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Chestnut ‘castanea sativa mill.’ honey potential in preventing memory decline on alzheimer’s disease model mice: in vitro enzyme activities, behavioural, memorial and histopathological studies

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Abstract

The primary purpose of this research is to investigate, both *in vitro* and *in vivo* studies, whether Chestnut '*Castanea sativa* Mill.' Honey consumption, can counteract the neurodegeneration occurring in Alzheimer's model mice brains caused by aluminium chloride combined with D-galactose. Within the scope of *in vitro* studies. The antioxidant activity of chestnut honey at a non-toxic concentration was then evaluated by DPPH and CUPRAC methods, indicating its radical scavenging and copper (II) ion reduction abilities, respectively. Finally, the modified Ellman method was used to measure its anti-acetylcholinesterase activity. For the *in vivo* studies, two chestnut honey doses were used, 150 mg/kg and 300mg/kg. As for the neurodegeneration, it was induced by Aluminium chloride at 100 mg/kg combined with D-galactose at 120 mg/kg. Following the neurological tests, the brain were subjected to histopathological study. The dose of chestnut honey, which has no effect on fibroblast cell growth following the 24-hour treatment was detected as 0.25% -w/v-, this dose was used for further *in vitro* assays. DPPH scavenging activity of the honey was 10.98 ± 1.20 g/mL -IC50-, while CUPRAC value was 0.57 ± 0.27 mM TEAC/g. The neurological tests, reported significant positive improvement in learning ability, these results were confirmed by the histopathological findings, in which the tissues section taken from the brain showed that, the honey markedly reduced hippocampal atrophy and neuronal loss. The results suggests that Chestnut honey acceptable daily-inake can reduce the burden of Alzheimer's disease, by preventingmemory impairment and brain alteration.

Introduction

Alzheimer's disease (AD) is a commonly occurring ailment, affecting a global population of more than 35 million individuals characterized by a gradual decline in memory and cognitive function that is correlated with aging [1–2]. The main neuropathological criteria for diagnosing (AD) continue to be the presence of extracellular β-amyloid deposition as neuritic plaques and intracellular accumulation of hyperphosphorylated tau as neurofibrillary tangles [3]. Moreover, the postulation that oxidative stress is a pivotal factor in the development of (AD) is substantiated by scientific findings indicating the abnormal elevation of oxidative stress indicators in conditions associated with the disease [4–5].

Given the complexity of (AD), the treatment remains challenging [6]. Therefore, given the multiple benefits that natural products offer. Honey bee products used in apitherapy are currently preferred over synthetic medicine due to their high safety margin, reduced cost, and broad bioactivities [7].

The biological properties of honey, be ascribed to its abundant nutrient composition, encompassing carbohydrates, polyphenols, minerals, and vitamins [8]. Nowadays, monofloral honey, which is characterized by predominantly containing pollen from a single floral source, such as Manuka, Chestnut, and Acacia honey, has garnered consumer interest due to its potential health benefits [9].

Castanea sativa Mill., commonly known as European Chestnut, belongs to the genus *Castanea* of the (Fagaceae family) [10]. Chestnut honey (CH) (monofloral honey), is identifiable from other honey varieties

by its deep color and its distinctive, enduring, acrid, and potent taste, which exhibits a more significant antioxidant potential than alternative honey types, including lavender, linden, sunflower, and multifloral blossom honey [11–12]. Moreover, it is a well-known source of phenolic bioactive substances, including L-ascorbic acid, carotenoids, phenolic substances like gallic and ellagic acid, and flavonoids notably high in kaempferol and quercetin [13].

Numerous studies have demonstrated that chestnut honey exhibits biological activities, such as antioxidant, antimicrobial, anticancer, anti-acetylcholinesterase, neuroprotective, and DNA damage inhibitory effects [14–15]. The available literature reveals that only two *in vivo* studies have been conducted to investigate the potential preventive effects of chestnut honey on brain damage and neurodegeneration [2–16]. In recent years, studies focused on preventing nervous system diseases by utilizing the antioxidant properties of (CH). The activity was due to its rich content of phenolic compounds [15].

Materials and Methods

Honey

Chestnut honey sample used in both *in vitro* and *in vivo* studies was provided from Balparmak R&D Center, Altıparmak Food Industry and Trade Inc. in 2020. Chestnut honey was analyzed for its physico-chemical composition and pollen content at the Balparmak Research and Development Center. A stock solution of Chestnut honey with a concentration of 40% (w/v) was prepared using dH_2O.

In Vitro Tests

Cytotoxic Activity

Embryonic mouse fibroblast (NIH3T3) cells in the animal cell culture collection of Istanbul University, Faculty of Science, Department of Molecular Biology and Genetics were used for cytotoxicity assessments. The MTT assay, a commonly employed method for assessing cell viability, was utilized to examine the effects of different concentrations of chestnut honey on the viability of NIH3T3 cell line. Briefly, the NIH3T3 cells were introduced into 96-well culture plates with a seeding density of $3x10^4$ cells per well, using 200 µL of culture medium. After a 24-hour incubation period (5% CO_2 and 37°C), the cells were subjected to various concentrations of chestnut honey (0.25-20%). At the end of the incubation period, the medium on the cells was removed, 30 µL of MTT [3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide] stock solution (5 mg/mL, prepared in Dulbecco's phosphate-buffered saline solution) was added to each well, and the culture plate was kept in an incubator for 4 hours. To dissolve the formed formazan crystals, 150 µL of DMSO (dimethyl sulfoxide) was added to each well and the plate was shaken at room temperature until the solution became homogeneous. The absorbance of the formed colored product at a wavelength of 540 nm was measured with a microplate reader and the

absorbance values were compared with the control. The results were calculated using the formula for the percentage of cell viability = (Absorbance of treated cells / Absorbance of control) x 100 [17].

