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Phytochemical Evaluation of *Lagerstroemia indica* (L.)Pers. Leaves as Anti-Alzheimer's

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Abstract

The present study aims to evaluate phytoconstituents of *Lagerstromia indica* (L.) and their effect on Alzheimer's disease (AD). HPLC was (carried out) for identification of phenolics, flavonoids and natural pigments. Seven phenolic acids were identified in this investigation for the first time; ferulic, cinnamic, vanillic, *p*-hydroxy benzoic *p*–coumaric, caffeic and *p*-amino benzoic acids. The result of this investigation stated a promising effect of the 80% ethanolic extract (total extract) of *L. indica* as anti-Alzheimer's agent. *In vivo* experiment using rats animal model to induce AD using aluminium cholride (Al Cl_3) indicated disturbances in neurotransmitter levels including norepinephrine (NEp), acetylcholine esterase (AChE), dopamine (DA) and serotonin (5-HT). In addition, elevation in oxidative stress protein carbonyl (PC) and apoptotic markers caspase -3 were also recorded. Histopathological investigation of AD induced rats showed cerebral cortex with necrosis, atrophy, pyknosis of neurons and focal gliosis. Meanwhile, hippocampus of AD rats showed necrosis of pyramidal cells. On the other hand, hippocampus of AD rats treated with *L. indica* showed necrosis of some pyramidal cells. Treatment of cerebral cortex or hippocampus of AD rats treated with *L. indica* total extract showed necrosis of some sporadic neurons and pyramidal cells respectively. Hence, it could be concluded that, *L. indica* exhibited neuro-modulating effect in Al–induced neurotoxicity, which could be used in a future as a promising candidate nutraceuticals.

1. Introduction

Alzheimer's disease (AD) is a neurodegenerative disorder characterized by the deposition of extracellular amyloid-beta peptide (Aβ) and intracellular neurofibrillar tangles, alongwith loss of neurons in the brain and consequent memory and learning deficits. oxidative stress play a role in AD and their suppression or reduction via antioxidant use could be a promising preventive or therapeutic intervention for patients by protect brain from Aβ neurotoxicity. [1]. Increasing number of studies demonstrated the efficacy of polyphenolic antioxidant compounds from plant origin to reduce the neuronal death occurring in the pathophysiology of the disorders. These studies revealed that other mechanisms beside the antioxidant activities could be involved in the neuroprotective effect of these phenolics. On the other hand, earlier studies indicated the strenuous influence of many of these compounds on neurodegenerative disorders such as Alzheimer's and Parkinson's diseases [2]; [3] and [4]. During the latest couple decades, plant bioactive metabolites were source of attention as part of the search for prophylaxis and treatment agents as well [5].

Lagerstromia indica, Family Lythraceae, is commonly known as Indian Crape myrtle. It has been ranked as one of the most significant ornamental woody species, endemic to China. It has been introduced due to its ease of cultivation in many other regions of the world [6]. Genus *Lagerstroemia* includes mainly tropical plants except four species only including our plant of interest; *L. indica* can grow in temperate regions. They generally have small white to purple flowers with little ornamental appeal and are used commercially, primarily for lumber [7].

The aqueous methanol leaf extract of *L. indica*, showed significant activities *e.g.* anti- inflammatory, antipyretic, analgesic, antihyperglycemic, antioxidant and hepatoprotective. Many phenolic were identified in this plant; e.g. brevifolin, and quercetin [8].

Our main target in this study was to evaluate the phenolic and flavonoid content, which the plant rich in, as treatment for AD's disease and to discover a new source of plant origin to conquer this disease.

2. Material and Methods

2.1. Material for phytochemical study

2.1.1. Collection and Identification of Plant Material

Fresh plant leaves of *L. indica* were collected in January 2016 from the National Research Centre garden, Dokki, Giza, Egypt. The plant leaves specimens were kindly identified by Trease Labib head specialist for plant identification in Orman botanical garden, Giza, Egypt. The collected plant leaves were air-dried, powdered and kept in tightly-closed amber glass containers until needed.

2.1.2. Material for Phytochemical Screening:

Ferric chloride reagent for tannins [9]
Mayer's reagent for alkaloids [10].
Iodine/KI reagent [10].
Molisch's reagent for carbohydrates and /or glycosides [11].
Lieberman-Burchard reagent for tritrpenes and steroids [10].

2.1.3. Flavonoids and phenolics Authentic Reference Material

Rutin for HPLC assay from United Pharma Industries Co. Ltd. quercetrin, quercetin and caffeic acid for HPLC assay from NODCAR, chlorogenic acid: Strasbourg (France). Gallic acid from Sigma Aldrich. Apigenin: Nur Fur Laborzwechegepruft Carl Roth Germany. Luteolin and kaempferol from Agricultural Research Center.

2.1.4. Chemicals

All chemicals used in the present study were of high analytical grade, produced by Sigma (USA), Merck (Germany), BDH (England), Riedel de Hàen (Germany) and Fluka (Switzerland). Aluminum chloride ($AlCl_3$) from BDH Laboratory Supplies, Poole (UK). *Eserine* was purchased from Sigma Co (USA).

2.2. Techniques of phytochemical study

2.2.1. Preparation of 80% alcoholic Extract

The air dried powdered leaves of *L. indica* (50g) were extracted by heating under reflux with 80% ethanol until complete exhaustion, filtered, concentrated using a rotary evaporator at 45°C under reduced pressure and kept in closed tight container.

2.2.2. HPLC Analysis of Carotenoid Content

HPLC Agilent Packared (series1200) equipped with autosampling injector, solvent degasser, ultraviolet (UV) detector set at 461 nm and quarter HP pump (series 1050). used for the chromatographic separation. Sample injection volumes (50 to 100 µL), were dispensed with injector. A solvent delivery system was used to deliver the mobile phase <acetonitrile-methanol-ethyl acetate, 88:10:2, v/v) at the rate of 1 mL/min. Procedure adopted according to T. Siong ,L.C. Lam. (1992) [12]. Peak areas were quantitated by comparing with reference standards using mean values obtained from at least three injections.

2.2.3. HPLC Analysis of Flavonoids and Phenolics

HPLC Agilent Packared (series1200) equipped with autosampling injector, solvent degasser, ultraviolet (UV) detector set at 254 nm and quarter HP pump (series 1050). The column temperature was maintained at 25° C. Gradient separation was carried out with methanol and acetonitrile as a mobile phase at flow rate of 1 ml/min. Standard flavonoids from sigma Co. were dissolved in the mobile phase and injected into HPLC. The flavonoids content of *L. indica* was determined according to the procedure adopted byP. Mattila,et al., (2000) [13], while phenolics determination was assigned following the technique applied by P. Goupy et al., (1999) [14].

2.3. Biological Experiment

2.3.1. Animals

Male albino rats (180-200 g) were obtained from Central Animal House, National Research Centre (NRC). Animals were acclimatized to the laboratory conditions at room temperature prior to the experimentation. Animals were kept under standard conditions of a 12 h light/dark cycle with food and water in plastic cages with soft bedding. All the experiments were carried out between 9.00 and 15.00 h.

