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Anti-hyperuricemic and neuroprotective effects of *Populus nigra* L. (*Saliacaceae*) flower buds used in Algerian folk medicine

[Efectos antihiperturicémicos y neuroprotectores de los brotes de *Populus nigra* L. (*Saliacaceae*) utilizados en la medicina popular argelina]

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Abstract

Context: *Populus nigra* L., is a species of cottonwood poplar that belongs to the *Salicaceae* family, is cultivated in multiple areas in Algeria, with an Algerian name of Safsaf.

Aims: To evaluate the *Populus nigra* flower buds extract for its effects on brain modifications against aluminum-induced neurotoxicity in mice. The hypouricemic action of the extract was also evaluated.

Methods: To evaluate the effect of extract on $AlCl_3$-induced neurotoxicity on mice a battery of tests was performed to assess a spectrum of learning and memory functions. The hypouricemic action of the extract was studied by the measure of uric acid levels and liver xanthine oxidoreductase activity in normal and hyperuricemic mice.

Results: Co-administration of *P. nigra* extract (200 mg/kg) and $AlCl_3$ (100 mg/kg/day) combined with D-galactose (200 mg/kg/day) during four weeks was found to antagonize the harmful effects of $AlCl_3$ by restoring all the test parameters. Moreover, extract restored the pyramidal cells to near normal in cerebral cortex of mice. Extract, when administered three times orally to the normal and oxonate-induced hyperuricemic mice, was able to elicit significantly hypouricemic effect comparable to that of allopurinol.

Conclusions: *P. nigra* flower buds extract can be considered as food enabling to antagonize $AlCl_3$ toxicity and to control serum uric acid levels.

Keywords: aluminum toxicity; Alzheimer diseases; hyperuricemia; *Populus nigra*; xanthine oxidoreductase.

Resumen

Contexto: *Populus nigra* L. es una especie de álamo que pertenece a la familia *Salicaceae*, se cultiva en múltiples áreas en Argelia, con un nombre argelino de Safsaf.

Objetivos: Evaluar el extracto de yemas florales de *Populus nigra*, por sus efectos sobre las modificaciones cerebrales frente a la neurotoxicidad inducida por aluminio en ratones. La acción hipouricemic del extracto también fue evaluada.

Métodos: Para determinar el efecto del extracto sobre la neurotoxicidad inducida por $AlCl_3$ en ratones, se realizó una batería de pruebas para evaluar un espectro de funciones de aprendizaje y memoria. La acción hipouricemia del extracto se estudió mediante la medición de los niveles de ácido úrico y la actividad xantina oxidorreductasa del hígado en ratones normales e hiperuricémicos.

Resultados: La administración conjunta del extracto de *P. nigra* (200 mg/kg) y $AlCl_3$ (100 mg/kg/día), combinado con D-galactosa (200 mg/kg día), durante cuatro semanas, antagonizó los efectos nocivos de $AlCl_3$ al restaurar todos los parámetros de prueba. Además, el extracto restauró las células piramidales a casi normal en la corteza cerebral de ratones. El extracto, cuando se administró tres veces por vía oral a los ratones hiperuricémicos normales e inducidos por oxatos, fue capaz de provocar un efecto significativamente hipouricémico, comparable al del alopurinol.

Conclusiones: El extracto de los brotes de flor de *P. nigra* puede considerarse como un alimento que permite antagonizar la toxicidad de $AlCl_3$ y controlar los niveles séricos de ácido úrico.

Palabras Clave: enfermedad de Alzheimer; hiperuricemia; *Populus nigra*; toxicidad del aluminio; xantina oxidorreductasa.

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INTRODUCTION

Hyperuricemia, characterized by high levels of uric acid in the blood and caused by overproduction or under excretion of uric acid, is a common metabolic disorder. It has been considered as an important risk factor for gout and may be associated with oxidative stress conditions, impaired renal function and cardiovascular diseases (Ramirez and Bargman, 2017).

Allopurinol, a xanthine oxidase inhibitor, is commercially available as an anti-gout drug; however, adverse effects limit its use (Pacher et al., 2006). The availability of a new agent, with improved tolerability and efficacy would represent an important advancement in the management of hyperuricemia in patients with gout. At present, attention has been focused on phytochemicals. Several compounds present in a wide variety of plants, have been investigated for their potential to protect neuronal injury and to counteract cerebral aging (Douichene et al., 2012; Lalkovičová and Danielisová, 2016). *Populus nigra*, an evergreen tree that belongs to the Salicaceae family, is grown in multiple areas in Algeria, with an Algerian name of Safsaf. It has been one of the popular traditional Algerian medicine, the remedies were prepared primarily by infusion. *P. nigra* flower buds possess multiple biological activities, the ethanol extract of *P. nigra* flower buds has demonstrated a potent anti-inflammatory activity (Karawya et al., 2010; Debbache et al., 2014). In another study, aqueous extract has regulated genes involved in antioxidant defenses (Dudonné et al., 2011) and was effective against several free radicals (Debbache et al., 2014).