Antioxidant Activity

The measurement of the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity of chestnut honey was conducted by the procedure outlined by Brand-Williams and coworkers [18]. The reagent consisted of 0.2 mM DPPH dissolved in methanol and was kept in an opaque bottle. Ascorbic acid solutions (1-1000 µg/mL) were used for references while blank samples consisted of deionized water (dH_2O) and methanol (MeOH). First, the absorbance of 0.2 mM DPPH solution was measured at 517 nm by using MeOH as a blank. Following the introduction of 40 µL of varying concentrations of chestnut honey and ascorbic acid standard into the wells of the 96-well plates, 160 µL of 0.2 mM DPPH was subsequently added. The plates were subjected to a period of darkness lasting 10 min, after which absorbance measurements were taken at 517 nm. The experiments were performed in triplicate and the mean values were used for calculation. The capacity to scavenge DPPH radical was determined using the formula for the percentage of inhibition (DPPH radical scavenging activity %) = [1-(Absorbance of Sample /Absorbance of Blank)] x 100%.

The total antioxidant capacity of chestnut honey was measured using the Copper (II) ion-reducing antioxidant capacity (CUPRAC) assay described by Apak and coworkers [19]. Various concentrations of Trolox (TR $1x10^{-3}$ M) were prepared as standard solutions. Assay mixture consisting of $1x10^{-2}$ M $CuCl_2$ (250 µL), $7.5x10^{-3}$ M CUPRAC reagent [the copper (II)- neocuproine (2,9-dimethyl-1,10-phenanthroline)] (250 µL), ammonium acetate buffer, pH 7 (250 µL), sample or standard solution (25 µL), and distilled water dH_2O (250 µL) were added into each well of a 24- well plate, successively. The plate was incubated at RT for 1 hour, then absorbances were read at 450 nm. The results were expressed as CUPRAC value (mM TEAC/g, millimolar Trolox Equivalent Antioxidant Capacity per gram) calculated by the formula given in the reference coworkers [19].

The Folin-Ciocalteu method was used to determine the total phenolic content (TPC) of CH [20]. The absorbance of the samples was measured at 765 nm. The curve equation of the graph between the concentration values of the gallic acid (GA) standard and the absorbance was used for interpolation. The total phenolic content of CH was expressed as milligrams of gallic acid equivalent per 100 grams of sample, mg GAE/100 g.

The total flavonoid content (TFC) of CH was measured according to the method described by Shraim et al. [21]. The total flavonoid content of CH was expressed as milligrams of catechin equivalents per 100 grams, (mg CE/100 mg), using the curve equation derived from the graph depicting the relationship between the concentration values of the catechin (CE) standard and the corresponding absorbance values.

Anti- AChE Activity

The *in vitro* acetylcholinesterase (AChE) enzyme activity was measured by the modified Ellman GL et al. (1961) method [22]. Donepezil (prepared by dissolving in methanol containing 10% DMSO) was used as a reference medication. The absorbance of the samples was measured at 412 nm. The inhibitory effect of the chestnut honey on AChE enzyme was calculated and expressed as an IC_{50} value.

In Vivo Experimental Design

A total of 25 female NMRI albino mice were purchased, from the Institute Pasteur – Algeria- (weightning 20 ± 3 g) was used for the present study. They were elevated in the Animal House (Part of the laboratory "LPAP") of the University Abdelhamid Ibn Badis -Mostaganem- Algeria. And access to food and water ad libitum (temperature 25°C ; 12 h light/dark cycle).), following the approved instructions and the recommendations described by the Institutional Animal Care and Use Committees (IACUCs) -2022-.

The mice were divided into five groups (n = 5) according to their body weight. Our experiment is divided into two phases, 7 weeks each. (The experimental protocol is based, on the Alzheimer model described by Feng et al. (2018) method [23]. Treatment with both CH at 150mg/kg (Alz-D1) and 300mg/kg (Alz-D2) as well as Rivastigmine at 1.5mg/kg (Alz-STD) were given by intragastric gavage. Followed by the induction of Alzheimer's disease with D-gal + $AlCl_3$, In which Alzheimer model mice (ALZ), and the treated groups received D-galactose (120 mg/kg BW/day) intraperitioneally and $AlCl_3$ (100 mg/kg BW/day) orally, both dissolved in saline solution (0.9%). The mice, were weighed every week, and the dose volumes adjusted according to body weight. At the end of the experimental protocol, the mice were subjected to neurological test to evaluate spatial reference and working memory effect of (CH).

Memory tests

Eight-arm radial maze (8-RAM)

Position distinction test

During this test six arms of the maze are used, three with food (baited arms) and the others without food (unbaited arms).The score being recorded each time by connecting the number of baited arms chosen by each mouse. Spatial reference memory test; only two arms of the maze are used in this test. One arm is illuminated with food at its end, and the other arm is dark. Thus, the residence time in the illuminated arm is measured.

Morris Water Maze (MWM)

Spatial working memory test; In which the mouse is placed in a container 147 cm in diameter by 25 cm in height, containing lukewarm water maintained at 21°C. An exposed platform is placed in the pool, which is surrounded by visual cues.

Both 8-RAM and MWM tests lasted for five days; four training days and the fifth day considered as the test day. Each (essay) session lasts 5 minutes.