Ethics

Anesthetic procedures and handling with animals were complied with the ethical guidelines of Medical Ethical Committee of the National Research Centre in Egypt and performed for being sure that the animals not suffer at any stage of the experiment (Approval no: 14147).

2.3.2. Drug and Treatment Schedule

$AlCl_3$ was dissolved in water at the beginning of the experiment and administered in a dose of 100 mg/kg to rats daily for 6 weeks [15]. *Eserine* (0.3 mg/kg b.wt. /day) as reference drug for AD diluted in bi-distilled water daily for 6 weeks.

Diet: Control groups were fed with standard diet (El- Kahira Co. for Oil and Soap).

Doses of plant extract were calculated according to the results of LD_{50} of investigated extracts and Had been orally administered. Intoxicated rats will receive Oral doses of the total extract depending on the LD_{50} on daily basis for 3 months and their effects will be determined 24 h after the administration of the last dose. Animals were randomized into five groups (fifty adult male albino rats) based on their body weight Where each group consists of 10 rats and will be distributed as follows:

- Group (1): Control non-treated aged rats.
- Group (2): Normal healthy animals receiving *L.indica total extract*
- Group (3): $AlCl_3$-intoxicated rats (AD induced).
- Group (4): $AlCl_3$-intoxicated rats receiving total extract 500 mg /Kg b.wt
- Group (5): $AlCl_3$-intoxicated rats receiving *eserine* (0.3mg/kg b.wt./day) as reference commercial drug for AD

2.3.2. Blood Sampling:

At the end of the experimental duration, rats were CVB fasted overnight, with free access to water. Under light anesthesia with diethyl ether, rats will be sacrificed by cervical decapitation and the blood was divided into two parts; one part collected into heparinized tubes and then centrifuged at 1400x g for 10 min at 4°C the separated serum, while the other part will be aliquoted and stored at -20° until further biochemical analysis.

2.3.3. Brain Tissue Sampling and Preparation

At the end of the experiment, rats were anesthetized and sacrificed. The whole brain of each rat rapidly dissected and divided sagitally into two portions. The first portion was weighed and homogenized in ice-cold saline solution (0.9% NaCl) to give a 10% (w/v) homogenate. This homogenate was centrifuged and the supernatant to be used for biochemical analyses. In addition, brain total protein concentration was measured to express the concentration of different brain parameters per mg protein. The second portion of the brain was fixed in 10 % formalin for histological investigation

2.3.4. Biochemical Analyses:

2.3.4.1. Estimation of Brain Neurotransmitters

2.3.4.1.1. Determination of epinephrine

Epinephrine (Ep) or adrenaline (A) activity was measured by a quantitative enzyme-linked immunosorbent assay (ELISA) technique according to the manufacturer's instructions [16]. This assay employed the competitive inhibition enzyme immunoassay technique. The microtiter plate provided in this kit were pre-coated with goat-anti-rabbit antibody. Standards or samples were added to the appropriate microtiter plate wells with an antibody specific for Ep and horseradish peroxidase (HRP) conjugated EPI. The competitive inhibition reaction was launched between HRP labeled EPI and unlabeled EPI with the antibody. A substrate solution was added to the wells and the color develops in opposite to the amount of EPI in the sample. The color development was stopped and the intensity of the color was measured.

2.3.4.1.2. Determination of Norepinephrine, Dopamine and Serotonin

The concentrations of brain norepinephrine (NEp) or noreadrenaline (NA), dopamine (DA) and serotonin (5-HT) were determined using high performance liquid chromatography with electrochemical detection (HPLC-ED) technique according to J. Zagrodzka et al., (2000) [17]. The mobile phase comprised a 0.15 M sodium dihydrogen phosphate, 0.1 mM EDTA, 0.5 mM sodium octane sulphonic acid, 10-12% methanol (v/v) and 5mM lithium chloride. The mobile phase was adjusted to pH 3.4 with phosphoric acid, filtered through 0.22 m filter and degassed with helium. A column temperature of 32 ºC and a flow rate of 1.4 mL/min were used.

2.3.4.1.3. Estimation of Acetylcholine Esterase (AChE) Activity

Serum AChE was measured by a quantitative ELISA technique according to G. Wen et al., (2009) [18]. An antibody specific for AChE has been pre-coated onto a microplate. A competitive inhibition reaction is launched between HRP labeled AChE and unlabeled AChE (standards or samples) with the pre-coated antibody specific for AChE. After incubation the unbound conjugate is washed off. The amount of bound HRP conjugate is reverse proportional to the concentration of AChE in the sample. After addition of the substrate solution, the intensity of color developed is reverse proportional to the concentration of AChE in the sample.

2.3.4.1.4. Estimation of Protein Carbonyl:

Protein carbonyl was determined in tissue homogenate by colorimetric assay method according to P.L. Zusterzeel et al., (2000)[19]. Cayman's protein carbonyl colorimetric assay kit utilizes the Dinitro-phenylhydrazine (DNPH) reaction to measure the protein content in tissue homogenates in a convenient 96-well format. The amount of protein-hydrozone produced is quantified spectrophotometrically at an absorbance between 360-385nm. The carbonyl content can then be standardized to protein concentration.

2.3.4.1.5. Estimation of Caspase 3

The activity of caspase enzyme in the brain tissue homogenate is measured, according to the method of [20]. The cells that are suspected or have been induced to undergo apoptosis are first lysed to collect their intracellular contents. The cell lysate can then be tested for protease activity by the addition of a caspase-specific peptide that is conjugated to the color reporter molecule *p*-nitroaniline (*p*NA). The cleavage of the peptide by the caspase releases the chromophore *p*NA, which can be quantitated spectrophotometrically at a wavelength of 405 nm. The level of caspase enzymatic activity in the cell lysate is directly proportional to the color reaction.

2.3.4.2. Histological Examination:

The brain tissue was fixed in 10 % formalin for one week, washed in running tap water for 24 h and dehydrated in ascending series of ethanol (50–90 %), followed by absolute alcohol. The samples were cleared in xylene and immersed in a mixture of xylene and paraffin at 60 ºC. The tissue was then transferred to pure paraffin wax of the melting point 58 ºC and then mounted in blocks and left at 4 ºC. The paraffin blocks were sectioned on a microtome at thickness of 5 µm and mounted on clean glass slides and left in the oven at 40 ºC to dryness. The slides were deparaffinized in xylene and then immersed in descending series of ethanol (90–50 %). The ordinary haematoxylin and eosin stain was used to stain the slides [21].

3. Result and discussion

3.1 Results of Preliminary Phytochemical Screening

The total extract yielded 11.50 g. On the other hand, the phytochemical study revealed that the air dried powdered leaves of *L. indica* are rich in carbohydrates and/or glycosides flavonoids, tannins alkaloids and/or nitrogenous compounds, sterols and triterpenes. Low levels of saponins and anthraquinones were reported while coumarins were totally absent Table 1.

