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Identification of New Alkaloids from Algerian Purslane by HPLC-QTOF-MS and Beneficial Effect of Purslane Enriched with Zinc on Experimental Alzheimer Disease in Rats

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Abstract

Our study aimed to investigate the impact of purslane (*P. oleracea)* and zinc as a therapeutic approach to physiological and biochemical alterations induced by Alzheimer disease (ALZ) in rats. For this purpose, 18 males albino Wistar rats were randomly divided into 4 groups (n=6); healthy rats (Control), untreated Alzheimer rats (Exp ALZ), Alzheimer rats treated with aqueous purslane extract (APE) and Alzheimer rats treated with purslane extract and zinc (PE-Zn).Various neurological and biological parameters were estimated and brain Histopathology was observed. Results of study showed an alteration in passive avoidance learning (PAL) and a significant increase in AChE activity ($P<0.01$) and protein, WBC, Monocyte, LYM, platelet and MDA levels and decreasing the GSH levels, GST and SOD activities in Exp ALZ group compared to control. In the other hand, histopathological analysis recorded a deep modification in brain tissues of Alzheimer rats group compared to control. However, the treatment of ALZ rats by APE and zinc ensured a partial amelioration and correction of the previous parameters. We conclude that the use of purslane seems to be the powerful limited of Alzheimer disease development or its complications.

Key words: Alzheimer, Purslane, Zinc, HPLC-QTOF-MS, Amyloid B, AChE, Oxidative stress,

Introduction

Alzheimer disease is an age-related neurodegenerative disorder, which progressive by nature, where the symptoms of dementia continue to increase and quality of life deteriorates gradually over a number of years (1). The amyloid hypothesis proposes β-amyloid (Aβ) among the major pathological hallmark of AD of the disease and suggests that misfolding of the extracellular Aβ protein accumulated in senile plaques and the intracellular deposition of misfolded tau protein in neurofibrillary tangles cause memory loss, confusion and result in personality and cognitive decline over time (2). The disordered functioning of neurotransmitters in brain, including acetylcholine (Ach), also belongs to AD pathological (3). Many of recent landmarks in scientific research have shown that in human beings, oxidative stress is an im-

portant factor causing physiological alterations and various diseases in the body (4). The oxidative stress may be a crucial link in AD initiation and development. The imbalance between the generation and elimination of reactive oxygen species (ROS) can cause extensive and lasting damage in the central nervous system (CNS), which finally accelerates the occurrence and development of AD (5). The medicinal plants have been reported to enhance the memory and learning process that normally decline with AD. The phytochemicals have been clinically proven the significant potentials anti-AD by different mechanism of action (6). Purslane (*Portulaca oleracea*) listed in the World Health Organization as one of the most used medicinal plants (7). It possesses a wide spectrum of pharmacological properties such as neuroprotective, antioxidant, anti-inflammatory, and anticancer activities (8). Zinc known as potential metal involved in metabolic organism regulation, where the zinc is considered as one of the most promising and magic materials because of its unique catalytic properties as well as its extensive applications in diverse biological areas (9). Due to the complexity of Alzheimer disease and inability researchers to obtain an appropriate and effective treatment, the present study was carried out to investigate the therapeutic efficiency of purslane and zinc against metabolic, physiological and histological alteration induced by experimental Alzheimer disease in rats.

Materials and Methods

Plant material and extract preparation

The purslane (*Portulaca oleracea* L.) was collected in August from a village in Touggourt of Ouargla state, Algeria. The leaves were washed with distilled water, and then dried at room temperature. The completely dried purslane leaves was powdered by using a mechanical grinder. The powder stored at room temperature in the airtight containers until the use. Aqueous extract was preparing by putting 10 g of dried leaves powder of *Portulaca oleracea L* with 100 ml of distilled water was boiled over low heat (50 c°) for 2 hours. After cooled and macerated to room temperature for 24 hours, then filtered through Whatman filter paper, the extract was then evaporated using a rotary evaporator and was drying using oven.

HPLC-QTOF-MS/MS analysis

The HPLC analysis was conducted on an Agilent 1290 UHPLC system (Waldbronn, Germany) hyphenated with a G1316A thermostatted column compartment, a G4226A auto-sampler, a G1330B thermostat and a G1312B binary pump. The HPLC separation was achieved on an XBP phenyl column (c18 (100mmx3mm, 2.7µm); Venusil, Agela Technologies, Tianjin, China). The mobile phase, which consisted of a 0.1% formic acid aqueous solution (A) and a mixture of acetonitrile methanol (1:1, v/v; B), was delivered at a flow rate of 0.4ml/min under the following gradient programme: 10–35% B for 0–25 min, 35–40% B for 25–35 min, and 40–85% B for 35–40 min. Mass spectra are obtained from a TripleTOF 5600+ system (Applied Biosystems Sciex, Framingham, MA, USA) equipped with an ESI interface in positive ionisation mode. The parameters of the “TOFMS” scan type were as follows: source temperature, 550°C; ion source gas 1 and ion source gas 2, 50 psi; curtain gas, 35 psi; ion spray voltage,5500 V; collision energy, 10 V; declustering potential, 80 V. In the “product ion” scan type, the parameters were the same as those of the “TOFMS” scan type except that collision energy was set as 35 V with a collision energy spread of 15 V. Data acquisition and processing were performed by using Peakview® software (version 2.0, Applied Biosystems Sciex) (10).