Behavioural tests

Locomotor activity

In this test the apparatus consists of a chamber surrounded by high walls (32 X 32 cm2), and the floor of the chamber is divided into squares (from 1 to 16). Crossings refer to the total number of square crossings during the test period, each move counts as a score [24].

Anxiety test

The light–dark box test (LBD)

The test consists of putting the mouse in the middle of the apparatus, in which the the apparatus consists of a dark and a bright lit compartments (L = 80cm; l = 30cm; h = 30cm). This test evaluates the mice behaviour in the dark compartment.

The elevated plus maze (EPM)

The apparatus used for the elevated plus maze test is in the configuration of a + and comprises two open arms across from each other and perpendicular to two closed arms with a center platform. The entire apparatus is 50 cm above the floor. The mice are placed on the central platform, and allowed to move freely in the experimental device. The duration of time spent in the protected arm is calculated for this test for 5 minutes in four consecutive phases.

Hematoxylin and Eosin (H&E) Staining

The day followed the end of the neurological tests, the mice were euthanatized. The brain, were taken out and washed with ice-cold saline (0.9%), and fixed in 10% paraformaldehyde. The histopathological study was carried out at the laboratory "LPAP" of Abd El Hamid Ibn Badis University -Mostaganem- Algeria, according to the manual of anatomo cytopathology techniques. The sections of the brain, were obtained following dehydration, dewaxing, embedding, sectioning, and staining. At 10X and 40X magnification, the slides were examined using an optical microscope.

Statistical Analysis

The *in vitro* experimental data were reported as means with SD. Statistical analysis was performed using SPSS software. The statistical significance of differences between groups was evaluated using an analysis of variance (ANOVA) followed by the Student Newman-Keuls post hoc test. A significance level of 0.05 or lower was used as a threshold to determine statistical significance. The statistical analysis of the *in vivo* experimental data obtained during the tests carried out, was realise using XLstat software. Results were expressed as average ± STDV ($P \leq 0.05$, $P \leq 0.01$, $P \leq 0.001$).

Results

In Vitro Cell Viability Activity

Treatment of NIH3T3 cells with chestnut honey (CH) in a concentration of 1% (w/v) significantly increased the viability by 43.8 ± 7.07% while the doses of 7.5%, 10%, and 20% (w/v) reduced viability by 72.32 ± 2.54%, 79.54 ± 3.73%, and 81.85 ± 1.67%, respectively, when compared to the control group (****$p < 0.0001$). Based on the data presented in Fig. 1, the dose of CH, which exhibited no observable toxic or proliferative effect on NIH3T3 cells, was 0.25% (w/v). This concentration was used in activity tests.

In Vitro Antioxidant Activity

In this study, the results of the antioxidant activities (DPPH and CUPRAC) as well as the total phenolic and flavonoid content of the monofloral chestnut honey sample, whose pollen analysis was given as supplementary data (*Cestanea sativa* pollen content 78%), are presented in **Table.1**. Additionally, the TPC and TFC of this sample were determined as 131.3 ± 28.7 mg GAE/100 g and 7.7 ± 2.5 mg CE/100 g, respectively. This research also reported the range of total phenolic content in chestnut honey water extract samples, which varied from 46.3 to 101.3 mg GAE/100 g. Likewise, the total phenolic content of chestnut honey methanol extract samples was between 39.9 to 102.5 mg GAE/100 g. When examining the literature on DPPH IC_{50} values of chestnut honey, it is shown that there exists a sample (6.32 ± 0.35 mg/mL) with higher efficacy compared to the value obtained in current research (10.98 ± 1.20 mg/mL). Conversely, there is also a sample (17.06 ± 1.30 mg/mL) that exhibits lower effectiveness. This difference between the IC_{50} values of the samples can be explained by the difference in their total phenolic content.

Table 1
The results of the antioxidant activities, the total phenolic and flavonoid content of the chestnut honey

	DPPH* (IC_{50} mg/mL)	CUPRAC* (mM TEAC/g)	TPC* (mg GAE/100 g)	TFC* (mg CAE/100 g)
Chestnut honey (CH)	10.98 ± 1.20	0.571 ± 0.27	107.719 ± 2.50	13.333 ± 0.04

*Values were the means of three replicates ± standard deviation.

Anti- AChE Activity

Table.2 shows the anti-acetylcholinesterase (AChE) activity of chestnut honey (CH). The anti-AChE activity of CH was found to be IC_{50} 3.16 ± 2.36 mg/Ml

Table 2
Anti-acetylcholinesterase activity results of chestnut honey

	IC_{50} as mg/mL*
Chestnut honey (CH)	3.16 ± 2.36
Donepezil	6.20 ± 0.92

*Values were the means of three replicates ± standard deviation.

Memory tests

Eight-arm radial maze (RAM)

a- Position distinction (RAM)

Besides the first day of the training days, the results of this test demonstrate that the number of visits to the baited arms in control mice is superior to that in the D-gal/$AlCl_3$-induced (AD) model mice. Same results were observed in the treated group with (CH) at 150mg/kg (Alz-D1) **(**Fig. 2.**A).** On the fifth day (Test day), The control group recorded fewer visits to the baited arms compared to the (ALZ) group **(**Fig. 2.**B).**

b- Spatial reference memory (SRM)