Table1: Results of Preliminary Phytochemical Screening of *L. indica* Leaves

Constituents	*L. indica*
Carbohydrates&/or glycosides	++
Flavonoids	++
Carotenoids	++
Sterols &/or Triterpenes	++
Alkaloids &/or nitrogenous compounds	++
Tannins	++
Saponins	+
Anthraquinones	+
Coumarins	-

(+): Present (-): Absent (++): Appreciably present

3.2 *Determination of Flavonoids and Phenolics Content Using HPLC*

Five grams of the 80% ethanolic extract of *L. indica* was separately mixed with methanol then centrifuged at 10000 rpm (10 min) and the supernatant was filtered through 0.2 µm Millipore membrane filter then 1-3 ml was collected in a vial for injection into HPLC Agilent Packared (series1200). Retention time alongwith peak area were used to calculate flavonoids and phenolics concentration by data analysis of W-Agilent software resulting in the determination of constituents as shown in Tables 2&3.

Table2: Flavonoids identified from the total alcoholic extract (80%) of *L. indica* leaves

Compound	***L. indica* leaves extract**	
	Rt (min.)	**Conc. (mg/g dry weight)**
Luteolin-6-arabinose-8-glucose	6.48	2.53
Luteolin-6-glucose-8- arabinose	10.78	0.30
Apigenin-6-arabinose-8-glactose	11.70	0.43
Apigenin-6-rhamnose-8-glucose	12.05	0.49
Apigenin-6-glucose-8- rhamnose	12.14	3.13
Luteolin-7-glucose	12.26	0.86
Naringeen	12.49	0.80
Hisperidin	12.58	4.86
Rutin	13.27	0.92
Apigenin-7-O-neohespiroside	13.47	0.33
Kampferol-3,7-dirhamnoside	13.89	1.51
Quercetrin	14.01	1.54
Rosemarinic	14.19	0.13
Quercetin	14.91	0.22
Naringenin	15.10	0.30
Kampferol-3-(2- *p*-comaroyl)glucose	15.27	1.18
Hespertin	15.46	0.31
Kampferol	16.24	0.23
Rhamnetin	16.38	0.06
Apigenin	16.53	0.13
Apigenin-7-glucose	17.27	1.25
Acacetin	18.88	6.20
Total identified flavonoids	**27.71mg/g**	

Rt: Retention time in minutes.

Table 3: Phenolics identified from the total alcoholic extract (80%) of *L.indica* Leaves.

Compound	***L. indica* leaves extract**	
	Rt (min.)	**Conc. (mg/g dry weight)**
Pyrogallol	6.97	03.10
Gallic acid	7.11	00.03
4-Amino-benzoic acid	7.56	00.08
Protocatchuic acid	8.28	00.80
Catechin	8.37	00.37
Catechol	8.44	01.43
Epicatechin	8.66	00.15
p -Hydroxy benzoic acid	9.77	00.66
Chlorogenic acid	10.09	00.27
Vanillic acid	10.20	00.99
Caffeic acid	10.32	00.13
p -Coumaric acid	11.59	00.53
Ferulic acid	1.80	00.29
Iso-ferulic acid	12.41	00.56
e-Vanillic acid	12.87	17.40
α-Coumaric acid	13.24	00.70
Benzoic acid	13.31	03.65
Ellagic acid	13.47	31.48
3,4,5,-Methoxy-cinnamic acid	14.03	1.24
Cinnamic acid	15.26	00.03
Salycilic acid	16.39	00.68
Total identified Phenolics	64.75 mg/g	

Rt: Retention time in minutes.

In our investigation the total identified flavonoids were 22 compounds representing 27.71 mg/g dry weight of the total extract of which acacetin is first to be identified from *L. indica* recording (6.20 mg/g) as the major compound of the total flavonoid content followed by hesperidin (4.86 mg/g) and apigenin-6-O-glucose-8-rhamnose (3.13 mg/g). Regarding the results of total identified phenolics, 22 compounds representing 64.75 mg/g dry weight from whilemany phenolic acids were identified for the first time in this study from *L. indica*; ferulic acid, vanillic *p* -coumaric acid acid, cinnamic acid, *p* -hydroxy benzoic acid, caffeic acid, and *p* -amino benzoic acid. Ellagic acid was the main compound representing alone 31.48 mg/g followed by e-vanillic acid 17.40 mg/g alongside with other many phenolic compounds. These recordable numbers of compounds with generous amounts are very encouraging for the Anti-Alzheimer study as most of these compounds have very powerful antioxidant activities [22].

3.3 *Determination of Natural Pigment Content Using HPLC*

Moreover, Table 3 showed the total identified pigment content of the 80% ethanolic extract which recorded (112.22 mg/g), of which β-carotene was the major component (117 mg/g). It was previously proven in many studies that carotenoids has a crucial role in prevention of many neurodegenerative diseases of which Alzheimer's disease was the main target [23].

Table 4: Natural pigments identified from the total extract of *L. indica* Leaves

Compound	***L. indica* leaves extract**	
	Conc. (mg/g dry weight)	**Rt (min.)**
β-carotene	117	40.45
Chlorophyll A	3.58	16.07
Chlorophyll B	1.64	19.52
Total identified carotenoids	**112.22 mg/g**	

Rt: Retention time in minutes.

3.4 Biochemical analyses

Table 5 revealed insignificant change in NA, A and AChE levels in normal rats treated with total extract of *L. indica* as compared to normal untreated rats. AD induced rats showed significant decrease in NA and A levels by 52.90 and 54.88%, respectively, comparing to control rats. However, AD induced rats exhibited significant increase in AChE by 84.80 %, comparing to control rats. On the other hand, treatment of AD induced rats with total extract of *L. indica* declared improvement by 22.10, 30.04 and 50.60%, respectively, for NA, A and AChE comparing to standard drug which showed improvement percentages reached to 31.40, 34.44 and 55.96%, respectively.

Table 5: Effect of total extract of *L. indica* leaves on brain NA, A and AChE levels in AD induced rats.

Groups/parameters	**Noradrenaline (NA)**	**Adrenaline (A)**	**Acetylcholine esterase (AChE)**
Normal control	195.50±7.90^{a}	342.89±6.98^{a}	101.83± 2.12^{a}
Normal control treated total extract	195.28± 9.90^{a}	326.61± 4.89^{a}	94.12±2.90^{a}
% Change	0.11%	4.74	7.57
Alzheimer's disease	92.01± 2.87^{b}	154.70±4.90^{b}	188.21±3.54^{b}
% Change	52.9%	54.88	84.80
Alzheimer treated total extract	135.38±3.11^{c}	257.75±6.85^{c}	136.66±5.82^{c}
% Change	30.75	24.8	34.15
% Improvement	22.1	30.04	50.6
Alzheimer treated standard drug	153.44±5.55^{c}	272.98±5.87^{c}	131.22±2.22^{c}
% Change	21.51	20.18	29.9
% Improvement	31.4	34.49	55.96

- Data are expressed as ng/gm for NA and A.
- Data are expressed as ng/ml for AChE
- Data were analyzed by one way analysis of variance (ANOVA) using the Statistical Package for the Social Sciences (SPSS) program, version combined with co-state computer program and least significant difference (LSD) to compare significance between groups, where unshared letters are significant at P ≤0.05.

