good source for phenolic compounds which has a great impact in prevention against liver injuries. These effective substances present in the leaves encourage consideration of *P. atlantica* as a new natural medicinal source.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

ACKNOWLEGEMENT

This research was supported by the Directorate General for Scientific Research and Technological Development (DGRSDT, MESRS, and Algeria).

AUTHOR CONTRIBUTIONS

FB performed the experiments, data analysis and also wrote the manuscript. FK, SB, AD, provided scientific advice, DS contributed to the experimental design and OK contributed to the manuscript revision. All authors read and approved the final version.

REFERENCES

Abd Elghani, S. A., Oda, S. S., Tohamy, H. G., & Hashem, M. A. 2020. Hematological, Biochemical and Histopathological Studies on the Prophylactic Effect of Zinc Sulphate Against Mercuric Chloride Toxicity in Rats. *Alexandria Journal for Veterinary Sciences*, *64*(2).

Achili, I., Amrani, A., Bensouici, C., Gül, F., Altun, M., Demirtas, I., ... & Benayache, S. 2020. Chemical constituents, antioxidant, anticholinesterase and antiproliferative effects of Algerian Pistacia atlantica Desf. extracts. *Recent patents on food, nutrition & agriculture*, *11*(3), 249-256.

Aebi H. Catalase. In: Berg Meyer H., editor. Methods of enzymatic analysis. 2nd ed. Weinheim: Verlag Chemie. 1974; p. 673-84

Agarwal AS, Goel R and Beharia J. 2010. Detoxification and antioxidant effects of curcumin in rats experimentally exposed to mercury. J Appl Toxicol, 30: 457-468.

Apak R., Özyürek M., Güçlü K et. Çapanoğlu E. 2016. Antioxidant Activity/Capacity Measurement. 1. Classification, Physicochemical Principles, Mechanisms, and Electron Transfer (ET)-Based Assays. *J.Agric.Food.Chem*. 64(5): 997-1027.

Awa D., Konan Y., Youssouf S., Honora T B F., Adama B et. Witabouna K M. 2018. Pouvoir antioxydant et teneurs en composés phénoliques de deux espèces du genre Albertisia: Albertisia cordifolia (Mangenot & J. Miège) Forman et Albertisia scandens (Mangenot & J. Miège) Forman (Menispermaceae). *European Scientific Journal, ESJ*. 14(30):128-144

Aziz M A., Diab A S et.Mohammed A A.2019.Antioxidant Categories and Mode of Action. *Intechopen*

Bakka C., Hadjadj M., Smara O., Dendougui H et. Mahdjar S.2019. In vitro antioxidant activities and total phenolic content of extracts from Pistacia atlantica desf. *J.Pharm.Sci.&Res*.11(11):3634-3637

Baldisserotto A., Demurtas M., Lampronti I., Tacchini M., Moi D., Balboni G., Pacifico S., Vertuani S., Manfredini S et. Onnis V. 2020. Synthesis and evaluation of antioxidant and antiproliferative activity of 2-arylbenzimidazoles.*Bioorganic Chemistry*.94.

Belaïch R., Boujraf S et. Benzagmout M. 2015. Impact du stress oxydatif et de l'inflammation sur les patients hémodialysés. *Médecine thérapeutique*. 21(2):95-103.

Ben Ahmed Z., Yousfi M., Viaene J., Dejaegher B., Demeyer K, Mangelings D et. Vander H Y. 2017. Seasonal, gender and regional variations in total phenolic,flavonoid, and condensed tannins contents and in antioxidant properties from *Pistacia atlantica* ssp. leaves. *Pharm. Biol*. 55(1):1185–1194.

Benahmed, F., Rached, W., Kerroum, F., & El Belhouari, H. F. Z. 2020. Protective effect of Pistacia atlantica Desf leaves on Mercury-Induced toxicity in Rats. *South Asian Journal of Experimental Biology*, *10*(3), 152-161.

Blois MS. Antioxidant determinations by the use of a stable free radical. Nature 1958; 181: 1199-200.

Bodeker, G., & Graz, B. 2020. Traditional medicine. In *Hunter's Tropical Medicine and Emerging Infectious Diseases* (pp. 194-199).

Bouyahya A., Abrini J., Bakri Y et. Dakka N. 2017. Screening phytochimique et évaluation de l'activité antioxydante et antibactérienne des extraits d'Origanum compactum. *Phytothérapie*.15 :379–383

Budnik LT and Casteleyn L. 2019. Mercury pollution in modern times and its socio-medical consequences. Sci Total Environ, 654:720–734.