As shown in Fig. 3.**A**, the residence time in the lighted arm is greater in(ALZ) group compared to the controls group. The results obtained for the Alzheimer mice treated with both Rivastigmine at 1.5mg/kg (Alz-STD) and (CH) at 150 mg/kg, 300mg/kg (Alz- D2, Alz-D2) had shown a significant ($p < 0.001$; $p < 0.01$; $p < 0.05$) stay preference in the lighted arm compared with the (ALZ) group on the training days. However, on the test day, there was a significant difference between the control group and the (ALZ) group, in which the control group showed more residence time in the lighted arm than the (ALZ) group, while the treated groups (Alz-STD, Alz-D2) indicated a residence time in the lighted arm practically close to that recorded in the control group **(**Fig. 3.**B).**

Morris water maze (MWM)

a- Spatial working memory (MWM)

After the four days of training, the results of the (ALZ) group, shows a very ($p < 0.01$) and a highly significant ($p < 0.001$) time to find the visible platform compared to the control group, who showed a short time into finding the platform. As for the Alzheimer mice treated with CH at 150mg/kg, 300mg/kg, they recorded a very significant short time to detect the visible platform compared to (ALZ) **(**Fig. 4.**A).** As

shown in Fig. 4.**B** same results were observed on the test day, in which, **(**Alz-D2) group had significantly shorter escape latency compared with the (ALZ) group, with a very significant difference (*P < 0.01*).

Behavioural test

a-Locomotor activity

The results obtained from the locomotor activity, showed hyperactivity in the control group compared with the (ALZ). However, the Alzheimer's mice treated with (CH) (Alz-D1, Alz-D2) showed hyperactivity compared with the other groups. Moreover, the Alzheimer's mice treated with Rivastigmine (Alz-STD) showed a hypoactivity compared with the other groups **(**Fig. 5**).**

b-Anxiety test

To measure the anxiety-like behaviour in the mice, two tests were performed. In which, the average of the four phases recorded for the light-dark box reveals a significant (*P < 0.05*) preference for the illuminated compartment for the (ALZ) group compared with the control mice, who showed a preference for the dark compartment. The treated groups (Alz-D1, Alz-D2 and Alz-STD), showed similar results to that observed in the control group **(**Fig. 6.**A).** As for the second test, the results of the elevated plus maze showed that the control group showed a preference stays in the protected arms compared with (ALZ) with a significant difference (*P < 0.05*). Moreover, the treated Alzheimer's mice (Alz-D1 and Alz-D2) showed a preference for the protected arms like the Control group compared with the (ALZ) with a very significant difference (*P < 0.01*) **(**Fig. 6.**B).**

Histopathological findings

Brain tissus : Hippocampus

The brain histological examination was carried out at different levels of the brain tissues. After exposition to $AlCl_3$ + d-gal for 7 weeks, The (ALZ) group showed a decreased cell density represented in the deposition of both amyloid plaques (AP) and neurofibrillary tangles (NFTs) at the CA1 region of the hippocampus, when compared with the control group **(**Fig. 7.**a, b).** While the chronic administration of (CH) markedly reduced hippocampal atrophy and neuronal loss in the CA1 region of the hippocampus for both (Alz-D1 and Alz-D2) groups. Same results were observed in the Alz-STD **(**Fig. 7.**c,d,e).**

Cerebral cortex

As shown in Fig. 7.**F, G**; when compared with the control group, the cortex histological examination of the (ALZ) shows perivascular haemorrhage on certain parts, neuronal degeneration and neuronophagia. However, the Alzheimer treated mice with Rivastigmine (Alz-STD) and (CH) (Alz-D1, Alz-D2) exhibited normal histological architecture of the cerebral cortex **(**Fig. 7.**H, I, J).**

Cerebelllum

After the cerebellum histological examination, the results had shown that except the Alzheimer model mice, all the groups has normals cells structure, when compared to the control group, the (ALZ) group cells were damaged, and Purkinje cells necrosis was detected after $AlCl_3$ and

D-gal exposure. In the control group, the Purkinje cells are situated between the granular and molecular layers **(**Fig. 7.**k, l).** As for the Alzheimer-treated groups with (CH) and Rivastigmine, the cerebellum cells have normal structure, revealing normally appearing molecular layer, Purkinje cell layer **(**Fig. 7.**m, n, o).**

Discussion

In another study investigating the cytotoxic effects of different concentrations (ranging from 0.015–5% v/v) of Kellut honey and Acacia honey on HGF fibroblast cells, the findings indicated that the IC50 value for both types of honey was determined to be 2% [25]. It is not possible to make a one-to-one comparison with the results obtained in this study due to the differences in honey type, application dose, and fibroblast cell type, even though the application time is the same (24 hours). The IC80 value of chestnut honey for NIH3T3 cells has been determined to be 20% (w/v). There is a limited number of studies in the literature revealing the toxic effects of chestnut honey on healthy cell lines [26–27]. There are several reports in the scientific literature on the toxic effects of honey, particularly on cancer cells. The cytotoxicity of honey is primarily attributed to its phytochemical constituents, including phenolic or flavonoid compounds such as chrysin, campherol, catechin, galangin, myricetin, caffeic acid, syringic acid, chlorogenic acid, gallic acid, and ferulic acid. It has been proposed that the mechanisms underlying this cytotoxicity involve cell cycle arrest and activation of the mitochondrial pathway. Specifically, these mechanisms encompass mitochondrial outer membrane permeability, induction of apoptosis, generation of oxidative stress, modulation of insulin signaling, inflammation, and augmentation of estrogenic activity [28–29 In this study, it is predicted that the toxic doses observed during dose determination studies may be due to the high phenolic and flavonoid content as stated in the literature. Additionally, it was shown that honey's high sugar content (fructose and glucose, 57.1%) may have a toxic impact by increasing the formation of advanced glycation end products (also known as "AGEs," which stands for "advanced glycation end products") [30–31].