Table 6 demonstrated insignificant change in serotonin and dopamine levels in normal rats treated with *L. indica* comparing to control untreated rats. Significant reduction in serotonin and dopamine levels was detected by 59.84 and 45.81 %, respectively in AD induced rats. Treatment of AD rats with total extract of *L. indica* exhibited marked improvement comparing to AD induced rats with amelioration percentages 30.56 and 19.12%, respectively. Obvious improvement in serotonin and dopamine levels upon treating AD rats was observed with percentages 35.70 and 34.68%, respectively.

Table 6: Effect of total extract of *L. indica* leaves on serotonin and dopamine levels in AD induced rats

Groups/parameters	**Serotonin**	**Dopamine**
Normal control	91.84±4.76[a]	68.51±3.43[a]
Normal treated total extract	94.23±8.55[a]	67.63±4.77[a]
%Change	2.6	1.28
Alzheimer 's disease	36.88±2.86[b]	37.12±1.90[b]
%Change	59.84	45.81
Alzheimer treated total extract	64.95±3.61[c]	50.22±3.55[c]
%Change	29.27	26.69
% Improvement	30.56	19.12
Alzheimer' s treated standard drug	69.70±5.87[c]	60.88±5.12[c]
%Change	24.10	11.13
% Improvement	35.70	34.68

- Data are expressed as ng/gm.
- Data were analyzed by one way analysis of variance (ANOVA) using the Statistical Package for the Social Sciences (SPSS) program, version combined with co-state computer program and least significant difference (LSD) to compare significance between groups, where unshared letters are significant at $P \leq 0.05$.

Table 7 clearly demonstrated that, insignificant change in caspase -3, protein carbonyl and amyloid β in sera of normal control rats treated with *L.indica* total extract comparing to untreated control rats. AD induced rats declared significant incease in caspase -3, protein carbonyl and amyloid β by 95.00, 144.40 and 500.00%, respectively. AD treated rats with total extract of *L. indica* showed significant decrease in caspase -3, protein carbonyl as well as amyloid β by improvement percentages 39.04, 179.27 and 216.00%, respectively, comparing to 56.19 , 180.77 and 274.40%, respectively, for standard drug.

Table 7: Effect of total extract of *L. indica* leaves on caspase -3, protein carbonyl and amyloid β in AD induced rats

Groups/ parameters	Caspase-3	Protein carbonyl	Amyloid β
Normal control rats	8.09±0.87[a]	4.68±0.45[a]	3.85±0.45
Normal control total extract	7.95±0.60[a]	4.94± 0.22[a]	4.00±0.03[a]
%Change	1.73	5.55	3.89
Alzheimer 's disease	15.78±1.10[b]	11.44±1.33[b]	23.12±2.12[b]
%Change	95.00	144.40	500.50

Data are expressed as ng/gm.

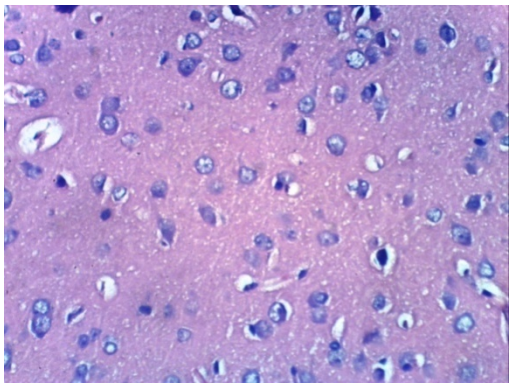
Data were analyzed by one way analysis of variance (ANOVA) using the Statistical Package for the Social Sciences (SPSS) program, version combined with co-state computer program and least significant difference (LSD) to compare significance between groups, where unshared letters are significant at $P \leq 0.05$.

3.5 Histopathological Examination of Cerebral Cortex:

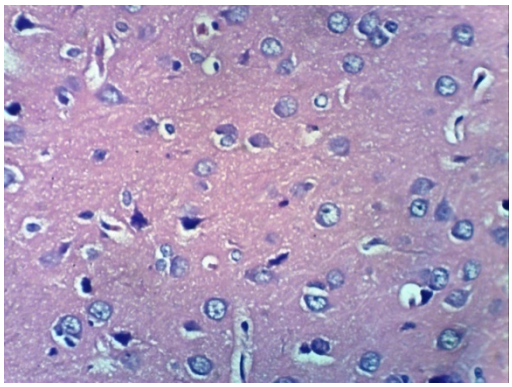
Microscopically, cerebral cortex of control, untreated rats revealed no histopathological changesas compared to normal control rats (Photomicrographs 1). Also, the cerabral cortex of treated rats with *L. indica* showed no histopathological changes (Photomicrographs 2) comparing to normal control rats. Meanwhile, AD induced rats showed necrosis, atrophy and pyknosis of neurons in two different brain sections (Photomicrograph 3a. and 3b.) and focal gliosis (Photomicrograph 4). Cerebral cortex of rat treated with *L.indica* total extract showed necrosis of sporadic neurons (Photomicrographs 5a. and 5b.); cerebral cortex of AD rats treated with standard drug showed necrosis of sporadic neurons (Photomicrograph 6).

3.6 Histopathological Examination of Hippocampus:

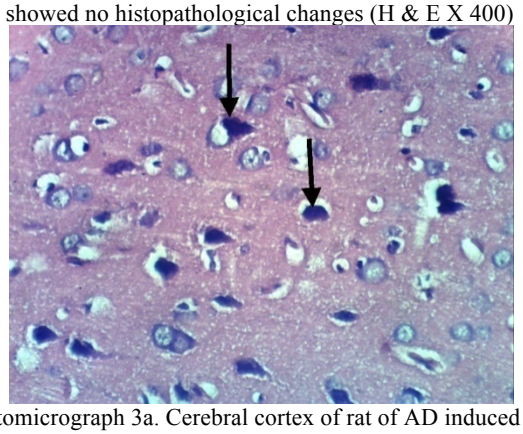
Photomicrograph 2 showed hippocampus of control treated rats with total extract of *L. indica* showed no histopathological changes. Photomicrograph 3 Hippocampus of AD rats showed necrosis of pyramidal cells. Meanwhile, Hippocampus of AD rats treated with *L. indica* showed necrosis of some pyramidal cells (Photomicrographs 4a.-4c.). Hippocampus of AD rats treated with standard drug showed necrosis of some pyramidal cells (Photomicrographs 5a.and 5b.).