Animals care and experimental design

In this study, thirty-six males albino Wistar rats at the age of 8 weeks old, weighting 131.28 ± 5.58 g, obtained from the Institute Pasteur of Algiers, and were housed in plastic cages at animal room of molecular and cellular biology department, of nature and life sciences faculty, in Echahid Hamma Lakhdar-El-Oued

University, Algeria. With a temperature of 25 ± 2°C, the rats were given a free access to their standard diet and tap water during the study. The animals were adapted to an inverse 12:12 h light/dark cycle. All experimental procedures employed, as well as rat care and handling, were in accordance with guidelines provided by El-Oued University Committee of Animal Care and Use. This study was carried out in compliance with the Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines.

After two weeks of acclimatization, the animals were divided into six groups, 6 in each of, Alzheimer disease was induced by oral administration of 200 mg/kg b.w in drinking water for 76 days, the rats treated during 21 days as following: Group 01 (Control): Healthy rats received distilled water. Group 02 (Exp Alzheimer): Experimental Alzheimer rats received distilled water. Group 03 (APE): Experimental Alzheimer rats treated orally by aqueous extract of *P.oleracea*. Group 04 (PE-ZnNPs): Experimental Alzheimer rats treated orally by *P.oleracea* + zinc

Alzheimer state was induced by oral administration 200 mg/kg b.w of both aluminum trichloride ($AlCl_3$) and D-galactose in drinking water during 76 days (11). The doses of *P.oleracea* (400mg/kg b.w) (12) and zinc (7mg/Kg b.w/wk) (13) by oral for 21 day.

At the end of each treatment, and after 12 hours of fasting, animals were anesthetized by inhalation of isofurane (2%) for 2 min and dissected immediately to obtain the blood by cardiac puncture and the brain. blood samples were transferred into EDTA tubes to carried the hematological parameters and dry tubes previously labeled and numbered for each rat. The blood was separated by centrifugation for 3000 revolutions/min during 15 min, the obtained serum stored at -20°C until the use for biochemical analysis; fasting blood glucose level obtained by glucometer for each rat. Brain was carefully removed, rinsed in NaCl 0.9% then weighed. Also, the organs stored in the freezer at -20°C until the preparation of homogenates for the determination of neurotransmitter and oxidative stress parameters.

Behavioural analysis

Passive avoidance training

During the treatment period, the control and experimental groups were subjected to passive avoidance learning which mainly depend on the shuttle box (PAL apparatus) that has two chamber light and dark with transparent and dark opaque plastic walls, respectively has a same size (30 x 20 x 20 cm each) which separate by an opaque door a rectangular opening between these two compartments. The dark chamber has a floor contained a very cold water (fig.1A). After five second, from placing the subject rodent in the light chamber, the door was opened until the entrance of rat to the dark side it closed, the rat passed into the floor possessed a very cold water. Subsequently, 60 seconds later the rat was delivered to the cage. In the next day of training if, the animal didn't enter the dark in 120 seconds, a successful acquisition of a passive avoidance behavior (14).

Retention test

Twenty-four hours following PAL acquisition, similar to what we did in Passive avoidance training but with one deferent which to keep the door opened after five seconds from placing the animal in the light chamber (fig.1B). Step-through latency; STL (the latency to enter the dark side) and time spent in the dark chamber (TDC) were reported for up to 120 seconds if the rodent last in the light compartment in 120 second, the retention test was ended, and the maximum score of 120 seconds was reported (14).

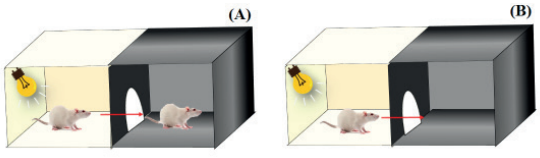


Fig. 1. Step-through passive avoidance, Train (A) and Test (B).

Biological and Oxidative stress parameters analysis

About 1g of heart was homogenized in 9 ml of buffer solution of Tris buffer saline (pH=7.4). Homogenates were centrifuged at 4000xg for 20 min +4C° and the obtained supernatant was used for the determination of amyloid protein, acetyl choline esterase and oxidative stress markers levels. AChE activity was determined by the method of Ellman et al., (15). Protein Aβ was determined in the brain tissue homogenate using ELISA Kit. MDA was measured according to the method described by Yagi et al., (16). The level of reduced Glutathion is determined according to Weckbecker & Cory (17). About the assay method of SOD activity which using the NBT by the superoxide anion (O2.), is used as a basis for detecting of presence of SOD by measuring the spectrophotometrically absorbance at 560 nm (18). The method used in this study to measure the GSTs is that of Habig et al., (19). The determination of hematological parameters performed using fully Auto Blood Cell Counter (ERMA).

Brain histopathological study

After the sacrifice of rats, brain and liver were removed and immersed for 48 h in a fixative solution (solution 4% formaldehyde, in phosphate buffer, pH=7.6), dehydrated in ascending graded series of ethanol, cleaned with toluene, immersed in paraffin, and colored with hematoxylin and eosin. Histopathological evaluation was performed with a light microscope.