According to the data of another study, the chestnut (*Cestanea sativa* Mill.) honey samples with the greatest DPPH radical scavenging activity IC_{50} value was 7.9 mg/mL in the methanol extract and 10.2 mg/mL in the water extract [14]. In a recent study, IC_{50} values of chestnut honey samples, whose pollen amount was between 51–81%, were found between 6.32 ± 0.35 and 17.06 ± 1.30 mg/mL in the DPPH radical scavenging activity test [12]. In a study, it is reported that the CUPRAC value in chestnut honey collected from the Eastern Black Sea region (TURKEY) varies between 1.37 ± 0.13–3.40 ± 0.01 µM TEAC/g [32]. In another study, the CUPRAC value of a sample collected from Kaz Mountains (Çanakkale) was found as 1.952 ± 0.08 mM TEAC/g [33]. Accordingly, the CUPRAC activity of the CH used in this study is lower than the chestnut honeys examined in previous studies conducted in different regions of

Turkey. In general, honey with high radical scavenging activity also has high total phenolic and/or flavonoid content. The CUPRAC value of chestnut honey used in this study (0.571 ± 0.27 mM TEAC/g) is within the range of values reported in previous studies [32–33]. This result might arise from several factors such as pollen content, environmental factors, and the breed of honey bees.

Few researches has examined chestnut honey's anti-AChE activity [12–15]. According to the findings of a research, chestnut honey samples with pollen concentrations ranging from 51–81% had AChE inhibitory activity ranging from 7.18 ± 0.83 mg/mL to 9.95 ± 1.25 mg/mL at their greatest and their lowest levels, respectively [12]. The fact that the CH sample used in this study is a more effective AChE inhibitor may be due to its different chemical composition caused by the flora diversity in the regions where it was obtained.

The results of the neurological tests revealed that, spatial memory was affected by D-gal/$AlCl_3$, in which during the training session, the working memory errors (WMEs) and reference memory errors (RMEs), the Alzheimer model mice showed a decline memory when compared with the control group with a very significant difference ($P < 0.01$). However, the Alzheimer mice treated (CH) at 150mg/kg and 300mg/kg revealed a significant improvement of learning and spatial memory. According to a study by [34], Aluminum exposure leads to impairment of spatial working memory in mice, in which they explained that, memory deficit could be associated with oxidative stress in the cortical and subcortical brain tissues.

In many studies, Aluminum Chloride ($AlCl_3$) exposure was proven to induce anxiety-depression-like behaviour in rodents animals. In one of [35] study, the rats were living in a permanent stress, the psychological state was due to a neurophysiological impairment caused by $AlCl_3$. Same results were observed from our study in the (ALZ) groups.

In contrast to the control group, (ALZ) group in the current study displayed histopathological abnormalities that were defined by a reduction in cell density, the deposition of amyloid plaques (AP) and neurofibrillary tangles (NFTs), in both, the CA1 region of the hippocampus, and the cortex. Results reported in previous studies, exposition to D-gal/$AlCl_3$ showed cognitive impairments and marked neuronal loss in hippocampal conus ammonis 1 (CA1) [36–37]. Chronic honey administration significantly decreased hippocampus and cortical atrophy and neuronal death when administered at 150 mg/kg and 300 mg/kg. Honey has the ability to reduce neurodegeneration especially the prefrontal cortex and hippocampus, which is linked to its neuroprotective effects on the brain [38].

In conclusion, Exposition to metal like ($AlCl_3$) may lead to memory decline, and dementia, which is a serious health problem worldwide. *In vitro* antioxidant and anti-cholinesterase activities of chestnut honey point out its potential protective effect against (AD). According to the findings from *in vivo* experiments conducted in an Alzheimer's mice model in this study, the impairment is closely related to the deposition of Aβ plaques on the brain cells and neurofibrillary tangles (NFTs) found in the hippocampus and cortex region. Administration of chestnut honey has been shown to improve cognition and memory, probably due to its powerful compounds such as flavonoids (quercetin as well as kaempferol) and tannin

(gallic acid) as well as its radical scavenging activity. However, further studies, such as pharmacokinetics and toxicological assessments, are needed for a better understanding of the mode of action, active principles, and side effects. The use of chestnut honey for the protection from and treatment of Alzheimer's disease can be possible after these detailed studies and clinical trials with volunteers.

Abbreviations

AChE : Acetylcholinesterase

AD : Alzheimer's disease

$AlCl_3$: Aluminium chloride

ALZ: Alzheimer model mice

Alz-D1 : Alzheimer mice treated with chestnut honey at 150 mg/kg

Alz-D2: Alzheimer mice treated with chestnut honey at 300 mg/kg

Alz-STD: Alzheimer mice treated with Rivastigmine at 1.5 mg/kg

AP: Amyloid plaques

CH : Chestnut honey

D-gal: D-galactose

dH_2O: Deionized water

DMSO: Dimethyl sulfoxide

DPPH : 2,2-diphenyl-1-picrylhydrazyl

GA : Gallic acid

MeOH: Methanol

NFTs: Neurofibrillary tangles

RMEs: reference memory errors

TFC : Total flavonoid content

TPC : Total phenolic content

WMEs: working memory errors

Declarations

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Competing interests

No competing interests declared.

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Conflict of Interests

The authors of this article declare that they have no competing interest.

Author contribution

Noueddine Djebli and Nazli ARDA was responsible for the conceptualization of this work (The ideas and our research aims), also the software and any formal analysis. While both Amani YAGOUB and Gizem SÖNMEZ OSKAY carried out the experiments and the practical hands-on work in the laboratory under the supervision of Noureddine DJEBLI and Nazli ARDA. All the authors had parts in writing and correcting this manuscript. All authors reviewed the manuscript.