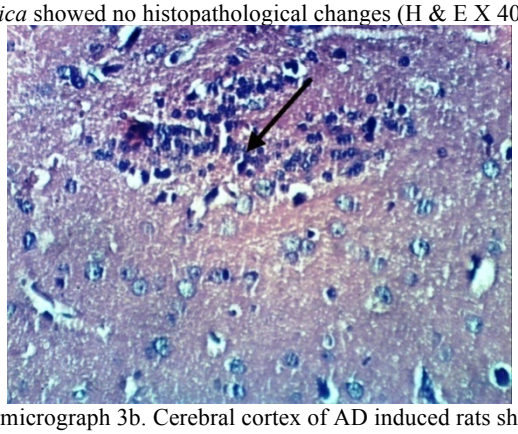
Photomicrograph 1: Cerebral cortex of control, untreated rat showed no histopathological changes (H & E X 400)



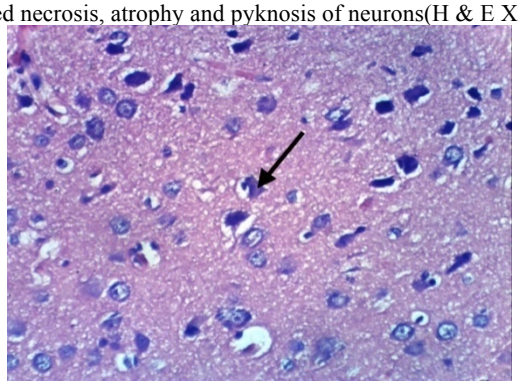
Photomicrograph 2: Cerebral cortex of control treated rats with *L. indica* showed no histopathological changes (H & E X 400).



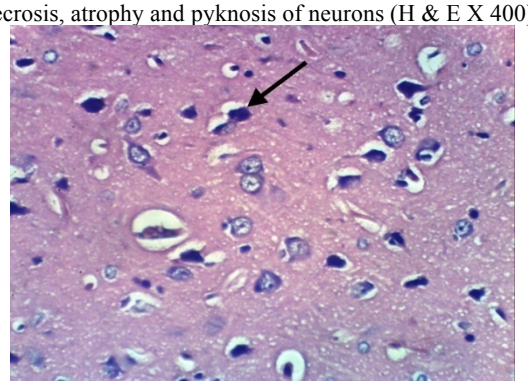
Photomicrograph 3a. Cerebral cortex of rat of AD induced rats showed necrosis, atrophy and pyknosis of neurons(H & E X 400).



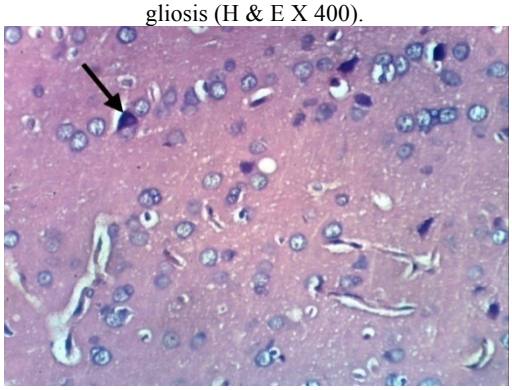
Photomicrograph 3b. Cerebral cortex of AD induced rats showed necrosis, atrophy and pyknosis of neurons (H & E X 400).



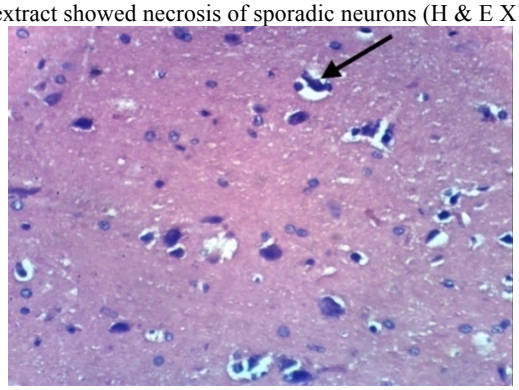
Photomicrograph 4: Cerebral cortex of AD rats showed focal gliosis (H & E X 400).



Photomicrograph 5a. Cerebral cortex of rat treated with *L. indica* total extract showed necrosis of sporadic neurons (H & E X 400).

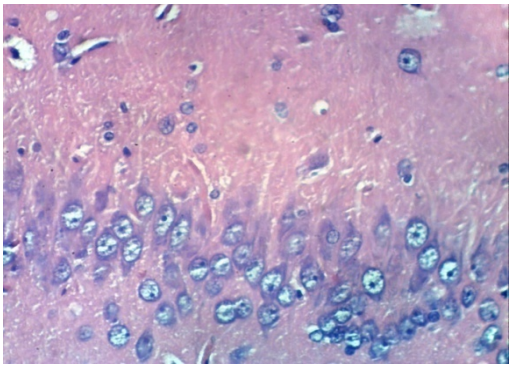


Photomicrograph 5b. Cerebral cortex of AD rats treated with *L. indica* total extract) showed necrosis of sporadic neurons (H & E X 400).

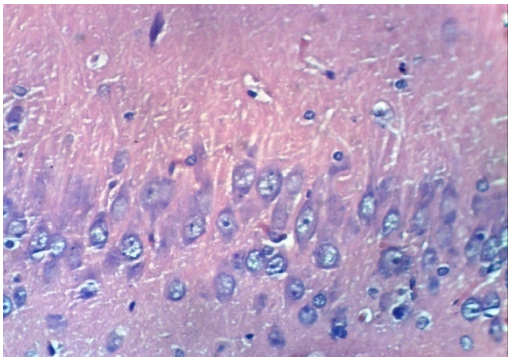


Photomicrograph 6: Cerebral cortex of AD rats treated with standard drug showed necrosis of sporadic neurons (H & E X 400).

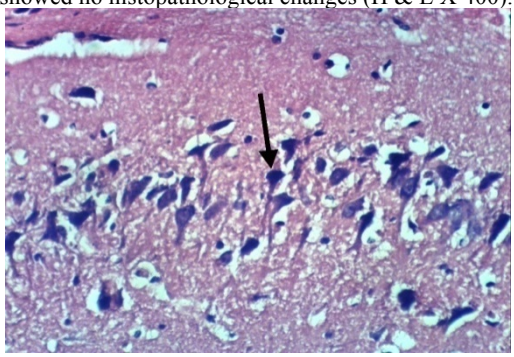
Histopathological examination of hippocampus:



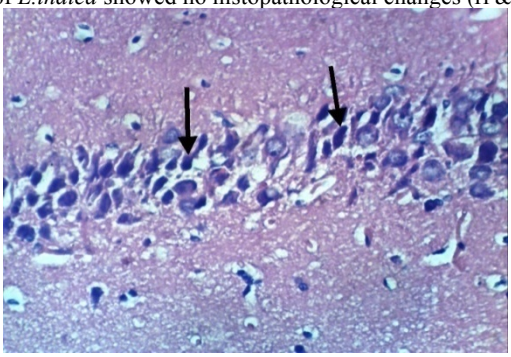
Photomicrograph 1: Hippocampus of control, untreated rat showed no histopathological changes (H & E X 400).