Previously, we have reported hepatoprotective and vasorelaxant effects of an ethanol extract (Debbache-Benaida et al., 2013). *P. nigra* is widely used to treat gout and hyperuricemia in folklore medicine, however, its use in modern medicine as anti-gout and as a protective agent against neurodegenerative disorders lack the scientific evidences. In the present study, the effect of *P. nigra* buds extract on the serum uric acid levels and liver xanthine oxidoreductase activity in normal and hyperuricemic mice was screened. Moreover, these investigations aim to clarify the role of *P. nigra* buds extract as a therapeutic agent against Alzheimer's disease (AD). For this purpose, the neuroprotective effect of *P. nigra* buds extract, in mice administered with D-galactose and $AlCl_3$ represented by behavior and memory tests, were confirmed by the histological study of mice's brains.

MATERIAL AND METHODS

Drugs and chemicals

All the reagents and chemicals were of analytical grade or of purest quality and were obtained from Sigma (Germany), represented by Prochima Sigma Tlemcen (Algeria) and from Biochemchemopharma (France).

Instrumentation

The instruments used in this study were analytical balance, Sartorius (Germany), centrifuge, Sigma, 2-16 PK, (Germany), electric mill Kika Labortechnik, Staufen, Germany; Heidolph homogenizers SilentCrusher, Sigma (Germany), micropipettes (1-10 µL, 10-100 µL, and 100-1000 µL) eppendorff, (Germany), magnetic stirrer, microscope Optika B380 Brightfield (Italy), rotary evaporator (IKA-Werke, M20, Germany).

Plant material

Fresh flower buds of *P. nigra* were collected in March 2015 from the forest of Azru'n Bechar in the county of Amizour (suburbs of Bejaia, Algeria). The Global Positioning Systems (GPS) location is 36.644° N, 4.921° E and the collected plant was identified in the Laboratory of Botany, University of Bejaia, Algeria. A voucher specimen number ph001_43 assigned for a spicemen deposited in the National Institute of Agronomy, Algiers (Algeria). http://gdebelair.com/tax/zzpz.html#Salicaceae.

The fresh flower buds of *P. nigra* were air-dried in the shade and ground to fine powder of 63 µm of diameter, using an electric mill (Kika Labortechnik, Staufen, Germany).

P. nigra powder (600 mg) was extracted with ethanol (70%) (1: 8, w:v) at room temperature for 24 h, followed by a decantation for 24 h. The supernatant was centrifuged at 3000 *g*/10 min. The extractive liquid was evaporated, and extract was concentrated in a rotary evaporator (IKA-WERKE, M20, Germany). The residual solid extracts (3.62%) were stocked at -20°C.

Experimental animals

A total of 80 male albino mice (body weights: 18-25 g) were obtained from the Pasteur Institute Algiers, Algeria. Each group containing six mice. They were fed with a commercial laboratory diet and allowed food and water ad libitum for an acclimatization period of two weeks prior to the experiment. All animals were maintained on a 12 h/12 h light/dark cycle and the temperature and humidity were kept at 25 ± 1°C and 50%, respectively. Animals were handled according to the recommendation of the International Ethic Committees Directive 2010/63/EU, which updated and replaced the 1986 Directive 86/609/EEC and the ethical exigencies of the Faculty of Life and Nature Sciences of the University of Bejaia, Algeria.

Anti-hyperuricemic activity of extract

Hypouricemic activity of extract in normal and hyperuricemic mice

Experimentally-induced hyperuricemia in mice (due to the inhibition of uricase by potassium oxonic acid) was used to study anti-hyperuricemic, anti-xanthine oxidase (XO) and anti-xanthine dehydrogenase (XDH) effects of ethanol extract. Food, but not water, was withdrawn from the animals 1.5 h prior to drug administration. Briefly, for hyperuricemic groups, 250 mg/kg of potassium oxonate (PO) dissolved in 0.9% saline solution was administered intraperitoneally (IP) to each animal 1 h before oral administration of extract or allopurinol (Zhu et al., 2004; Haidari et al., 2009).

The animals were randomly divided into ten equal groups (n = 6).

Group 1: Control (vehicle) received saline solution (0.9%).

Groups 2, 3, 4: Normal animals given 100, 200 and 400 mg/kg of extract, respectively.

Group 5: Normal animals given 10 mg/kg allopurinol.

Group 6: Hyperuricemic animals (250 mg/kg oxonic acid).

Groups 7, 8 and 9: Hyperuricemic animals given 100, 200 and 400 mg/kg of extract, respectively.

Group 10: Hyperuricemic animals given 10 mg/kg allopurinol.

The volume of the suspension administered was based on body weight measured immediately prior to each dose administration (10 mL/kg). All drugs were given orally once daily for three days.

Sample preparation

At the end of experiment, mice were anesthetized using inhalant anesthetics conducted in an induction chamber, then they were sacrificed. Blood samples was collected from jugular mice vein using catheter as described by (Parasuraman et al., 2010) and the blood was deposited in a commercial EDTA blood collection tubes. The blood samples were centrifuged at 2500 *g* for 10 min (Centrifuge: Sigma, 2-16 PK, Germany) and the obtained serum was stored at -20°C until use.

Mice livers were removed, homogenized in ice cold isotonic potassium chloride solution (1.15% KCl w/v) containing 0.1 mM EDTA for 3 mn (Homogenizer: Heidolph, Silent Crusher S, Germany). The livers homogenate was then centrifuged at 3000 *g* for 10 min (Centrifuge: Sigma, 2-16 PK, Germany), the lipid layer was carefully removed, and the resulting supernatant fraction was further centrifuged at 1500 g for 60 min at 4°C. The supernatant was stored at -20°C until use.