Statistical analysis

The results obtained are expressed as the mean ± standard error of the mean (Mean ± SEM). The analysis of the data was carried out by application of the Student's T test, which is based on the comparison between two means, using the MINITAB software (Version 13) and EXCEL (Version 2019) which helps us to do the tests and the curves.

Results and Discussion

HPLC-QTOF-Mass spectroscopy of pursulane alkaloids

The structural information of all peaks in the HPLC chromatograms (figure2). In the Purslane leaves, the presence of the following alkaloids was classified depending on the polarity into positive such as Betaine, Trigonelline, L-Phenylalanine, Galactaric acid and Citric acid, N-Acetyl-L-phenylalanine, according to negative polarity as what presented in table 1.

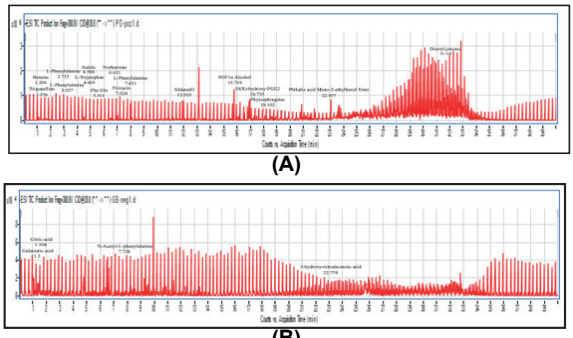
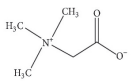
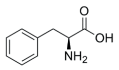
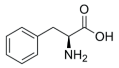
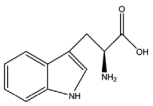
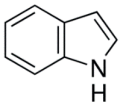
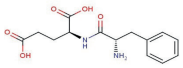
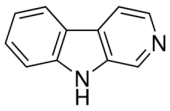
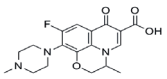
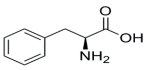
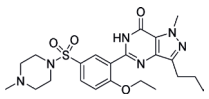
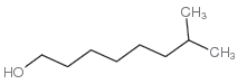
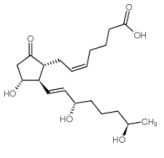
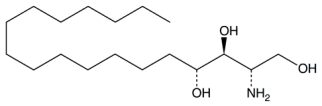
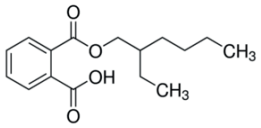
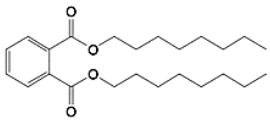
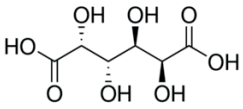
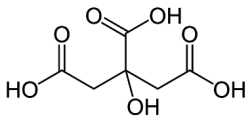
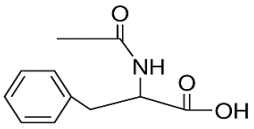
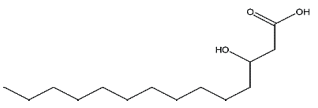


Fig. 2. LC-Q-TOF-MS chromatogram in positive ion (A) and negative ion (B) mode for tentative assigned alkaloids compounds

Table 1. Different of positive and negative alkaloids existed in purslan aqueous extract

Polarity	Retention Time (min)	Name	Formula	Structure
	1.209	Betaine	C5H12NO2	
	1.276	Trigonelline	C7H8NO2	

Positive	2.733	L-Phenylalanine	C9H11NO2	
	3.027	L-Phenylalanine	C9H11NO2	
	4.495	L-Tryptophan	C11H-12N2O2	
	4.506	Indole	C8H7N	
	5.511	Phe Glu	C14H-18N2O5	
	6.651	Norharman	C11H8N2	
	7.024	Ofloxacin	C18H20F-N3O4	
	7.431	L-Phenylalanine	C9H11NO2	
	12.015	Sildenafil	C22H-30N6O4S	
	15.719	PGF1α Alcohol	C20H38O4	
Positive	16.735	19(R)-hydroxy-PGE2	C20H32O6	

	18.102	Phytosphingo-sine	C18H-39NO3	
	22.957	Phthalic acid Mono-2-ethyl-hexyl Ester	C16H22O4	
	31.754	Dioctyl phtha-late	C24H38O4	
Negative	1.2	Galactaric acid	C6H10O8	
	1.504	Citric acid	C6H8O7	
	7.738	N-Acetyl-L-phenylalanine	C11H-13NO3	
	22.779	3-hydroxy-te-tradecanoic acid	C14H28O3	

Step-through latency and time spent in the dark compartment

The step-through latency **(STL)** results illustrated in figure 18, which revealed that there were significant decrease in STL level (P<0.001) in experimental Alzheimer group as compared to control group. However, we consulted a very high significantly increased (P<0.001) of STL in the different treatment groups in comparison to experimental Alzheimer rats. Time spent in the dark compartment (TDC) for experimental Alzheimer, PE-Zn group were significantly increased (P<0.001) as compared to the control. In the other side, the comparison with Alzheimer disease rats scored a significant decrease in TDCs for APE (P<0.001) and PE-Zn (P<0.001) treated animals (figure 3).