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Figures

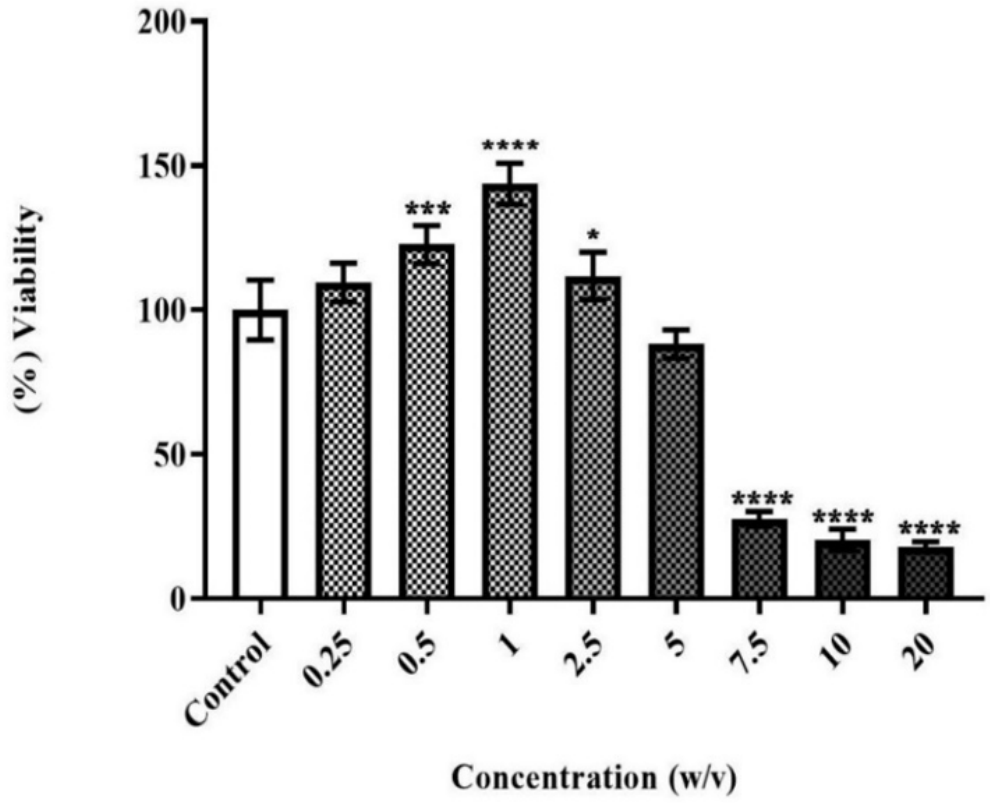

Figure 1

Effect of chestnut honey (CH) treatment for 24-hour on NIH3T3 cell viability.

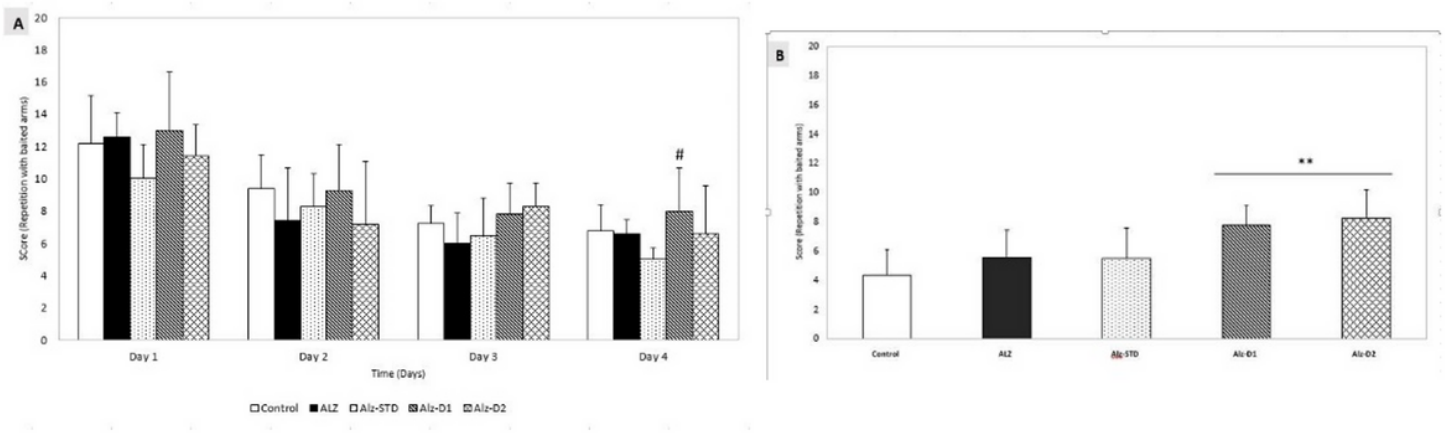

Figure 2

Radial arm maze- Position distinction, **(A)** 4 days- of training, **(B)** Test day. Values were represented as means ± SD for each group (n=5). **P≤ 0.01 vs, control group. #P≤0.05 vs, ALZ group.