Uric acid assay

The serum uric acid (UA) levels were measured using standard diagnostic kits (Spinreact, Spain) according to the manufacturer's instructions. Each assay was performed in triplicate.

Livers XO and XDH activities determination

The XO and XDH activities were measured spectrophotometrically (Shimadzu UV-1800 spectrophotometer) by monitoring the production of uric acid from xanthine according to Prajda and Weber's method (Prajda and Weber, 1975; Zhu et al., 2004). In the case of XDH, the assay mixture consisted of 50 µM xanthine, 50 µM phosphate buffer (pH 7.4), 200 µM NAD+. After pre-incubation at 37°C for 15 min, the reaction was initiated by the addition of 100 µL of liver homogenate solution. After 10 min, the reaction was terminated by adding 0.5 mL HCl (0.6 M), and the absorbance was measured at 290 nm using a Shimadzu UV-1800 spectrophotometer.

XO activity was measured using a similar method described for XDH with the difference being that molecular oxygen was used in place of NAD+ as electron acceptor (Zhu et al., 2004).

One unit (U) of activity was defined as 1 nmol of uric acid formed per minute per mg of protein at 37°C and pH = 7.4.

Proteins quantification

Determination of microgram quantities of protein in liver homogenate by the Bradford Coomassie brilliant blue using bovine serum albumin (BSA) as standard (Bradford et al., 1976).

Evaluation of neuroprotective effect of *P. nigra* extract

Neurotoxicity was induced by $AlCl_3$ (100 mg/kg) administered orally and D-galactose (D-Gal) (200 mg/kg) injected intraperitoneally. After 10 min, extract (200 mg/kg) was injected intraperitoneally. All drugs were dissolved in physiological saline solution and administered once daily for four weeks. The mice were randomly divided into four groups; each group containing six mice:

Group I: Control group received only vehicle.

Group II: Alzheimer model group received $AlCl_3$ (100 mg/kg) with D-Gal (200 mg/kg).

Group III: Control treated group received only extract (200 mg/kg).

Group IV: Intoxicated/Alzheimer's treated group received extract and $AlCl_3$ with D-Gal.

Behavioral tests

Functional behavioral assessment was required as part of testing the nervous status (manifest determination).

The open field test measured behaviors in mice to profile their locomotor activity. The platforms 72 x 72 cm with 36 cm walls, lines were drawn on the into sixteen 18 x 18 cm squares (Seibenhener and Wooten, 2015). A camera wired to an automated tracking system (Panasonic WV-CP280, Japan) was used to quantify the total distance travelled and time spent in the center of the open-field.

In head-dipping test, the number of mice head-dipping was recorded for 20 min with interval of 5 min in an apparatus (60 × 39.5 × 20 cm) with sixteen holes (25 mm in diameter) (Boissier and Simon, 1964). A camera wired to an automated tracking system (Panasonic WV-CP280, Japan) was used to quantify the total head dipping.

Black and white test box, described by Crawley and Goodwin, (1980) was based on the total time spent in each chamber was recorded for 20 min with intervals of 5 min using an apparatus consists of a box (80 × 30 × 30 cm) divided into two equal compartments, one compartment colored black and the second white. A camera wired to an automated tracking system was used to quantify the total time spent in dark box.

In forced swim test, the cylindrical tanks (30 cm height x 20 cm diameters) was required. The mice were placed in an inescapable transparent tank that was filled with water (level: 15 cm from the bottom) (Petit-Demouliere et al., 2005). An escape related mobility behavior was measured using a camera wired to an automated tracking system (Panasonic WV-CP280, Japan).

Memory tests

Morris water maze test, established by Morris (1984). Animals were placed in a pool of water that is colored opaque with powdered non-toxic tempera paint, where they must swim to a hidden es-

cape platform (a circular white platform for 10 cm in diameter was submerged 0.5 cm under the water level). The effects of $AlCl_3$ administration on reference memory (SRM) and working memory (SWM) were expressed as the average latencies of the mice to reach an escape platform. In the SRM test, the platform was placed in a fixed location (NE quadrant of the pool) throughout the experiment. In the SWM test, the escape platform was placed from the edge of the pool in one of the four possible locations. A camera wired to an automated tracking system was used to quantify the total time (in seconds) to locate the hidden platform.

Histological analysis

After the step-through avoidance test, the mice were sacrificed. Removed brains were impressed in 10% neutral buffered formaldehyde. Serial coronal paraffin sections were cut at 4 µm thickness with a Leica rotary microtome (RM2245 a semi-motorized rotary microtome, US) for hematoxylin and eosin (HE) staining (Fischer et al., 2008) followed by the microscopic examination (microscope Optika B380 Brightfield (Italy).

Statistical analysis

Data were expressed as mean ± standard error means (SEM) of n=6 tests. Experimental data were analyzed using GraphPad Prism 5.3 Software. Analysis of variance (ANOVA) was used, followed by Student's Newman–Keul's test. P values ≤ 0.05 were considered statistically significant.

RESULTS

Effects of *P. nigra* extract and allopurinol on serum urate levels in normal and hyperuricemic mice

The hypouricemic effects of *P. nigra* extract and allopurinol in normal mice are shown in Fig. 1A. The administration of extract (100, 200 and 400 mg/kg) significantly ($p<0.01$) reduced UA level in normal mice by 38 - 43%. Oral administration of allopurinol decreased it by 90.91% (Table 1).