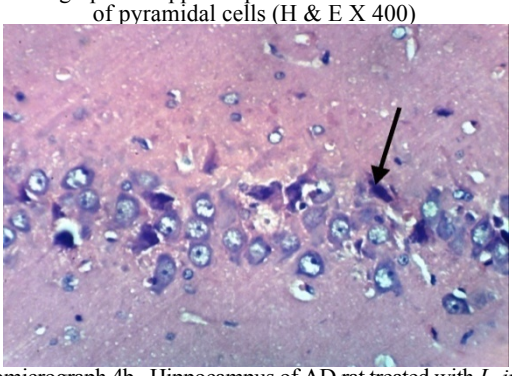
Photomicrograph 2: Hippocampus of control treated rats with total extract of *L.indica* showed no histopathological changes (H & E X 400).



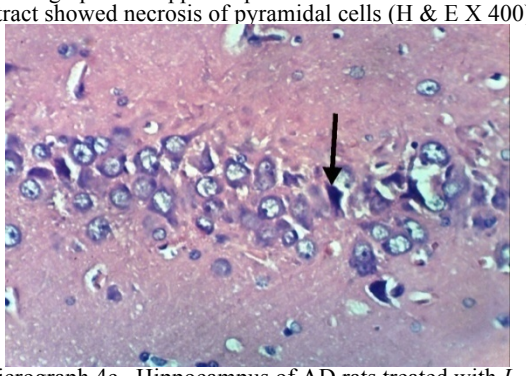
Photomicrograph 3: Hippocampus of AD rats showed necrosis of pyramidal cells (H & E X 400)



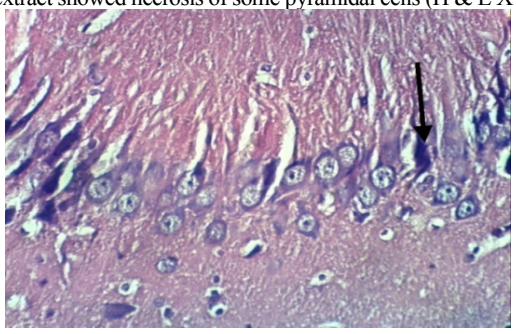
Photomicrograph 4a. Hippocampus of AD rats with *L. indica* total extract showed necrosis of pyramidal cells (H & E X 400).



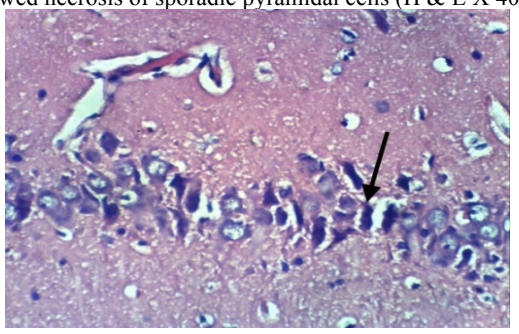
Photomicrograph 4b. Hippocampus of AD rat treated with *L. indica* total extract showed necrosis of some pyramidal cells (H & E X 400).



Photomicrograph 4c. Hippocampus of AD rats treated with *L. indica* showed necrosis of sporadic pyramidal cells (H & E X 400).



Photomicrograph 5a. Hippocampus of AD rats treated with standard drug showed necrosis of some pyramidal cells (H & E X 400).



Photomicrograph 5b. Hippocampus of AD rats treated with standard showed necrosis of some pyramidal cells (H & E X 400).

Discussion

Aluminium (Al) represents an important risk factor for several age-associated neurodegenerative disorders, including AD; ($AlCl_3$) is a neurotoxicant that accumulates in hippocampus and negatively affecting ionic, cholinergic, and dopaminergic neurotransmission [24], as well as it stimulates functional alterations in the cholinergic, dopaminergic and noradrenergic neurotransmission; therefore, it causes impaired cholinergic transmission by affecting the synthesis and release of neurotransmitters. Impaired cholinergic transmission occurs by two pathways: One of them includes elevated AchE activity at synapse by accelerated decomposition of available ACh, this degradation of ACh is abolished by (AChE inhibitor) and, thus, is found effective in AD

through improvement in cholinergic transmission [25]. Virtually, Al exposure increased ROS formation and thus the levels of free radicals thus decreased AChE activity [26]. The current data showed that administration of $AlCl_3$ induced cholinergic impairment in AD-induced rats; represented by marked decrease in brain and serum Ach content, while, brain and serum AChE activity in the AD-group significantly increased, as compared to control group. These results run in concord with [27].

Two different biomarkers of free-radical damage versus protein have been suggested: protein oxidation that leads to the production of protein carbonyls and protein peroxidation that causes the production of nitrated protein [28], [29], [30] and [33]. As a peripheral marker of oxidative stress in the brain, higher serum/plasma protein carbonyls level in AD is demonstrated in several studies despite varying patient selection criteria [31] and [32]. The present results declared significant increase in PC tissue of AD induced rats. Protein carbonyl level in CSF is universally significantly higher in AD than in controls [34]. This present study showed significant increase in AChE, and reduction in NA, AD, DA, 5-HT in AD induced rats. [35] reported increased AChE activity in Al-overloaded rats. [36] suggested that Al exposure increased AChE activity *via* allosteric interaction between Al and the peripheral anionic site of the enzyme molecule, leading to the etiology of AD pathological deterioration. All exerts cholinotoxic effects by impairing the activities of choline acetyl transferase (ChAT) itself or blocking the provision of acetyl-CoA, which is required for Ach synthesis [37]. The high activity of acetylcholine esterase, has been found to increase AchE activity and accelerate the assembly of β-amyloid into fibrils as well as lowering the acetylcholine level in brain tissues [38].

In addition, the current results showed a significant decrease in catecholamine level in AD induced rats as compared to normal control one. In agreement with the present study [39] declared that there has been an interaction between catecholamine and pathological amyloid proteins in neurodegenerative diseases. In addition, [40] hypothesized that dopamine is depleted by 60–70%, due to dopaminergic neuronal degeneration. Therefore, it has been suggested that oxidative stress–induced degeneration in adrenergic neurons is the main cause of down-regulation of adrenaline level in the brain as shown in the present results. The dysfunctions that occur in the cholinergic system in AD's disease are accompanied by selective degeneration of cholinergic neurons in the cortex, hippocampus and base of the forebrain. This has high significance because such acetylcholine-containing neurons play a key role in memory and affect the highest levels of cognitive functioning. The brains of Alzheimer's patients contain low dopamine, serotonin and norepinephrine than those of controls [41]

Concerning neurotransmitters function, researchers have focused on the basal forebrain cholinergic system and some of the neurotransmitters that originate in the midbrain [42]. The authors added that decreased choline acetyltransferase activity increased accumulation of extracellular β-amyloid plaques and increased accumulation of neurofibrillary tangles were demonstrated as neuropathologic factors of AD's disease. Chronic neuroinflammation may contribute to the initial stages of cellular dysfunction, lead to a decline in choline acetyltransferase activity, deplete acetylcholine, and determine the neuroanatomy of the pathology or the appearance of activated microglia [42] and [37].