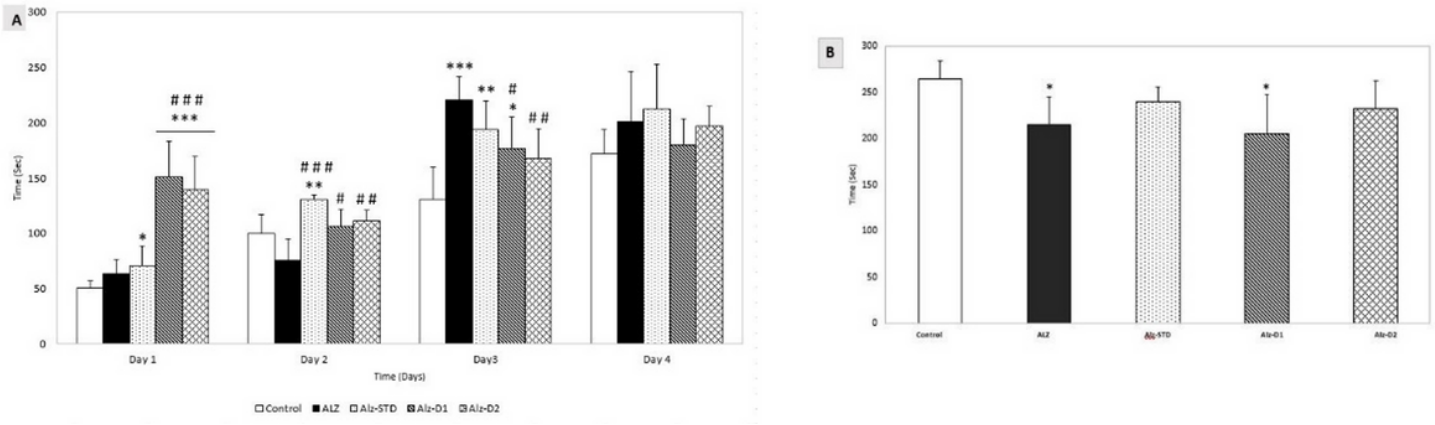

Figure 3

Radial arm maze- Spatial reference memory, **(A)** 4 days- of training, **(B)**Test day. Values were represented as means ± SD for each group (n=5). *P≤ 0.05, **P≤ 0.01, ***P≤ 0.001 vs, control group. #P≤0.05, ##P≤0.01, ###P≤0.001 vs, ALZ group.

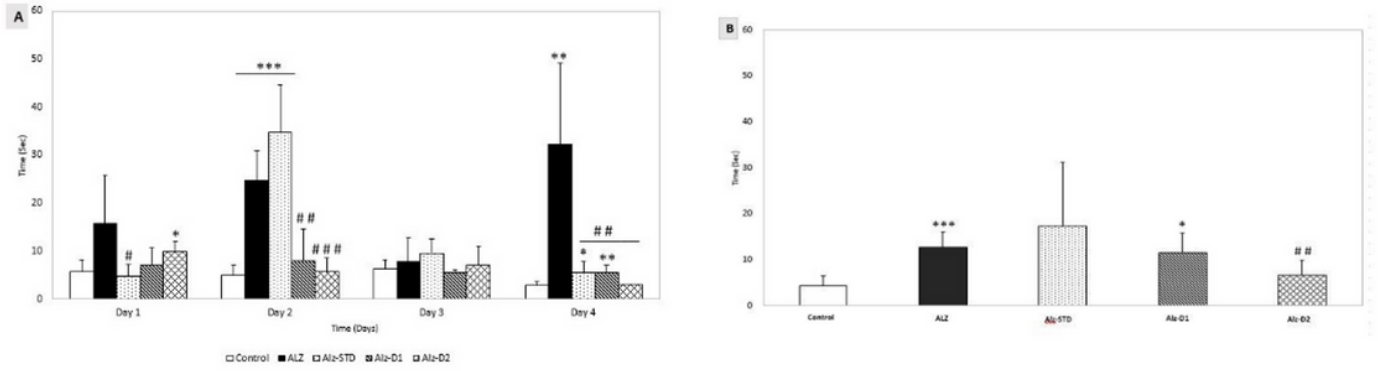

Figure 4

Morris water maze- Spatial working memory. **(A)** 4 days- of training, **(B)**Test day. Values were represented as means ± SD for each group (n=5). *P≤ 0.05, **P≤ 0.01, ***P≤ 0.001 vs, control group. #P≤0.05, ##P≤0.01, ###P≤0.001 vs, ALZ group.