As shown in Fig. 1A, in control mice, serum UA levels were 3.11 ± 0.35 mg/dL. In hyperuricemic mice, serum UA levels were elevated significantly ($p<0.01$) to 4.21 ± 0.10 mg/dL, 1 h after intraperitoneal injection of PO. These data revealed that PO successfully induced hyperuricemia.

The results indicated that *P. nigra* extract exerted an effective hypouricemic effect in 100, 200 and 400 mg/kg doses (Fig. 1B).

The serum UA levels of hyperuricemic mice treated with extract (100, 200, 400 mg/kg), were lowered significantly ($p<0.001$) by 42.88 - 63.36% (Table 1). At the same time, allopurinol significantly ($p<0.001$) reduced the urate levels (91.98 %), when compared with hyperuricemic control group.

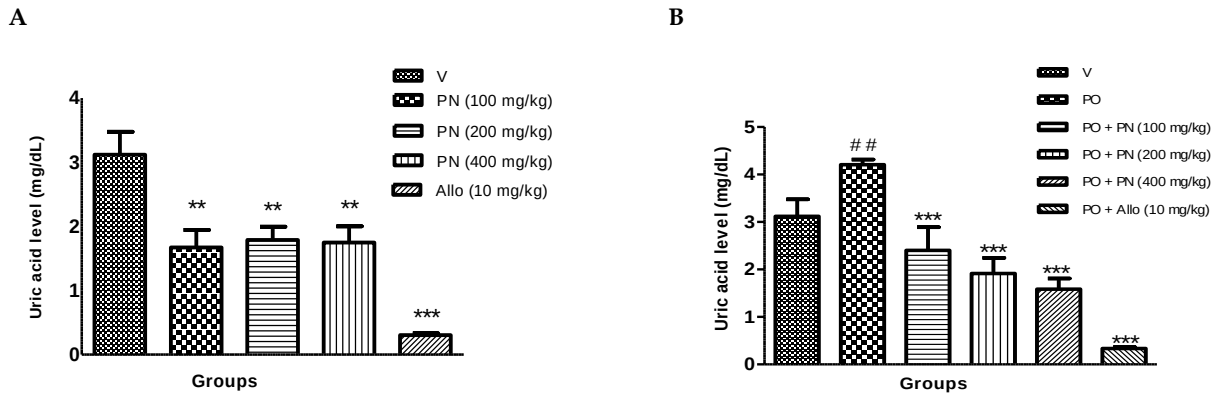

Figure 1. Effect of orally administration of *P. nigra* extract, and allopurinol on serum urate levels **(A)** in normal mice, and **(B)** in hyperuricemic mice pretreated with the potassium oxonate.

V: vehicle, PO: potassium oxonate, PN: *Populus nigra*, Allo: allopurinol. All values are expressed as mean ± SEM of six animals. One-way ANOVA followed by Student's Newman–Keul's test was used for statistical significance. ##P<0.01 compared to normal control group (V) and **p<0.01, ***p<0.001 compared to hyperuricemic control group (PO).

Table 1. Extract and allopurinol induce reduction of uric acid levels in normal and hyperuricemic mice.

Normal animals		**Hyperuricemic animals**	
Groups	**Uric acid reduction (%)**	**Groups**	**Uric acid reduction (%)**
Vehicle	---	Hyperuricemic (PO)	---
Vehicle + extract (100 mg /kg)	38.56	PO + extract (100 mg/kg)	42.88
Vehicle + extract (200 mg/kg)	42.62	PO + extract (200 mg/kg)	54.53
Vehicle + extract (400 mg/kg)	43.94	PO + extract (400 mg/kg)	63.36
Vehicle + allopurinol (10 mg/kg)	90.91	PO + allopurinol (10 mg/kg)	91.98

PO: potassium oxonate.

Table 2. Effects of *P. nigra* extract and allopurinol on livers XDH/XO activities in normal and hyperuricemic mice.

Groups	**Activity (nmol UA mn-1/mg protein)**		**Inhibition (%)**	
	XO	**XDH**	**XO**	**XDH**
Vehicle	2.78 ± 0.18	2.73 ± 0.19	---	---
Vehicle + extract 100 mg/kg	2.73 ± 0.75	3.35 ± 0.46	1.79	---
Vehicle + extract 200 mg/kg	2.67 ± 0.23	2.69 ± 0.33	13.30	13.46
Vehicle + extract 400 mg/kg	2.63 ± 0.22	2.15 ± 0.16	14.39	13.15
Vehicle + allopurinol 10 mg/kg	0.65 ± 0.1###	0.65 ± 0.1###	76.61	76.19
Hyperuricemic (OP) 250 mg/kg	2.59 ± 0.16	2.83 ± 0.17	---	---
PO + extract 100 mg/kg	2.56 ± 0.22	3.07 ± 0.04	1.16	---
PO + extract 200 mg/kg	2.54 ± 0.26	2.62 ± 0.21	1.93	7.42
PO + extract 400 mg/kg	1.72 ± 0.30*	2.31 ± 0.36	33.59	18.37
PO + allopurinol 10 mg/kg	0.62 ± 0.11***	0.6 ± 0.09***	71.55	78.79

U: Unit: nanomole uric acid per minute; V: vehicle; PO: potassium oxonate; XO: xanthine oxidase; XDH: xanthin dehydrogenase. All values are expressed as mean ± SEM of six animals. One-way ANOVA followed by Student's Newman–Keul's test was used for statistical significance. ###P<0.001 compared to normal control group (V) and *p<0.05, ***p<0.001 compared to hyperuricemic control group (PO).