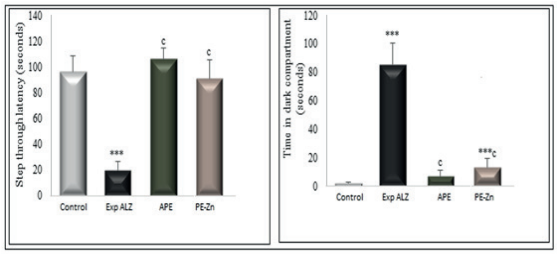


Fig. 3. STL and TSD in control and experimental groups. * p<0.05, ** p<0.01, *** p<0.001: significantly different from control group. c p<0.001 : significantly different from Exp Alzheimer group. Values are mean ± SEM, n=6

Determination of brain protein AB level and acetyl cholinesterase activity

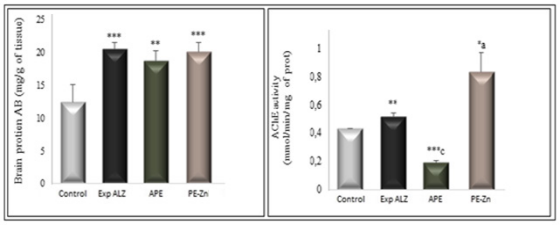


Fig. 4. Brain protein AB concentration and Acetyl cholinesterase activity in control and experimental groups. ** p<0.01, *** p<0.001: significantly different from control group. b p<0.01, c p<0.001: significantly different from Exp Alzheimer group. Values are mean ± SEM, n=6

As shown in figure 4; in the brain, as compared to the control the experimental Alzheimer and PE-Zn groups presented a significant increase in protein levels (P<0.001) of Alzheimer disease rats (P<0.001). In addition, a significant decrease of brain protein concentration in PE-Zn (P<0.001) rats as compared to Alzheimer group. The results illustrated in figure 4 demonstrated that, there were a significant increase in acetyl cholinesterase (AChE) activity for the experimental Alzheimer animals (P<0.01) and the treated group by PE-Zn (P<0.05) compared to the control. However, the APE groups presented a very high significantly decrease in AChE activity (P<0.001) compared to control and Exp Alzheimer rats.

Hematological parameters

Hematological parameters illustrated in table 2 showed that, there were a significant raise (P<0.01) of white blood cell (WBC), lymphocytes (Lymph), monocytes (Mono) and platelet levels and no significant change of red blood cell (RBC), hemoglobin (HBG) and basophile (BASO) in Alzheimer disease rats compared to the control . In the other hand all experimental treatment rats showed significantly reverse change (P<0.001) in WBC. Whether treatment with Zn significantly decreased (P<0.001) Lymph, Mono levels, however Platelet levels was significantly decline (P<0.001) by Zinc. Additionally, the treatment by APE were significantly reduced (P<0.01) the Lymph, Mono and Platelet levels as compared with Alzheimer group.

Table 2. Erythrocyte and Leukocyte line in blood of control and experimental groups

Parameters	Control (n=6)	Exp ALZ (n=6)	APE (n=6)	PE-Zn (n=6)
Red blood cell (10^6/µl)	10.01 ± 0.08	10.07± 0.11	10.27 ± 0.19	9.77±0.04^{***c}
Hemoglobin (g/dl)	15.27±0.24	15.66 ± 0.2	15.20 ± 0.42	14.87 ± 0.11
Platelet (10^3/µl)	959 ± 29.4	1020.3 ± 5.1***	775,8 ± 65.1^{*b}	915.5 ±5.48^{***c}
White blood cell (10^3/µl)	4.84 ± 0.255	7.01 ±0.63**	5,61 ± 0,26^{*c}	4,23 ±0,36^{c}
Lymphocytes (10^3/µl)	2.60 ± 0.23	5.000 ± 0.56**	3,89 ± 0,25^{***b}	2,86± 0,20^{c}
Monocytes (10^3/µl)	0.06 ± 0.009	0,25 ± 0,04***	0,13±0,03^{*b}	0.09 ± 0.02^{c}
Basophyle (10^3/µl)	0.0025±0.001	0.004 ± 0.001	0.008±0.001^{**a}	0.002± 0.001

Values are mean ± SEM. * p<0.05, ** p<0.01, *** p<0.001: significantly different from control group. a p<0.05, b p<0.01, c p<0.001: significantly different from Exp ALZ group.

Brain oxidative stress parameters

In comparison to control group, the results demonstrated that there were a significant increase (P<0.05) of MDA level and a significant decrease of GSH, GST and SOD activities in Exp ALZ group. In addition as compared to Exp ALZ rats, a significant decrease (P<0.05) of MDA level was observed in APE groups and no significant change of GSH level, GST and SOD activities in treatment groups (fig.5).