Histopathological examination of either cerebral cortex or hippocampus revealednecrosis, atrophy and pyknosis of neurons and focal gliosis. Also, hippocampus of AD rats showed necrosis of pyramidal cells. On the other hand, cerebral cortex of AD rats treated with *L. indica* total extract or standard drug showed necrosis of sporadic neurons and neuronophagia of necrotic neurons. Meanwhile, hippocampus of AD rats treated with *L. indica* or standard drug showed necrosis of pyramidal cells in hippocampus. The present results are in accordance with [38], who noticed the cerebellum and hippocampus of AD rats in the revealed necrosis of neurons and multifocal cerebral hemorrhage in addition to necrosis of pyramidal cells, neuronophagia of sporadic necrotic neurons and necrosis in the cerebellum as well as some necrotic pyramidal cells. In accordance with the present results, [37] studied the severe congestion in the blood vessels with oedema in the meninges of AD-induced rats. Beside, a micrograph of a brain section of AD-induced rats treated with standard drug, in our investigation, showed also that the same results as *L. indica* total extract treated AD rats. Hence, the amelioration effect of the total extract may be based upon preservation of the normal neuronal cells and normalization in blood congestion and maintaining the tissue architecture. This may be reflected on the improvement of all the studied biomarkers levels.

On the other hand, the ameliorative signs which are noticed in neurotransmitters, antioxidant and oxidative stress as a result of treatment of Alzheimer induced rats with *L. indica* as compared to untreated diseased one, may be explained on the basis of the active constituents of the extracts. A diversity of researches asserted the influential contribution of carotenoids, phenolics and flavonoids in protection and prohibition of many degenerative diseases controlled by oxidative stress [43] and [22]. Recent study proved the importance of the phenolic acids such as (ferulic acid, cinnamic acid, e-vanillic acid, *p* -hydroxy benzoic acid *p* -coumaric acid and caffeic acid) , which are firstly identified in our current study, as potent anti-AD's through inhibition of acetyl- and butyrylcholinesterase responsible for the rapid hydrolysis of acetylcholine in cholinergic synapses.

Also ferulic acid was established as an inhibitor for amyloid structures which are responsible for AD's disease [44]; [45] and [1]. Several studies have linked the intake of some flavonoids including hesperidin to dementia and Alzheimer's disease [46] and [47]. Acacetin which is the main identified flavonoid proved to have inhibitory effect of amyloid-beta (Aβ) that are crucially involved in Alzheimer's disease. Further, hesperidin and hesperetin have also demonstrated the ability to cross the blood brain barrier making them ideal candidates in the natural treatment of different central nervous system disorders [48]. The neuroprotective effect of hesperidin has been widely studied in the last decade and mainly attributed to its antioxidant and anti-inflammatory properties as seen by an increased level of antioxidant enzymes, decreased level of oxidative stress, inflammatory markers and pro-apoptotic proteins in neuron. The findings from *in vitro* and *in vivo* studies have demonstrated that hesperidin is able to attenuate reduction in levels of cellular antioxidant enzymes [49].

Many findings have proven that gallic acid has powerful antioxidant effect as well as ameliorating effects on behavioral impairments as GA pretreatment improved cerebral ischemia/reperfusion injury [50]. Moreover, apigenin suppressed mitogen-activated protein kinase with no effect on the activity of extracellular signal-regulated kinase. It also alleviated AD-associated memory impairment through relieving Aβ burden, suppressing amyloidogenic process, suppresing oxidative stress, so it protects neuronal cells from injury in middle cerebral artery occlusion rats and cerebroprotective action and represents an alternative medication for the AD prevention and therapy [51] and [52].

Catechin a well-known polyphenol, thought to be radical scavengers, is now considered to invoke a spectrum of cellular mechanisms of action related to their neuroprotective activity. Therefore, these compounds have proven to exert therapeutic cytoprotective effect for the treatment of neurodegenerative and other diseases [53]. The differential effects of quercetin and rutin on cerebral ischemia was determined as well as their effects on neuronal death and spatial memory impairment in rats. The effects of luteolin was deeply linked to the modulation of antioxidant, anti-diabetic, anti-AD, and anti-inflammatory effects of luteolin and some of its *C*-glycosides [54]. Many flavanols (catechin, epicatechin and epigallocatechin), gallic acid with its dimer (ellagic acid), flavonols (quercetin and kaempferol), flavonol glycosides (astragalin, quercitrin and isoquercitrin) and flavonol acylated glycosides (astragalin 6"-gallate and isoquercitrin6"-gallate) all inhibited acetylcholinesterase activity in scopolamine-induced memory impairment of mice, proving that they have anti-Alzheimer's activity [55].

Conclusion

According to this study, the curative effect of *L. indica* may be attributed to the strong antioxidant capacity related to the high carotenoid, phenolic and flavonoid contents. Hence, it could be concluded that, *L. indica* exhibited neuro-modulating effect in Al -induced neurotoxicity that could be used in a future as a promising candidate nutraceuticals.

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Conflict of interest - The authors declare that, there are no conflicts of interest.

References

1. A. Sgarbossa, D. Giacomazza, M. Di Carlo. *Nutrients*. 7 (2015) 5764-5782.
2. M.C. de Rijk, M.M. Breteler, J.H. den Breeijen, L.J. Launer, D.E. Grobbee, F.G. van der Meché, A. Hofman. *Arch Neurol* 54 (1997) 762-765.
3. M.J. Engelhart, M.I. Geerlings, A. Ruitenberg, J.C. van Swieten, A. Hofman, J.C. Witteman, M.M. Breteler. *Jama*. 287 (2002) 3223-3229.
4. M.C. Morris, D.A. Evans, J.L. Bienias, C.C. Tangney, D.A. Bennett, N. Aggarwal, R.S. Wilson, P.A. Scherr. *Jama*. 287 (2002) 3230-3237.
5. Y. Diab, K. Atalla, K. Elbanna. *Drug discoveries & therapeutics*. 6 (2012) 212-217.
6. N.O. Anderson, *Flower breeding and genetics: issues, challenges and opportunities for the 21st century*. Springer Science & Business Media. (2006)
7. D.R. Egolf. *HortScience (USA)*. (1987).
8. R. Labib, N. Ayoub, A. Singab, M. Al-Azizi, A. Sleem. *Phytopharmacology*. 4 (2013) 373-389.
9. I. Smith. *Chromatographic and electrophoretic techniques. Vol. II. Zone electrophoresis.* (1960).
10. E. Stahl ,E. Dumont. *J Chromatogr Sci*. 7 (1969) 517-525.
11. H. Molisch, (1886). Monatsh. Chem., 7, through Stank, J.; Gerny, M., Kocourek, J. and Pacak, J. , *The Monosaccharides", 872,* . Publishing House of Czechoslovak Academy of Science, Prague. (1963)
12. T. Siong ,L.C. Lam. *ASEAN Food Journal*. 7 (1992) 91-99.
13. P. Mattila, J. Astola, J. Kumpulainen. *J. Agric. Food Chem*. 48 (2000) 5834-5841.
14. P. Goupy, M. Hugues, P. Boivin, M.J. Amiot. *J. Sci. Food Agric.* 79 (1999) 1625-1634.