Liver xanthine oxidoreductase (XO + XDH) activities in normal and hyperuricemic mice treated with extract and allopurinol

Results (Table 2) revealed that, treating mice with *P. nigra* extract at a daily dose of 100, 200 and 400 mg/kg for three days caused lesser inhibition <15% towards XDH and XO, when compared with the vehicle group. Whereas, allopurinol (10 mg/kg) significantly (p<0.001) decreased liver XO and XDH with 76.61 and 76.19%, respectively.

There was no statistically significant difference in liver XO-XDH activities between normal control mice and hyperuricemic mice (Table 2) except the extract at 400 mg/kg dose caused 33.59 and 18.37% inhibition of liver XO and XDH, respectively. On the other hand, allopurinol potently inhibited the XO/XDH activities (p<0.001) with 71.55 and 78.79%, respectively.

Neuroprotective effect of extract against $AlCl_3$ and D-Gal-induced toxicity

In order to investigate the effect of extract on $AlCl_3$ and D-Gal-induced neurotoxicity and neurodegeneration, respectively in mice, several test parameters were used.

Behavioral tests

$AlCl_3$ duration of exposure, had no significant influence on the exploratory motor activity assessed in the open field test (Fig. 2A). Furthermore, extract treated mice did not develop any alterations in the locomotor activity.

The results of the mice exposed to $AlCl_3$ of novelty induced anxiety related behaviors regardless of $AlCl_3$ exposure time have been shown in Fig. 2B. The frequency of head-dipping differed significantly across trials, $AlCl_3$ significantly ($p<0.001$) modified head dipping behavior at 15 and 20 min. Extract has significantly decreased the number of heads-dips compared to $AlCl_3$ and D-Gal-treated group.

No significant difference was seen in black and white box test (Fig. 2C) in first 15 min. However, time spent in a dark box for Alzheimer groups was significantly ($p<0.001$) more important in the last training period. The increased number of times spent in dark box was significantly reversed by the extract ($p<0.001$).

In the forced-swimming test, as presented in Fig. 2D, $AlCl_3$-treated mice spent less time immobile in this test than control animals. Aluminum chloride has significantly decreased immobility time ($p<0.001$) while intoxicated mice-treated with extract showed shorter latencies and longer time spent in target quadrant.