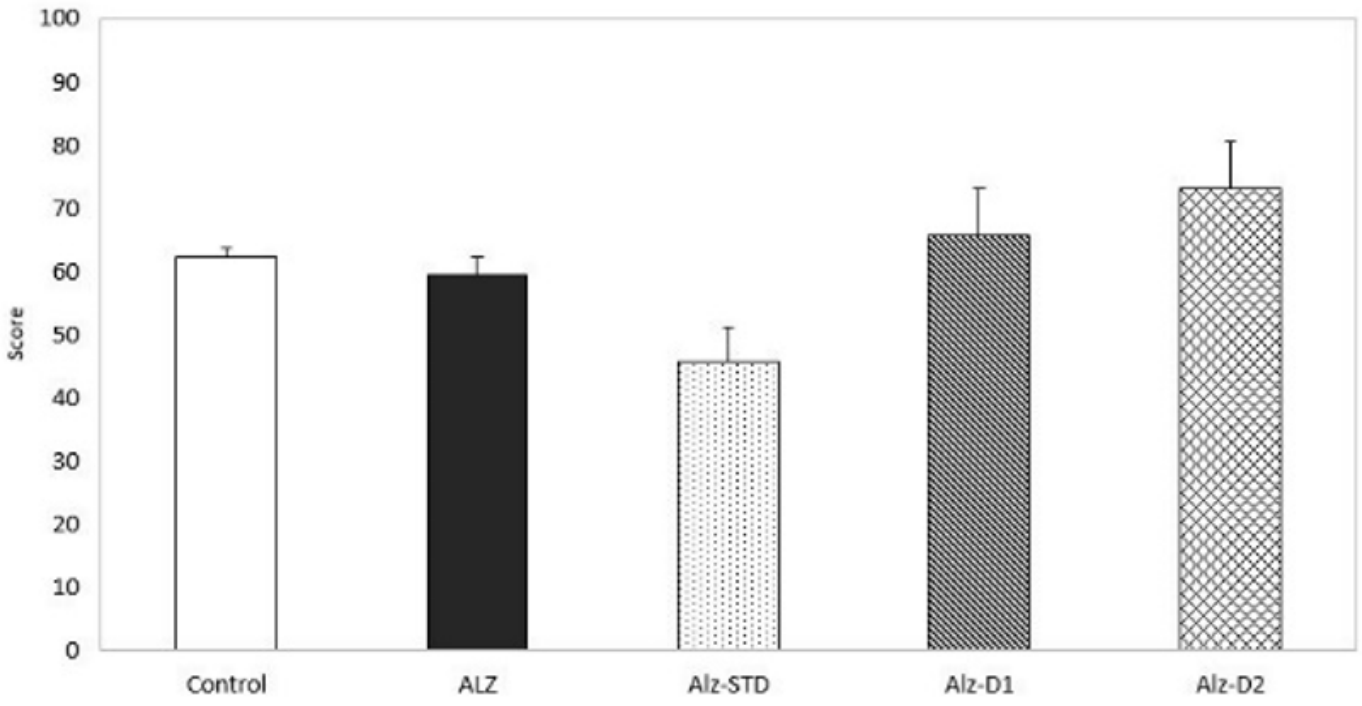

Figure 5

Average of the four phases of locomotor activity.

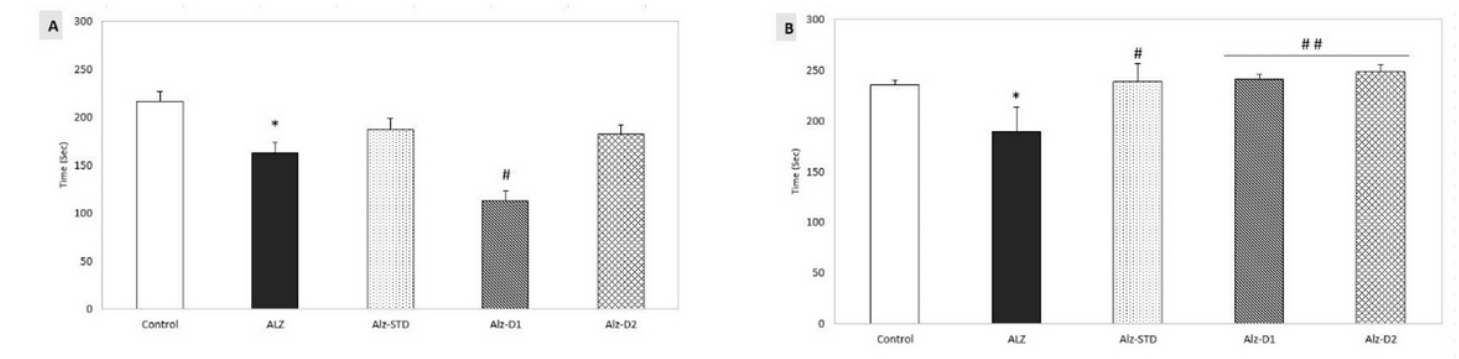

Figure 6

Anxiety test. **(A)**Average of the four phases of light-dark box, **(B)** Average of the four phases of the elevated plus maze. Values were represented as means ± SD for each group (n=5). *P≤ 0.05 vs, control group. #P≤0.05, ##P≤0.01 vs, ALZ group.

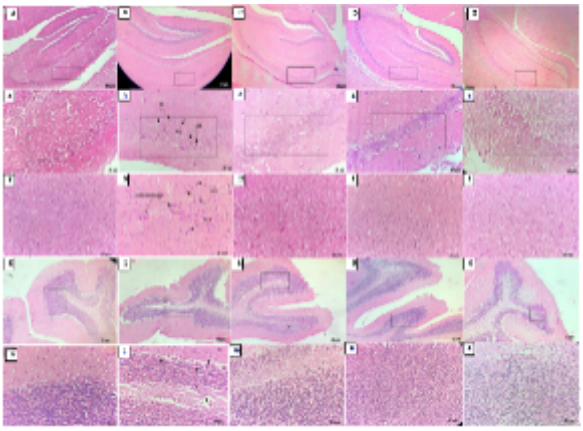

Figure 7

Photomicrograph of mice brain, **(A-E) Hippocampus region (H&E) stained**;(a,b,c,d,e **(X10)** are **CA1** part of A, B, C, D, E **(X40)** respectively). **(F-J) Cerebral cortex (H&E X40) stained**;**(K-O) Cerebellum, (H&E X10-X40) stained**. **(A, F, K)** Control group, **(B, G, L)**Alzheimer model mice, **(C, H, M)**Alzheimer mice treated with CH at 150mg/kg, **(D, I, N)** Alzheimer mice treated with CH at 300mg/kg, **(E, J, O)** Alzheimer mice treated with rivastigmine at 1.5mg/kg.**PA:** Plaque amyloïdes. **NFT:** Neurofibrillary tangles. **VC:** Vacuole cells. **PC**: Purkinje cells.