Dawidowicz A L et. Olszowy M. 2013. The importance of solvent type in estimating antioxidant properties of phenolic compounds by ABTS assay. *Eur.Food.Res.Technol*. 236:1099–1105

Dieng, S. I. M., Fall, A. D., Diatta-Badji, K., Sarr, A., Sene, M., Sene, M., ... & Bassene, E. 2017. Evaluation de l'activité antioxydante des extraits hydro-ethanoliques des feuilles et écorces de Piliostigma thonningii Schumach. *International Journal of Biological and Chemical Sciences*, *11*(2), 768-776.

Djeridane A, Yousfi M, Nadjemi B, Boutassouna D, Stocker P & Vidal N. 2006. Antioxidant activity of some Algerian medicinal plants extracts containing phenolic compounds. Food Chem, 97(4), 654-660.

El Guiche R.,Tahrouch S., Amri O.,El Mehrach K et. Hatimie A. 2015. Antioxidant Activity and Total Phenolic and Flavonoid Contents of 30 Medicinal and Aromatic Plants Located in the South of Morocco. *International Journal of New Technology and Research (IJNTR)*.1(3):07-11

Et-Touys A., Bouyahya A., Bourais I., Dakka Net. Bakri N. 2019. Étude in vitro des propriétés antioxydante, antiproliférative et antimicrobienne de Salvia clandestina du Maroc. *Phytothérapie.*

Evenamede S K., Kpegba K., Simalou O., Boyode P., Agbonon A et. Gbeassor M. 2017. Etude comparative des activités antioxydantes d'extraits éthanoliques de feuilles, d'écorces et de racines de Cassia sieberiana. *Int.J.Biol.Chem.Sci*. 11(6) : 2924-2935

George V C., Dellaire G et. Rupasinghe V H P.2017. Plant flavonoids in cancer chemoprevention: role in genome stability. *Journal of Nutritional Biochemistry* .45:1–14

Habig, W. H., & Jakoby, W. B. 1981. Assays for differentiation of glutathione S-Transferases. In Methods in enzymology (Vol. 77, pp. 398-405). Academic press.

Hallal N et. Kharoubi O. 2016. Evaluation of oxidative stress and neuroinflammation after mercuric-chloride and Artemisia absinthium L. administration. Toxicology Letters. (258), S246.

Hashemi L., Asadi-Samani M., Moradi M-T et. Alidadi S. 2017 Anticancer Activity and Phenolic Compounds of Pistacia atlantica Extract. *International Journal of Pharmaceutical and Pharmacological Research*. 7(2):26-31.

Hazelhoff MH, Torres AM (2018) Gender differences in mercuryinduced hepatotoxicity: potential mechanisms. Chemosphere 202: 330–338

Hęś M., Dziedzic K., Górecka D., Jędrusek-Golińska A et. Gujska E. 2019. Aloe vera (L.) Webb.: Natural Sources of Antioxidants –A Review. *Plant.Foods.Hum.Nutr*.74(3):255–265.

Joshi D., Sunil K S., Sateesh B et. Vaibhav D A. 2017. Zingiber officinale and 6-gingerol alleviate liver and kidney dysfunctions and oxidative stress induced by mercuric chloride in male rats: A protective approach. Biomedicine and Pharmacotherapy. 91:645–655.

Karimi A., Moradi MT et. Gafourian A. 2020. In vitro anti-adenovirus activity and antioxidant potential of pistacia atlantica Desf. Leaves.*Res.J.pharmacogn*.7(2) :53-60

Khiya Z., Oualcadi Y., Gamar A., Amalich S., Berrekhis F., Zair T et. EL Hilali F. 2019.In vitro Evaluation of Antioxidant Activity of the Methanol and Ethanol Extracts of *Pistacia atlantica* Desf from Morocco*. Phytothérapie*.17:321-333

Konieczynski P., Arceusz A et. Wesolowski M. 2016. Essential elements and their relations to phenolic compounds in infusions of medicinal plants acquired from different European regions. *Biological trace element research*. 170(2):466-475

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem., 1951; 193(1):265-75

Mahjoub F, Rezayat KA, Yousefi M, Mohebbi M and Salari R. 2018. *Pistacia atlantica* Desf. A review of its traditional uses, phytochemicals and pharmacology. J Med life, 11(3): 180.

Mahjoub F., Salari R., Yousefi M., Mohebbi M., Saki A et. Akhavan Rezayat K. 2018. Effect of Pistacia atlantica kurdica gum on diabetic

gastroparesis symptoms: a randomized, triple-blind placebo-controlled clinical trial. *Electronic Physician*. 10(7):6997–7007.