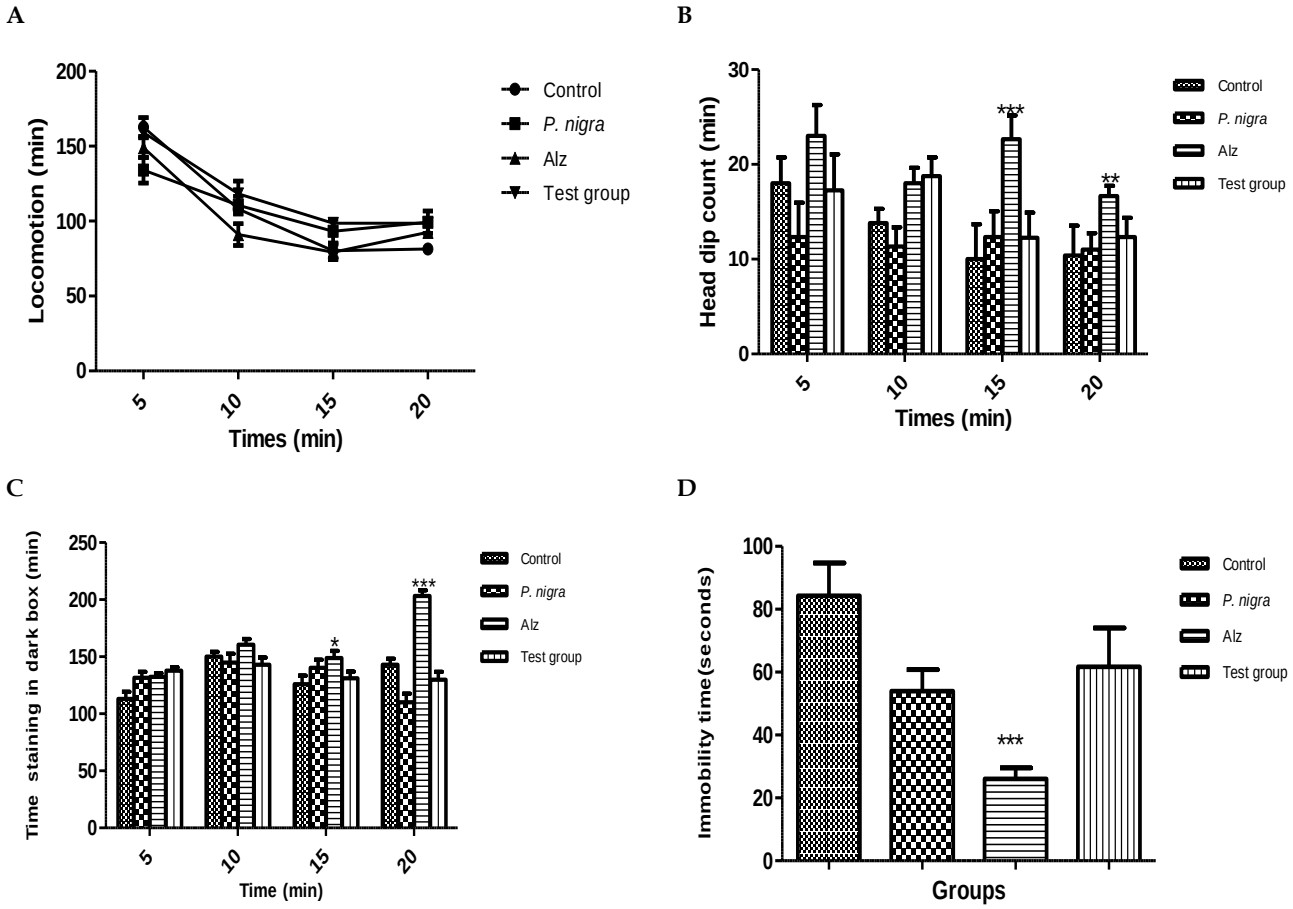

Figure 2. Effect of extract on exploratory behavior in $AlCl_3$-treated mice (**A)** on locomotion, (**B)** on the hole-board, (**C)** on the black and white shuttle box, and (**D)** forced-swimming test.

Each point represents the mean ± SEM (n=6) control group. Control: control group; *P. nigra:* control treated group received only extract; Alz: neurotoxicity and Alzheimer model; Test group: intoxicated/Alzheimer treated groups. Two-way ANOVA Followed by Student's Newman–Keul's test was used for statistical significance. *P<0.05, **p<0.01 and ***p<0.001 compared to normal control group.

Spatial memory in the Morris water maze

Learning and memory functions in mice were examined using the Morris water maze test.

Global analysis (Fig. 3A and B) revealed that in both tests, except the intoxicated groups, all mice reduced their escape latencies over the training period. In the first days, the results revealed that intoxicated mice demonstrated impaired ability in water maze learning, compared with the control group ($p<0.001$). However, the intoxicated mice treated with *P. nigra* extract have demonstrated improved learning ability on the first day (Fig. 3A). In both tests, for intoxicated groups, the reduced escape latency was observed only after three days, with significant differences ($p<0.001$) in the training period compared to other groups.

Throughout the training, mice treated with $AlCl_3$ and D-gal took longer ($p<0.001$) to reach the platform (28.9 s on day five) than those of the vehicle group, (Fig. 3B). There was no significant difference between control, treated and intoxicated treated mice learned to find the platform in a time of about <10 s from three days (data have not been shown).

Histological analysis

Hematoxylin and eosin stained sections of the cerebral cortex showed the general histological structure of the cerebral cortex layers. In the control groups, the neurons were full and arranged tightly, the nuclei were light stained (Fig. 4A). Histological analysis showed that there were typical neuropathological changes in the cerebral cortex of intoxicated/Alzheimer's model showing dilatation of blood capillary, shrinkage of the neuron cytoplasm and dark staining of nuclei (Fig. 4B) and less of pyramidal cells (Fig. 4C). *P. nigra* extract administration to tested group showed moderate neuropathological changes. The neurons recovered their characteristic shape, with prolonged neurofibrillary tangles (Fig. 4D).

DISCUSSION

Previously, the ethanol extract of *P. nigra* has demonstrated *in vitro* antioxidant (Debbache et al., 2014), hepatoprotective and vasorelaxant effects (Debbache-Benaida et al., 2013). These findings have prompted the authors to evaluate the hypouricemic effect of ethanol extract and its ability to counteract aluminum-induced neurotoxicity. This study is the first to reveal that *P. nigra* buds extract significantly reduced the accumulation of purine metabolites in blood of normal mice and following oxonate-induction. The obtained results indicated that PO successfully induced hyperuricemia, by inhibition of uricase and/or blocking of uric acid transporters (UAT) activity (Tang et al., 2017).

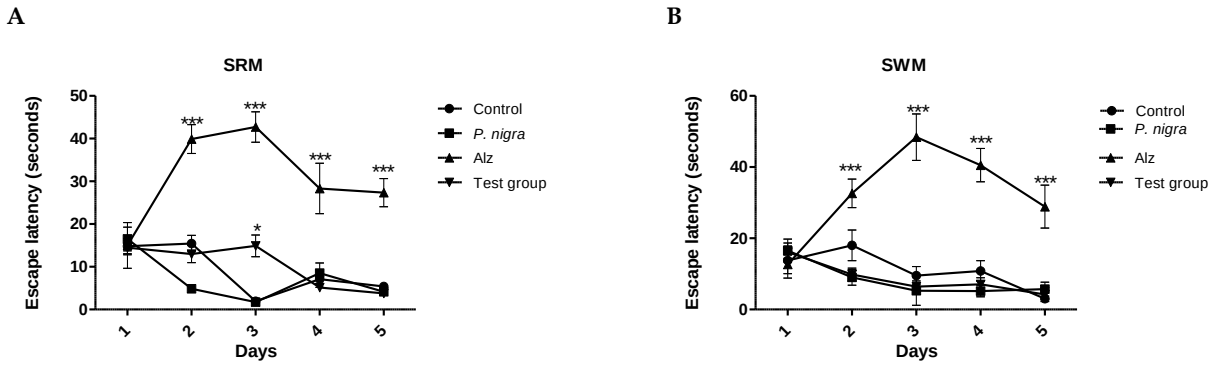

Figure 3. Reference **(A)** and working **(B)** memories in Morris water maze of mice.