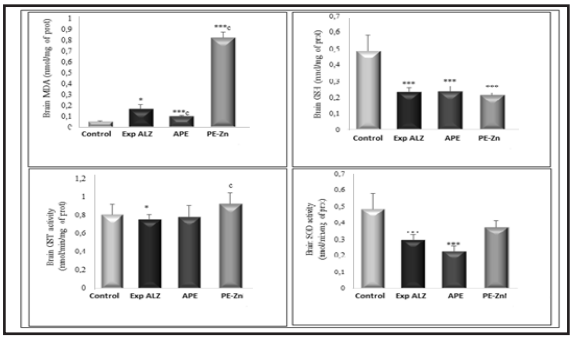


*Fig. 5***.** Brain MDA, GSH, GST and SOD levels in control and experimental groups *** p<0.001: significantly different from control group. a p<0.05, b p<0.01, c p<0.001: significantly different from Exp Alzheimer group. Values are mean ± SEM, n=6

Histological analysis

Performed the histopathological studies to determine the severity of Exp ALZ and neuroprotective effects of *P.oleracea* aqueous extract, copper and zinc oxide nanoparticles. Microscopic examination of the brain tissues representing normal histological structure in the control while a huge alteration from hemorrhagic, inflammatory to degeneration cells in brain tissue structure of Exp ALZ rats. Furthermore, the treatment by APE, and PE+Zn improved that these histological alterations had a structure not far from the control (figure 6).

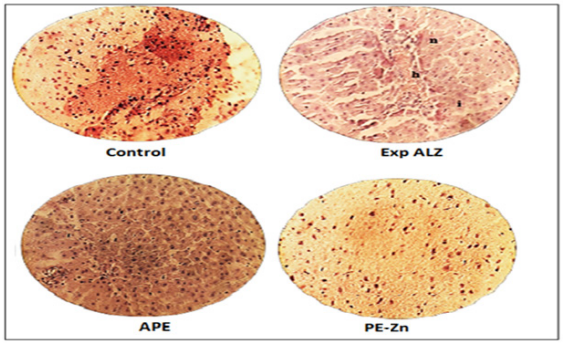


Fig.6. Photomicrographs of brain section of all experimental groups stained with hematoxylin and eosin (H&E), (A): x 40 n: necrosis; h: hemorrhage; i: inflammation

Discussion

The alkaloids compounds have a highly beneficial effect that according to their natural chemical compounds. HPLC-MS/MS method has become a rapid, sensitive, precise and convenient way to analyze chemical constituents such as alkaloids (20), which have been isolated from the plant. From the LC-Q-TOF-MS chromatograms and by comparing to previous reports the trigonelline, N-acetyl- L –phenylalanine, betaine (21), L-Phenylalanine and Norharman (22) are isolated from Chinese *P.oleracea* . Nevertheless citric acid is extracted from purslan of Greece and Pakistan (23, 24). However, each of indole, ofloxacin, sildenafil, PGF1α alcohol, (25) (R)-hydroxy-PGE2, phytosphingosine, phthalic acid mono-2-ethylhexyl ester, dioctyl phthalate, Galactaric acid and 3-hydroxy-tetradecanoic acid are new alkaloids, which not determined yet in this plant.

In order to investigate the phyto and metallotherapy treatment to improve behavior and learning-memory. The performance of animals in the step through show that the Exp Alzheimer rats and our experimental treatment groups demonstrated a remarkable difference. Various study(26, 27) revealed that the test of passive avoidance learning (PAL) was assessed by the time spending in a dark compartment (TDC), a high score showed in the Exp Alzheimer rats comparatively with the control group. While, the opposite is observed in the step-through latency (STL) results a similar finding with Haider et al., which confirmed the decreased learning and memory ability in Exp Alzheimer group (28). However, the treatment with *P. oleracea* ameliorate the memory of rats through the significantly change of (STL) and (TDC) when compared to Exp Alzheimer group. These results are consistent with the studies of Tabatabaei et al., (29) and Hafez & Gad (30). Therefore, the combination administration of AEPo, and Zn allows the beneficial effect, which presented in PE+Zn group. Short term memory is generally associated with the levels of acetylcholine (ACh) (31), which is the key neurotransmitter involved

in the learning and memory processes and the alterations on the cholinergic activity is the main event in the neurochemical changes of AD (32). Data from our study showed a significantly increase of brain AChE activities for the Exp Alzheimer rats. AD causes disturbances in cholinergic neurotransmission, which may be associated with altered memory and learning processes (33), in the other hand the increase of AChE activities has been linked to the genetic overexpression through oxidative stress (34). *Portulaca oleracea* leaves aqueous extract significantly decreased the activity of AChE, which reflect to their alkaloids compound, especially the indole and trigonelline has been considered as inhibitor of AChE (35). However, Zn apparently increased the AChE activity (36). Thus, due to the combination effect of the compounds existed in PE+Zn approach; the activity of this enzyme is decreased, according to down-regulation of its expression or by affecting the stereometric active site. The increase level of AB protein in Exp Alzheimer due to hyperphosphorylation and abnormal aggregation of tau protein as confirmed by Chiroma et al., an abnormally high expression of amyloidogenic proteins such as APP, β-secretase, γ-secretase and Aβ (37). Moreover, the presence of the two compounds together may inhibit the aggregation of Aβ. A significant decrease of AB protein level is a beneficial result of PE+Zn, which make it possible to say that this new approach may be has the ability to suppression the aggregation of Aβ and tau protein. That probably by a specific interaction with it, or by inhibition of the enzymes that responsible of its synthesis or activate the specific proteolytic system.