Maoka T. 2020. carotenoids as natural functional pigments. *Journal of Natural Medicines*.74:1-16.

Mbaïhougadobé S., Ngakegni-Limbili A C., Tsiba Gouollaly T., Koane J-N., Ngaïssona P., Loumpangou C N., Mahmout Y et. Ouamba J-M. 2017. Evaluation de l'activité anti-oxydante de trois espèces de plantes utilisées dans le traitement de la goutte au Tchad. *J.Biol.Chem.Sci*. 11(6): 2693-2703

Nachvak SM, Hosseini S, Nili-Ahmadabadi A, Dastan D & Rezaei M. 2018. Chemical composition and antioxidant activity of Pistacia atlantica subsp. Kurdica from Awraman. J report Pharm Sci, 7(3): 222-230.

Norasteh H, MohammadiSh, Nikravesh M, TarazJamshidi Sh. 2020 Protective Effect of Bene (*PistaciaAtlantica*) on Busulfan-Induced Renal-Liver Injury in Laboratory Mice. Journal of Babol University of Medical Sciences22: 17-23

O¨ ztu¨rk M, Duru ME, undefined Kivrak, N MD, A T, MA on the three most edible mushrooms. Food Chem Toxicol.

Ohkawa, H., Ohishi, N., & Yagi, K.. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal. Biochem., 1979; 95(2):351–358.

Organisation mondiale de la santé. Stratégie de l'OMS pour la médecine traditionnelle pour 2014-2023.Genève. *Organisation mondiale de la santé* ;2013.

Rajamanikandan S, Sindhu T, Durgapriya D, Sophia D,Ragavendran P., & Gopalakrishnan V. 2011. Radical scavenging and antioxidant activity of ethanolic extract of Mollugo nudicaulis by invitro assays. Ind J Pharm Educ Res.;45(4):310-316.

Rana N, Mishra S, Bhatnagar S, Paul V, Deorari AK & Agarwal R, 2011. Efficacy of zinc in reducing hyperbilirubinemia among at-risk neonates: a randomized, double-blind, placebo-controlled trial. Indian J. Pediatr, 78(9): 1073-1078.

Re R., Pellegrini R., Proteggente A., Pannala A., Yang M et. Rice-Evans C.1999.Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free.Rad.Biol.Med*.26:1231-1237

Rigane G., Ghazghazi H., Aouadhi C., Ben Salem R et. Nasr Z. 2016. Phenolic content, antioxidant capacity and antimicrobial activity of leaf extracts from *Pistacia atlantica*. *Natural Product Research*.1-4.

Roslan A S., Ando Y., Azlan A et. Ismail A.2019. Effect of Glucose and Ascorbic Acid on Total Phenolic Content Estimation of Green Tea and Commercial Fruit Juices by Using Folin Ciocalteu and Fast Blue BB Assays. *Pertanika Journal of Tropical Agricultural Science*.42 (2):545-556.

Sanchez-Moreno C. Methods used to evaluate the free radical scavenging activity in foods and biological systems. Revista de Agaroquimica y Tecnologia de Alimentos. 2002;8(3):121-137.

Sharma O P et. Bhat T J. (2009). DPPH antioxidant assay revisited.*Food Chemistry*. 113(4):1202-1205

Singleton VL, Orthofer R, & Lamuela-Raventós RM. 1999. Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. J Method Enzymol, 299: (152-178).

Snezhkina A., Kudryavtseva A V., Kardymon O L., Savvateeva M V., Melnikova N V., Krasnov G Set. Dmitriev A A. 2019. ROS Generation and Antioxidant Defense Systems in Normal and Malignant Cells. *Oxidative Medicine and Cellular Longevity*.17 p

Toul F, Belyagoubi-Benhammou N, Zitouni A & Atik-Bekkara F. 2017. Antioxidant activity and phenolic profile of different organs of Pistacia atlantica Desf. subsp. atlantica from Algeria. Nat Prod Res, 31(6): 718-723.

Wang T-Y., Li Q et. Bi K-S. 2018. Bioactive flavonoids in medicinal plants: Structure, activity and biological fate. *Asian Journal of Pharmaceutical Sciences*. 13(1):12-23

Xu DP, Li Y, Meng X, Zhou T, Zhou Y, Zheng Zhang J & Li HB. 2017. Natural antioxidants in foods and medicinal: Extraction, assessment and resources. Int J Mol Sci., 18(1): 96.

Zhou Y., Jiang Z., Lu H., Xu Z., Tong R., Shi J et. Jia G. 2019. Recent Advances of Natural Polyphenols Activators for Keap1-Nrf2 Signaling Pathway.*Chem.Biodiversity*.16.