Each point represents the mean ± SEM (n=6) control group. SRM: reference memory; SWM: working memory; Control: control group; *P. nigra:* control treated group received only extract; Alz: neurotoxicity and Alzheimer model; Test group: intoxicated/Alzheimer treated groups. Two-way ANOVA Followed by Student's Newman–Keul's test was used for statistical significance. *P<0.05 and ***p<0.001 compared to normal control group.

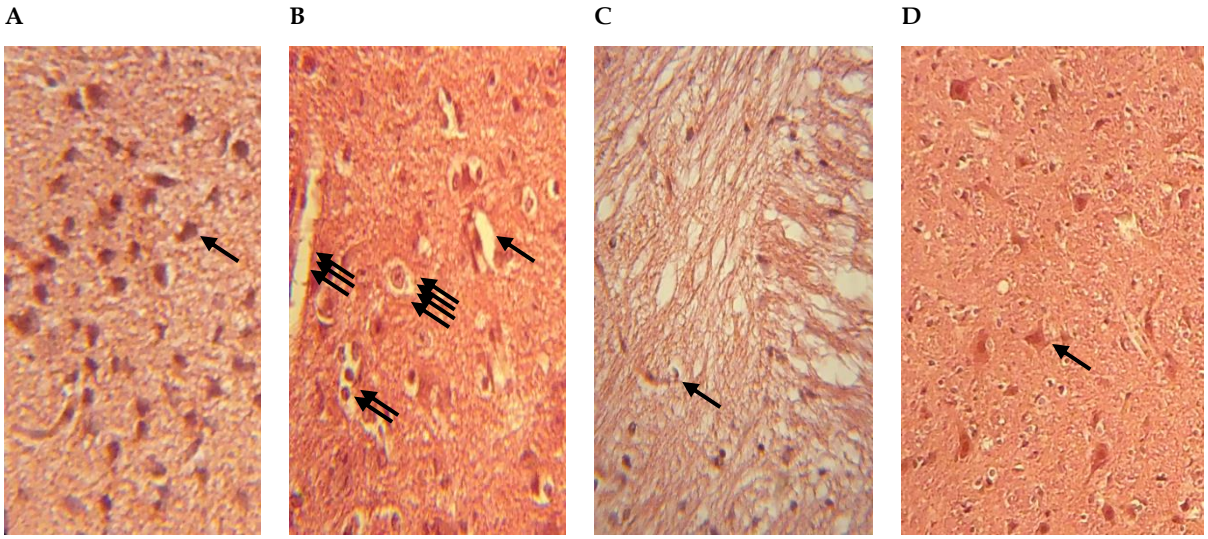

Figure 4. Light micrograph of cerebral cortex section of experimental groups.

(A) A photogram of a paraffin section from a cerebral cortex of control mice showed normal pyramidal cells with appropriate histological features (head, axon and dendrite). **(B)** Photomicrograph of cerebral cortex of brain section of mice daily treated with $AlCl_3$ and D-gal for 30 days. Undefined cell membrane and granulated cytoplasm and dense chromatin materials were observed, dilatation of blood and necrosis. **(C)** Cerebral cortex of mice administered $AlCl_3$ and D-gal for 30 days characterized by neuronal degeneration, deteriorating or negative adverse effects of $AlCl_3$ on the cortex. **(D)** A photomicrograph of a paraffin section from a cerebral cortex, pyramidal layer of mice administered 200 mg/kg $AlCl_3$, 100 mg/kg D-Gal and 200 mg/kg of *P. nigra* for a month, showed the pyramidal cells nearly to normal.

In this context, the *P. nigra* extract can be considered as food enabling the control of UA levels. XO and UAT play important roles in urate homeostasis (Zhu al., 2004).

The hypouricemic effect observed in experimental animals did not seem to parallel the change in livers XDH and XO activities, the extract significantly inhibited XO only at 400 mg/kg.

Interestingly, serum XO activity was correlated with serum UA levels, while liver XO activity has little effect (Huang et al., 2011). So, it can be suggested that *P. nigra* extract decreased UA levels, though the inhibition of serum XO. Similar results have been reported by other researchers (Zhu et al., 2004; Haidari et al., 2009). According to these findings, the involvement of others possible mechanisms such us enhanced uric acid excretion or action on other purine metabolizing enzymes can be proposed. Because, D-Gal-induced senescence is accompanied by neurodegeneration, when combined with neurotoxin aluminum treatment, it would be an ideal model for studying the molecular mechanisms involved with age-associated neuro-degeneration disease (Pan et al., 2008). Importantly, combined use of D-Gal and $AlCl_3$ is an effective way to establish the nontransgenic AD animal model and it is useful for studying the AD pathogenesis and therapeutic evaluation (Sun et al., 2009; Xiao et al., 2011).