Besides the brain's innate immune system, the resident microglia, peripheral macrophages (monocyte) as well as cells from the adaptive immune system (lymphocyte T), are increasingly recognized as being involved in AD pathology (38). Suggested that the abundance of leucocytes in general and lymphocyte in particular is a prominent response of body tissues facing injurious impact of AlCl3 (39). Additionally, immuno imbalance occurs mainly due to oxidative damage, which is responsible for ROS generation by enhancing the inflammatory response (40). The information obtained from Jarosz et al. suggest that zinc supplementation may lead to down-regulation of the inflammatory cytokines (IL-1β and TNF-α) through up-regulation A20 to inhibit induced NF-κB activation (41). The immunoregulated of WBC effect shown by PE+Zn approach, which probably due to its anti-inflammatory activity through the inhibition of cytokines either by decrease their synthesis or decrease their concentration in free active form or block their interaction with specific receptors. The Aβ promotes oxidative stress, which is known to play an important role in the pathogenesis linked to the etiology of Alzheimer's disease (42). In this study, the oxidative stress was highly remarked in Exp Alzheimer group by the decrease in SOD activity and GSH level. From all of this, we can say that the two nano-compound associated with an natural antioxidant power of plant in PE+Zn approach can give an ideal reduction, due to the presense of Zn as a cofactor of SOD enzymes. This serves as a first line of the antioxidant defense system, which converts superoxide anion to hydrogen peroxide (H2O2) and oxygen (43).

Additionally, the Zn2+ were shown to suppress the activity of γ secretases that, along with beta- site APP cleaving enzyme 1 (BACE1), are involved in the formation of Aβ (44), which can contribute to a reduction in the formation of this peptide that may explicate the reduction of oxidative stress. Our histological results indicated that there is a necrosis, hemorrhage and infiltration of immune cells in brain section of Exp Alzheimer group, similar finding with (45). This revealed that AD mediates progressive alterations, including neurons degeneration, apoptotic changes and necrosis, which related to oxidative damage of brain cells via free radical production (46). The ameliorative effect of our new approach PE+Zn may appear distinctly in its histological section, whether the presence of the three compound together may enhance ei-

ther its anti-inflammatory activities by inhibition the pro-inflammatory substances or inhibit the amyloidogenic activity and probably affect the BBB by decreasing vascular permeability.

We conclude that the treatment with purslane enriched with zinc could significantly rescue the impairment of spatial and negative avoidance memory in rats. It also reduces excess protein AB and improves acetylcholine performance. It is suggested that this therapy has potential therapeutic effects for neurodegenerative diseases, such as Alzheimer's disease. Further investigation is needed to extend these findings.

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References

1. Chiroma SM, Baharuldin MTH, Taib CNM, Amom Z, Jagadeesan S, Adenan MI, Mahdi O, Moklas MAM. Protective Effects of *Centella asiatica* on cognitive deficits induced by d-gal/$AlCl_3$ via inhibition of oxidative stress and attenuation of acetylcholinesterase level. *Toxics*. 2019; **7**: 19.

2. Chen, G Xu T, Yan Y, Zhou Y, Jiang Y, Melcher K, Xu HE. Amyloid beta: structure, biology and structure-based therapeutic development. *Acta pharmacologica sinica*. 2017; 38: 1205-1235.

3. Zhang Y, Pi Z, Song F, Liu Z. Ginsenosides attenuate d-galactose- and AlCl3 induced-spatial memory impairment by restoring the dysfunction of the neurotransmitter systems in the rat model of Alzheimer›s disease. J Ethnopharm. 2016; 194: 188-195.

4. Derouiche S, Degachi O, Gharbi K. Phytochemistry analysis and modulatory activity of *Portulacae oleracea* and *Aquilaria malaccensis* extracts against High-fructose and high-fat diet induced immune cells alteration and heart lipid peroxidation in Rats. *International* Res J Bio Sci. 2019 ; 8 : 6-11.

5. Wei Y, Liu D, Zheng Y, Li H, Hao C, Ouyang W. Protective effects of kinetin against aluminum chloride and D-galactose induced cognitive impairment and oxidative damage in mouse. Brain Res bul, 2017; 134: 262-272.

6. Rahimi, V B, Ajam, F, Rakhshandeh, H, & Askari, V R. A Pharmacological Review on *Portulaca oleracea* L.: Focusing on Anti-Inflammatory, Anti- Oxidant, Immuno-Modulatory and Antitumor Activities. J pharmacopuncture. 22, 7–15 (2019).

7. Leng F, Liu F, Yang Y, Wu Y, Tian, W. Strategies on nanodiagnostics and nanotherapies of the three common cancers. *Nanomaterials*. 2018; 8: 202.

8. Lombardo SM, Schneider M, Türeli AE, Türeli NG. Key for crossing the BBB with nanoparticles: the rational design. Beilstein J. Nanotechnol. 2020; 11: 866–883.