15. V. Kumar, A. Bal, K.D. Gill. *Toxicology*. 264 (2009) 137-144.
16. H.A. Fernando, H.-F. Chin, S.H. Ton, K. Abdul Kadir. *J Diabetes Res.* 2013 (2013), 18 pages, doi:10.1155/2013/190395.
17. J. Zagrodzka, A. Romaniuk, M. Wieczorek, P. Boguszewski. *Acta neurobiologiae experimentalis*. 60 (2000) 333-344.
18. G. Wen, W. Hui, C. Dan, W. Xiao-Qiong, T. Jian-Bin, L. Chang-Qi, L. De-Liang, C. Wei-Jun, L. Zhi-Yuan, L. Xue-Gang. *Acta histochemica et cytochemica*. 42 (2009) 137-142.
19. P.L. Zusterzeel, W. Visser, W.H. Peters, H.W. Merkus, W.L. Nelen, E.A. Steegers. *Obstet Gynecol* 96 (2000) 50-54.
20. C.B. Thompson. *Science*. 267 (1995) 1456-1462.
21. R. Drury ,W. EAC, *Coreleton's Histological technique 4th edition*. Oxford, Oxford University Press. (1980).
22. H. Kagechika ,K. Shudo. *J Med Chem*. 48 (2005) 5875-5883.
23. B. Shanmuganathan ,K. Pandima Devi. *Eur J Phycol*. 51 (2016) 482-490.
24. C. Kumar, A. Igbaria, B. D'autreaux, A.G. Planson, C. Junot, E. Godat, A.K. Bachhawat, A. Delaunay-Moisan, M.B. Toledano. *EMBO J.* 30 (2011) 2044-2056.
25. M. Nampoothiri, J. John, N. Kumar, J. Mudgal, G.K. Nampurath, M.R. Chamallamudi. *Behav Neurol*. vol. 2015, (2015) 9 pages, 2015. doi:10.1155/2015/210169.
26. N. Singla ,D. Dhawan. *J. Neurosci. Res.* 90 (2012) 698-705.
27. K. Taïr, O. Kharoubi, O.A. Taïr, N. Hellal, I. Benyettou, A. Aoues. *JAD*. 5 (2016) 470-482.
28. S. Aldred, S. Bennett, P. Mecocci. *Clinical biochemistry*. 43 (2010) 267-271.
29. F. Sinem, K. Dildar, E. Gokhan, B. Melda, Y. Orhan, M. Filiz. *Curr. Alzheimer Res*. 7 (2010) 463-469.
30. Y.-T. Chang, W.-N. Chang, N.-W. Tsai, C.-C. Huang, C.-T. Kung, Y.-J. Su, W.-C. Lin, B.-C. Cheng, C.-M. Su, Y.-F. Chiang. *BioMed Res. Int.* vol. 2014, (2014), 14 pages. doi:10.1155/2014/182303.
31. Z.-y. CAI, Y. Yong, Y. Ling, F.-y. Wang, H. Huang, Y.-l. Wang, Y. Zhao. *Journal of Medical Colleges of PLA*. 22 (2007) 352-356.
32. D.O. Cristalli, N. Arnal, F.A. Marra, M.J. de Alaniz, C.A. Marra. *J. Neurol. Sci.* 314 (2012) 48-56.
33. A. Nunomura, T. Tamaoki, N. Motohashi, M. Nakamura, D.W. McKeel Jr, M. Tabaton, H.-g. Lee, M.A. Smith, G. Perry, X. Zhu. *J Neuropathol Exp Neurol*. 71 (2012) 233-241.
34. M. Korolainen ,T. Pirttilä. *Acta Neurol Scand*. 119 (2009) 32-38.
35. J. Zhang, J.-Q. Yang, B.-C. He, Q.-X. Zhou, H.-R. Yu, Y. Tang, B.-Z. Liu. *Saudi Med. J.* 30 (2009) 760-66
36. R. Kaizer, M. Correa, L. Gris, C. Da Rosa, D. Bohrer, V. Morsch, M.R.C. Schetinger. *Neurochem. Res*. 33 (2008) 2294-2301.
37. H.F. Aly, F.M. Metwally, H.H. Ahmed. *Acta Biochim Pol*. 58 (2011) 513-520.
38. A.H.F. El-Baz F.K., Ali G. H. . *Int J Pharm PharmSci,* . 9 (2017) 198-206.
39. D.K.-. Murray I. *Alzheimers Association*. 7 (2011) 392-393.
40. H. Sawada, T. Oeda, K. Yamamoto. *Diagnostics*. 3 (2013) 210-221.
41. H.D. Foster. *J Orthomol Med*. 15 (2000) 21-51.
42. G.L. Wenk. *J Clin Psychiatry*. 64 (2003) 7-10.
43. H. Kagechika. *Curr. Med. Chem*. 9 (2002) 591-608.
44. A.A. Rao, G.R. Sridhar, U.N. Das. *Med Hypotheses* 69 (2007) 1272-1276.
45. D. Szwajgier. *Z. Naturforsch. C Bio. Sci*. 68 (2013) 125-132.
46. G.P. Gil, D.S. Ramirez, C.J. Ribera. *J Nutr Health Aging* 7(2003) 304-308.
47. X. Gao, A. Cassidy, M. Schwarzschild, E. Rimm, A. Ascherio. *Neurology*. 78 (2012) 1138-1145.
48. W. Dimpfel. *J Pharm Pharmacol* 58 (2006) 375-379.
49. K. Tamilselvam, J. Nataraj, U. Janakiraman, T. Manivasagam, M.M. Essa. *Int J Nutr Pharmacol Neurol Dis*. 3 (2013) 294.
50. Y. Farbood, A. Sarkaki, S. Hashemi, M.T. Mansouri, M. Dianat. *Avicenna J Phytomed* 3(2013) 329.
51. S.K. Ha, P. Lee, J.A. Park, H.R. Oh, S.Y. Lee, J.-H. Park, E.H. Lee, J.H. Ryu, K.R. Lee, S.Y. Kim. *Neurochem Int*. 52 (2008) 878-886.
52. L. Zhao, J.-L. Wang, R. Liu, X.-X. Li, J.-F. Li, L. Zhang. *Molecules*. 18 (2013) 9949-9965.
53. S. Mandel ,M.B. Youdim. *Free Radic Biol Med* 37 (2004) 304-317.
54. J.S. Choi, M.N. Islam, M.Y. Ali, Y.M. Kim, H.J. Park, H.S. Sohn, H.A. Jung. *Arch Pharm Res* 37 (2014) 1354-1363.
55. A. Nugroho, B.-M. Song, S.H. Seong, J.S. Choi, J. Choi, J.-Y. Choi, H.-J. Park. *Nat. Prod. Sci.* 22 (2016) 299-306