The results showed the effectiveness of the *P. nigra* extract in alleviating the toxicity of aluminum on body weight. Results showed that $AlCl_3$-treated mice did not develop any alterations in the locomotor activity as compared to control mice. Several investigations have previously examined the effects of Al on behavior in the open field test, the results were not consistent, i.e., locomotion behaviors were either did not alter (Azzaoui et al., 2008), or decreased (Rebai and Djebli, 2008) in $AlCl_3$-treated mice. It was reported that mice with combined treatment of D-Gal and Al has noticeable learning and memory deficits (Rebai and Djebli, 2008; Biasotto et al., 2016), and showed decrease in brain acetylcholine level (Zhang et al., 2011). The behavioral and pathological changes persisted

for at least six weeks after withdrawal of D-Gal and Al (Xiao et al., 2011).

The extract treated-intoxicated animals showed decrease in the number of head dips compared to groups treated with $AlCl_3$ only. In support of previous reports (Pan et al., 2008), the obtained results indicated that during the last training period the mouse entered the dark chamber was significantly increased in the Alzheimer model group than the control group. The forced swimming test showed that the immobility time recorded by intoxicated mice is less than the intoxicated treated group. Based on these results in intoxicated treated group during the hole board, black/white room and Porsolt tests activities may reflect the less anxiety response of an animal to an unfamiliar environment. The results of this study showed that *P. nigra* extract prevents the cognitive deficits produced by aluminum.

$AlCl_3$ combined with D-Gal interferes with memory and cognitive function and subsequently causes similar degrees of impairment in both reference and working memory in the Morris water maze test (Yang et al., 2014). This suggests a decreasing tendency of aluminum on memory effect due to reduced functional capacity of serotonergic neurotransmission, as suggested earlier (Abu-Taweel et al., 2012). *P. nigra* extract treatment on the $AlCl_3$-induced neurotoxicity in mice exhibited a significant shorter escape latency in daily 1st trial than the neurotoxic groups during a 4-consecutive days training periods. The results demonstrate that extract improved the impairment of spatial memory induced by $AlCl_3$ and D-Gal as indicated by the formation of long-term and/or short-term spatial memory. Several interesting behavioral phenomena were observed during the visual cue test. A majority of mice were able to locate the visible platform relatively quickly (escape latency ≤22 s) (Barnhart et al., 2015).

Lesions in distinct brain regions like hippocampus and cerebral cortex were shown to impair Morris water maze performance (D'Hooge and De Deyn, 2001). Aluminum accumulation produced histopathological lesions in cerebral cortex including reduced number of the pyramidal cells, neuronal degeneration and necrosis. Oversupply of D-Gal could contribute to generation of ROS through oxidative metabolism of D-Gal as well as through glycation end products (Parameshwaran et al., 2010). The results clearly demonstrate that *P. nigra* extract oral treatment 10 min before the administration of $AlCl_3$ resulted in a reverse action of neurotoxin and protect cerebral cortex region to deleterious effects of neurotoxic agents. AD model is based on the generation of free radicals in the brain, which increases lipid peroxidation and decreases the antioxidant glutathione leading to memory impairment (Kumar and Gill, 2009). *In vivo*, *P. nigra* extract may protect liver cells from $AlCl_3$-induced damage through the antioxidant pathway (Debbache-Benaida et al., 2013) as susceptible brain cells get exposed to less oxidative stress resulting in reduced brain damage. On the basis of previous studies, these findings suggested that *P. nigra* buds extract can cause cognitive improvement in $AlCl_3$ and D-Gal induced neurotoxicity in mice, at least in part, by the increase of antioxidant enzymes activities (Dudonnée et al., 2011), a reduction in lipid peroxidation (Debbache et al., 2014), and also by its anti-inflammatory activity (Karawya et al., 2010; Debbache-Benaida et al., 2013).

The presence of phenol compounds like caffeic acid, quercetin and salicylate, which were identified in *P. nigra* extracts (Vardar-Ünlü et al., 2008; Dudonnée et al., 2011) may be responsible of efficacy of extract in the protection of $AlCl_3$-induced toxicity and hyperuricemea.

Nevertheless, this early beneficial effect of *Populus nigra*. L buds extract needs to be further investigated as a useful strategy in neurodegeneration and gout prevention. Further characterization of the active compound(s) could lead to the identification of new drug candidate(s) to be useful as adjunct therapy to prevent hyperuricemia and neurodegeneration disorders.

CONCLUSIONS

The data reported in the present study indicated that oral administration of *P. nigra* flower buds extract has reduced serum urate levels of normal

and hyperuricemic mice. *P.nigra* extract has beneficial influences and could be able to antagonize $AlCl_3$ toxicity. The existence of pharmacological active compounds in *P. nigra* flower buds extract may present a new type of hypouricemic and neuroprotective agents that may have a potent effect in clinical use and functional food.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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AUTHOR CONTRIBUTION:

Contribution	Debbache-Benaida N	Berboucha M	Ayouni K	Atmani D	Cheraft N	Boudaoud H	Djebli N	Atmani D
Concepts or ideas	x	x	x	x			x	x
Design	x	x	x	x		x	x	x
Definition of intellectual content	x	x	x	x	x	x	x	x
Literature search	x	x	x	x				x
Experimental studies	x	x	x	x	x	x	x	x
Data acquisition	x	x	x	x	x	x	x	x
Data analysis	x	x	x	x	x		x	x
Statistical analysis	x	x	x	x	x		x	x
Manuscript preparation	x	x	x	x				x
Manuscript editing	x	x	x	x				x
Manuscript review	x	x	x	x	x	x	x	x

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