9. Rubilar O, Rai M, Tortella G, Diez MC, Seabra AB, Duran N. Biogenic nanoparticles: copper, copper oxides, copper sulphides, complex copper nanostructures and their applications. Biotechnol. Lett. 2013; 35: 1365-75.

10. Shangguan Y, He J, Kang Y, Wang Y, Yang P, Guo J, Huang J. Structural characterisation of alkaloids in leaves and roots of stephania kwangsiensis by LC-QTOF-MS. Phytochem Anal. 2018; 29 : 101–111.

11. Debbache-Benaida N, Berboucha M, Ayouni K, Atmani D, Nassima C, Boudaoud H, Djebli N, Atmani, D. Antihyperuricemic and neuroprotective effects of *Populus nigra L.* (*Saliacaceae)* flower buds used in Algerian folk medicine. J Pharm Pharmacogn Res. 2018; 6: 471-482.

12. Samir D, Sara C, Widad A. "The Effects of aqueous leaf extract of Portulaca oleracea on haemato-biochemical and histopathological changes induced by Sub-chronic Aluminium toxicity in male wistar rats. Pharmacol Res Modern Chinese Med. 2022; 100101.

13. Derouiche S, Laib I, Zeribit W. Protective effect of zinc acetate and Zinc - Aristolochia longa Extract Nanoparticles against nickel induced acute liver and kidney injury in rats. Annal RSCB. 2022; 26(1): 307-316.

14. Dehbani Z, Komaki A, Etaee F, Shahidi S, Taheri M, Komaki S, Faraji N. Effect of a hydro-alcoholic extract of *Melissa officinalis* on passive avoidance learning and memory. J HerbMed Pharmacol. 2019; 8: 120-125.

15. Ellman GL, Courtney KD, Andres JV, Featherstone RM. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem. Pharmacol. 1961; 7: 88-95.

16. Yagi, K. A simple fluorometric assay for lipoperoxide in blood plasma. Biochem Med. 1976; 15: 212-216.

17. Weckbecker G, Cory JG. Ribonucleotide reductase activity and growth of glutathione-depleted mouse leukemia L1210 cells in vitro. Cancer lett. 1988; 40: 257–264.

18. Beauchamp C, Fridovich I. Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. Anal Biochem. 1971; 44: 276287.

19. Habig WH, Pabst MJ, Jakoby WB. Glutathione S-transferase. The first enzymatic step in mercapturic acid formation. J Biol Chem. 1974; 249 : 7130-7139.

20. Jin TY, Li SQ, Jin CR, Shan H, Wang RM, Zhou MX, Xiang L. Catecholic isoquinolines from *Portulaca oleracea* and their anti-inflammatory and β2-adrenergic receptor agonist activity. J Nat Prod. 2018; 81: 768-777.

21. Petropoulos SA, Fernandes Â, Dias MI, Vasilakoglou IB, Petrotos K, Barros L, Ferreira ICFR. Nutritional Value, Chemical Composition and Cytotoxic Properties of Common Purslane (*Portulaca oleracea* L.) in Relation to Harvesting Stage and Plant Part. *Antioxidants (Basel)*. 2019; 8: 293.

22. Javed MT, Akram MS, Habib N, Tanwir K, Ali Q, Niazi NK, Gul H, Iqbal N. Deciphering the growth, organic acid exudations, and ionic homeostasis of Amaranthus viridis L. and *Portulaca oleracea* L. under lead chloride stress. Environ Sci Pollut Res. 2017; 25.

23. Bhat RS, Al-Daihan S. Phytochemical constituents and antibacterial activity of some green leafy vegetables. Asian Pac J Trop Biomed. 2014; 4: 189-193.

24. Iranshahy M, Javadi B, Iranshahi M, Jahanbakhsh SP, Mahyari S, Hassani FV, Karimi, G. A review of traditional uses, phytochemistry and pharmacology of *Portulaca oleracea* L. J Ethnopharmacol. 2017; 205: 158-172.

25. Dhandapani KV, Anbumani D, Gandhi AD, Annamalai P, Muthuvenkatachalam BS, Kavitha P, Ranganathan B. Green route for the synthesis of zinc oxide nanoparticles from *Melia azedarach* leaf extract and evaluation of their antioxidant and antibac-

terial activities. Biocatal Agric Biotechnol. 2020; 24: 101517.

26. Kumar SEP, Bairy KL, Nayak V, Reddy SK, Kiran A, Ballal A. Amelioration of aluminium chloride ($AlCl_3$) induced neurotoxicity by combination of rivastigmine and memantine with artesunate in albino Wistar rats. Biomed. Pharmacol. J. 2019; 12: 703-711.

27. Chogtu B, Arivazhahan A, Kunder, SK, Tilak, A, Sori, R, & Tripathy, A. Evaluation of acute and chronic effects of d-galactose on memory and learning in wistar rats. *Clin Psychopharmacol Neurosci*. 2018; 16: 153-160.

28. Haider S, Liaquat L, Ahmad S, Batool Z, Siddiqui RA, Tabassum S, Shahzad S, Rafiq S, Naz N. Naringenin protects AlCl3/D-galactose induced neurotoxicity in rat model of AD via attenuation of acetylcholinesterase levels and inhibition of oxidative stress. Plos One. 2020; 15: e0227631.

29. Tabatabaei SRF, Rashno M, Ghaderi S, Askaripour M. The aqueous extract of *Portulaca oleracea* ameliorates neurobehavioral dysfunction and hyperglycemia related to streptozotocin-diabetes induced in ovariectomized rats. Iranian J Pharm Res. 2016; 15: 561-571.

30. Hafez MH, Gad SB. Zinc oxide nanoparticles effect on oxidative status, brain activity, anxiety-like behavior and memory in adult and aged male rats. Pakistan Vet J. 2018; 38: 311-315.

31. Liaquat L, Muddasir S, Sadir S, Batool Z, Khaliq S, Tabassum S, Emad S, Madiha S, Shahzad S, Haider S. Development of AD like symptoms following co-administration of $AlCl_3$ and D-gal in rats: a neurochemical, biochemical and behavioural study. Pakistan J Pharm Sci. 2017; 30: 647-653.

32. Hampel H, Mesulam MM, Cuello AC, Farlow MR, Giacobini E, Grossberg GT, Khachaturian AS, Vergallo A, Cavedo E, Snyder PJ, Khachaturian ZS. The cholinergic system in the pathophysiology and treatment of Alzheimer's disease. *Brain.* 2018; 141: 1917-1933.

33. Thenmozhi AJ, Raja TRW, Manivasagam T, Janakiraman U, Essa, MM. Hesperidin ameliorates cognitive dysfunction, oxidative stress and apoptosis against aluminium chloride induced rat model of Alzheimer's disease. Nutrit Neurosci. 2016; 20: 360-368.

34. Walczak-Nowicka ŁJ, Herbet M. Acetylcholinesterase Inhibitors in the Treatment of Neurodegenerative Diseases and the Role of Acetylcholinesterase in their Pathogenesis. Int J Mol Sci. 2021; 22: 9290.

35. Khalili M, Alavi M, Esmaeil-Jamaat E, Baluchnejadmojarad T, Roghani M. Trigonelline mitigates lipopolysaccharide-induced learning and memory impairment in the rat due to its anti-oxidative and anti-inflammatory effect. Int Immunopharmacol. 2018; 61: 355-362.

36. Navaei-Nigjeh M, Gholami M, Fakhri-Bafghi MS, Baeeri M, Abdollahi M. Molecular and biochemical evidences for beneficial effects of zinc oxide nanoparticles in modulation of chlorpyrifos toxicity in human lymphocytes. Iranian J Pharm Res. 2018; 17: 927-939.

37. Chiroma SM, Baharuldin MTH, Taib CNM, Amom Z, Jagadeesan S, Adenan MI, Moklas MAM. *Centella asiatica* protects d-galactose/AlCl3 mediated Alzheimer's disease-like rats via PP2A/GSK-3β signaling pathway in their hippocampus. Int J Mol Sci. 2019; 20: 1871.

38. Unger MS, Schernthaner P, Marschallinger

J, Mrowetz H, Aigner L. Microglia prevent peripheral immune cell invasion and promote an anti-inflammatory environment in the brain of APP-PS1 transgenic mice. J Neuroinflam. 2018; 15: 274.

39. Saleh SMM, Elghareeb TA, Ahmed MAI, Mohamed IA, El-Din HAE. Hepato-morpholoy and biochemical studies on the liver of albino rats after exposure to glyphosate-Roundup®. J Basic Appl. Zool. 2018; 79: 48.

40. Dey A, Manna S, Kumar S, Chattopadhyay S, Saha B, Roy S. Immunostimulatory effect of chitosan conjugated green copper oxide nanoparticles in tumor immunotherapy. *Cytokine*. 2020; 127: 154958.

41. Jarosz M, Olbert M, Wyszogrodzka G, Młyniec K, Librowski T. Antioxidant and anti-inflammatory effects of zinc. Zinc-dependent NF-κB signaling. Inflammopharmacol. 2017; 25: 11-24.

42. Cheignon C, Tomas M, Bonnefont-Rousselot D, Faller P, Hureau C, Collin F. Oxidative stress and the amyloid beta peptide in Alzheimer's disease. Redox Bio. 2018; 14: 450-464.

43. Derouiche S, Zeghibe K, Gharbi S, Khelef Y. In-vivo study of stress oxidative and liver damage in rats exposed to acetate lead. Int Res J Biol Sci. 2017; 6 : 1-6.

44. Isaev NK, Stelmashook EV, Genrikhs EE. Role of zinc and copper ions in the pathogenetic mechanisms of traumatic brain injury and Alzheimer's disease. Rev Neurosci. 2020; 31: 233-243.

45. Amjad S, Umesalma S. Protective Effect of Centella asiatica against Aluminium-Induced Neurotoxicity in Cerebral Cortex, Striatum, Hypothalamus and Hippocampus of Rat Brain-Histopathological, and Biochemical Approach. J Mol Biomark Diagn. 2015; 6: 212.

46. Abd-Elghaffar SK, El-Sokkary GH, Sharkawy AA. Aluminum-induced neurotoxicity and oxidative damage in rabbits: protective effect of melatonin. Neuroendocrin lett. 2005 ; 26: 481-224.